

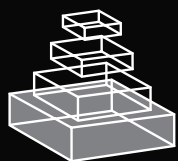
frontiers

RESEARCH TOPICS



THE MICROBIAL REGULATION OF GLOBAL BIOGEOCHEMICAL CYCLES

Topic Editors
Johannes Roušk and Per Bengtson



frontiers in
MICROBIOLOGY



frontiers

FRONTIERS COPYRIGHT STATEMENT

© Copyright 2007-2014
Frontiers Media SA.
All rights reserved.

All content included on this site, such as text, graphics, logos, button icons, images, video/audio clips, downloads, data compilations and software, is the property of or is licensed to Frontiers Media SA ("Frontiers") or its licensees and/or subcontractors. The copyright in the text of individual articles is the property of their respective authors, subject to a license granted to Frontiers.

The compilation of articles constituting this e-book, wherever published, as well as the compilation of all other content on this site, is the exclusive property of Frontiers. For the conditions for downloading and copying of e-books from Frontiers' website, please see the Terms for Website Use. If purchasing Frontiers e-books from other websites or sources, the conditions of the website concerned apply.

Images and graphics not forming part of user-contributed materials may not be downloaded or copied without permission.

Individual articles may be downloaded and reproduced in accordance with the principles of the CC-BY licence subject to any copyright or other notices. They may not be re-sold as an e-book.

As author or other contributor you grant a CC-BY licence to others to reproduce your articles, including any graphics and third-party materials supplied by you, in accordance with the Conditions for Website Use and subject to any copyright notices which you include in connection with your articles and materials.

All copyright, and all rights therein, are protected by national and international copyright laws.

The above represents a summary only. For the full conditions see the Conditions for Authors and the Conditions for Website Use.

ISSN 1664-8714

ISBN 978-2-88919-297-7

DOI 10.3389/978-2-88919-297-7

ABOUT FRONTIERS

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

FRONTIERS JOURNAL SERIES

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing.

All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

DEDICATION TO QUALITY

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.

Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.

By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

WHAT ARE FRONTIERS RESEARCH TOPICS?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area!

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

THE MICROBIAL REGULATION OF GLOBAL BIOGEOCHEMICAL CYCLES

Topic Editors:

Johannes Rousk, Lund University, Sweden

Per Bengtson, Lund University, Sweden



Earth - the scene for the microbial regulation of biogeochemical cycles. Credit: Visible Earth, NASA. Image by: Stöckli, Nelson, Hasler. Laboratory for Atmospheres, Goddard Space Flight Center <http://rsl.gsfc.nasa.gov/rsl>

Global biogeochemical cycles of carbon and nutrients are increasingly affected by human activities. So far, modeling has been central for our understanding of how this will affect ecosystem functioning and the biogeochemical cycling of carbon and nutrients. These models have been forced to adopt a reductive approach built on the flow of carbon and nutrients between pools that are difficult or even impossible to verify with empirical evidence. Furthermore, while some of these models include the response in physiology, ecology and biogeography of primary producers to environmental change, the microbial part of the ecosystem is generally poorly represented or lacking altogether.

The principal pool of carbon and nutrients in soil is the organic matter. The turnover of this reservoir is governed by microorganisms that act as catalytic converters of environmental conditions into biogeochemical cycling of carbon and nutrients. The dependency of this conversion activity on individual environmental conditions such as pH, moisture and temperature has been frequently studied. On the contrary, only rarely have the microorganisms involved in carrying out the processes been identified, and one of the biggest challenges for advancing our understanding of biogeochemical processes is to identify the microorganisms carrying out a specific set of metabolic processes and how they partition their carbon and nutrient use. We also need to identify the factors governing these activities and if they result in feedback mechanisms that alter the growth, activity and interaction between primary producers and microorganisms. By determining how different groups of microorganisms respond to individual environmental conditions by allocating carbon and nutrients to production of biomass, CO₂ and other products, a mechanistic as well as quantitative understanding of formation and decomposition of organic matter, and the production and consumption of greenhouse gases, can be achieved.

In this Research Topic, supported by the Swedish research councils' programme "Biodiversity and Ecosystem Services in a Changing Landscape" (BECC), we intend to promote this alternative framework to address how cycling of carbon and nutrients will be altered in a changing environment from the first-principle mechanisms that drive them – namely the ecology, physiology and biogeography of microorganisms – and on up to emerging global biogeochemical patterns. This novel and unconventional approach has the potential to generate fresh insights that can open up new horizons and stimulate rapid conceptual development in our basic understanding of the regulating factors for global biogeochemical cycles. The vision for the research topic is to facilitate such progress by bringing together leading scientists as proponents of several disciplines. By bridging Microbial Ecology and Biogeochemistry, connecting microbial activities at the micro-scale to carbon fluxes at the ecosystem-scale, and linking above- and belowground ecosystem functioning, we can leap forward from the current understanding of the global biogeochemical cycles.

Table of Contents

- 06 *Microbial Regulation of Global Biogeochemical Cycles***
Johannes Rousk and Per Bengtson
- 09 *Bacterial Chitin Degradation—Mechanisms and Ecophysiological Strategies***
Sara Beier and Stefan Bertilsson
- 21 *Field and Lab Conditions Alter Microbial Enzyme and Biomass Dynamics Driving Decomposition of the Same Leaf Litter***
Zachary L. Rinkes, Robert L. Sinsabaugh, Daryl L. Moorhead, A. Stuart Grandy and Michael N. Weintraub
- 35 *Dynamic Relationships Between Microbial Biomass, Respiration, Inorganic Nutrients and Enzyme Activities: Informing Enzyme-Based Decomposition Models***
D. L. Moorhead, Z. L. Rinkes, R. L. Sinsabaugh and M. N. Weintraub
- 47 *Environmental impacts on the Diversity of Methane-Cycling Microbes and their Resultant Function***
Emma L. Aronson, Steven D. Allison and Brent R. Helliker
- 62 *Controls on Bacterial and Archaeal Community Structure and Greenhouse Gas Production in Natural, Mined, and Restored Canadian Peatlands***
Nathan Basiliko, Kevin Henry, Varun Gupta, Tim R. Moore, Brian T. Driscoll and Peter F. Dunfield
- 76 *Metabolic Adaptation and Trophic Strategies of Soil Bacteria—C1- Metabolism and Sulfur Chemolithotrophy in Starkeya Novella***
Ulrike Kappler and Amanda S. Nouwens
- 88 *A Meta-Analysis of Soil Microbial Biomass Responses to Forest Disturbances***
Sandra R. Holden and Kathleen K. Treseder
- 105 *Microbial Responses to Multi-Factor Climate Change: Effects on Soil Enzymes***
J. Megan Steinweg, Jeffrey S. Dukes, Eldor A. Paul and Matthew D. Wallenstein
- 116 *Thermal Adaptation of Decomposer Communities in Warming Soils***
Mark A. Bradford
- 132 *Controls on Soil Microbial Community Stability Under Climate Change***
Franciska T. de Vries and Ashley Shade
- 148 *Plant Soil Interactions Alter Carbon Cycling in an Upland Grassland Soil***
Bruce C. Thomson, Nick J. Ostle, Niall P. McNamara, Simon Oakley, Andrew S. Whiteley, Mark J. Bailey and Robert I. Griffiths
- 160 *Off-Season Biogenic Volatile Organic Compound Emissions From Heath Mesocosms: Responses to Vegetation Cutting***
Riikka Rinnan, Diana Gierth, Merete Bilde, Thomas Rosenørn and Anders Michelsen

- 170** *Effects of **Spartina Alterniflora** Invasion on the Communities of Methanogens and Sulfate-Reducing Bacteria in Estuarine Marsh Sediments*
Jemaneh Zeleke, Qiang Sheng, Jian-Gong Wang, Ming-Yao Huang, Fei Xia, Ji-Hua Wu and Zhe-Xue Quan
- 183** *Rhizosphere Priming: A Nutrient Perspective*
Feike A. Dijkstra, Yolima Carrillo, Elise Pendall and Jack A. Morgan
- 191** *Stoichiometric Imbalances Between Terrestrial Decomposer Communities and their Resources: Mechanisms and Implications of Microbial Adaptations to their Resources*
Maria Mooshammer, Wolfgang Wanek, Sophie Zechmeister-Boltenstern and Andreas Anatol Richter
- 201** *Moss-Cyanobacteria Associations as Biogenic Sources of Nitrogen in Boreal Forest Ecosystems*
Kathrin Rousk, Davey L. Jones and Thomas H. DeLuca
- 211** *Microbes in Nature are Limited by Carbon and Energy: The Starving-Survival Lifestyle in Soil and Consequences for Estimating Microbial Rates*
John E. Hobbie and Erik A. Hobbie
- 222** *Specificity of Plant-Microbe Interactions in the Tree Mycorrhizosphere Biome and Consequences for Soil C Cycling*
Carolyn Churchland and Sue J. Grayston



Microbial regulation of global biogeochemical cycles

Johannes Rousk* and Per Bengtson

Department of Biology/Microbial Ecology, Lund University, Lund, Sweden

*Correspondence: johannes.rous@biol.lu.se

Edited by:

Lisa Y. Stein, University of Alberta, Canada

Reviewed by:

Pierre Offre, University of Vienna, Austria

Keywords: microbial ecology, biogeochemistry, stoichiometry, climate change, soil microbiology, elemental fluxes, respiration, aquatic microbiology

Global biogeochemical cycles of carbon and other nutrients are increasingly affected by human activities (Griggs et al., 2013). So far, modeling has been central for our understanding of how this will affect ecosystem functioning and the biogeochemical cycling of elements (Treseder et al., 2012). These models adopt a reductive approach built on the flow of elements between pools that are difficult or even impossible to verify with empirical evidence. Furthermore, while some of these models include the response in physiology, ecology and biogeography of primary producers to environmental change, the microbial part of the ecosystem is generally poorly represented or lacking altogether (Stein and Nicol, 2011; Treseder et al., 2012).

The principal pool of carbon and other nutrients in soil is the organic matter (Schimel, 1995). The turnover time of this reservoir is governed by the rate at which microorganisms consume it. The rate of organic matter degradation in a soil is determined by both the indigenous microbial community and the environmental conditions (e.g., temperature, pH, soil water capacity, etc.), which govern the biogeochemical activities of the microorganisms (Waksman and Gerretsen, 1931; Schmidt et al., 2011). The dependences of these biogeochemical activity rates on environmental conditions such as pH, moisture and temperature have been frequently studied (Conant et al., 2011; Schmidt et al., 2011). However, while various microorganisms involved in carrying out biogeochemical processes have been identified, biogeochemical process rates are only rarely measured together with microbial growth, and one of the biggest challenges for advancing our understanding of biogeochemical processes is to systematically link biogeochemistry to the rate of specific metabolic processes (Rousk and Bååth, 2011; Stein and Nicol, 2011). We also need to identify the factors governing these activities and if it results in feedback mechanisms that alter the growth, activity and interaction between primary producers and microorganisms (Treseder et al., 2012). By determining how different groups of microorganisms respond to individual environmental conditions by allocating e.g. carbon to production of biomass, CO₂ and other products, a mechanistic as well as quantitative understanding of formation and decomposition of organic matter, and the production and consumption of greenhouse gases, can be achieved.

In this Research Topic, supported by the Swedish research councils' program "Biodiversity and Ecosystem Services in a Changing Landscape" (BECC), we intend to promote an

alternative framework to address how cycling of carbon and other nutrients will be altered in a changing environment from the first-principle mechanisms that drive them—namely the ecology, physiology and biogeography of microorganisms. In order to improve the predictive power of current models, the alternative framework supports the development of new models of biogeochemical cycles that factor in microbial physiology, ecology, and biogeochemistry. Our ambition has been richly rewarded by an extensive list of submissions. We are pleased to present contributions including primary research targeting the microbial control of biogeochemistry, comprehensive reviews of how microbial processes and communities relate to biogeochemical cycles, identification of critical challenges that remain, and new perspectives and ideas of how to optimize progress in our understanding of the microbial regulation of biogeochemistry.

Our Research Topic presents new findings about the importance of the microbial community composition, their metabolic state, and the activity of enzymes for the fate and degradation of specific substrates such as chitin (Beier and Bertilsson, 2013), the degradation of more complex compounds such as those constituting plant litter (Moorhead et al., 2013; Rinkes et al., 2013), and the metabolism and biogeochemical cycling of one-carbon compounds (Aronson et al., 2013; Basiliko et al., 2013; Kappler and Nouwens, 2013). The environmental control and land-use perturbation of microbial communities and methane production were assessed in a comprehensive review (Aronson et al., 2013) as well as a case study (Basiliko et al., 2013) and a meta-analysis (Holden and Treseder, 2013). Other contributions have focused on how environmental variables that are affected by climate change can modulate microbial activities by e.g. their influence on the production and activity of enzymes (Steinweg et al., 2013), while Bradford (2013) has provided a comprehensive review of how microbial processes respond to warmer temperatures. These reviews are accompanied by a new suggestion for how we can achieve better predictions for microbial responses (and feedbacks) to climate change (de Vries and Shade, 2013), while Moorhead et al. (2013) identify knowledge gaps and provide important insights about how data on microbial communities, environmental conditions, and enzyme activities can be used to better inform enzyme-based models.

Several submissions have highlighted the importance for plant-microbial feedbacks for the regulation of organic matter decomposition and formation (Moorhead et al., 2013; Thomson

et al., 2013; Churchland and Grayston, under review), the production of biogenic volatile organic compounds (Rinnan et al., 2013), and the community composition of methanogens and sulfate reducing bacteria (Zelege et al., 2013). A very active research area in soil microbial ecology is presently how small amounts of labile carbon sources can trigger, or “prime,” the decomposition of soil organic matter. A route toward a more general understanding of the regulation of plant-soil interaction for biogeochemistry, that may well facilitate our understanding of “priming effects,” could be the incorporation of stoichiometric concepts (Dijkstra et al., 2013; Mooshammer et al., 2014). Stoichiometric variations in the concentration of nutrients, combined with variations in carbon and nutrient demands of different decomposer groups, also seems to be reflected in the degradation rate of plant litter (Rinkes et al., 2013). A comprehensive review of biogenic fixation of nitrogen demonstrates the importance of interactions between different biogeochemical cycles for nitrogen fixation in ecosystems with nitrogen-limited plant productivity (Rousk et al., 2013). These contributions emphasize that stoichiometric variations in nutrient concentrations are of importance for both factors that could determine the propensity for organic matter to accumulate in an ecosystem, and thus for carbon to be sequestered.

Some contributions to this Research Topic have also highlighted methodological challenges that urgently need attention. For instance, the ability of contemporary isotopic tracer methods to estimate microbial contributions to biogeochemical processes could be systematically overestimated (Hobbie and Hobbie, 2013), suggesting that estimates of the turnover of low molecular weight organic compounds, and possibly also for estimations of nitrogen transformation rates, need to be revised. Additionally, there is a need to move from laboratory-based estimations of the microbial role in ecosystem level processes, often omitting crucial components such as the presence of plants, to field-based assessments in intact systems (Rinkes et al., 2013).

The contributions to our Research Topic have opened up new horizons and stimulated conceptual developments in our basic understanding of the regulating factors of global biogeochemical cycles. Within this forum, we have begun to bridge Microbial Ecology and Biogeochemistry, connecting microbial activities at the microcosm scale to carbon fluxes at the ecosystem-scale, and linking above- and belowground ecosystem functioning. We are hopeful that we have initiated conceptual developments that can reach far beyond this Research Topic. It is a mere first step, but we are confident it is directed toward a predictive understanding of the microbial regulation of global biogeochemical cycles.

ACKNOWLEDGMENTS

This Research Topic was supported by the action-group “MICROGLOBE” within the “Biodiversity and Ecosystem Services in a Changing Landscape” (BECC) environment funded by the Swedish Research Council. We are grateful to the Frontiers team support and editorial endorsement of our ambitions.

REFERENCES

- Aronson, E. L., Allison, S. D., and Helliker, B. R. (2013). Environmental impacts on the diversity of methane-cycling microbes and their resultant function. *Front. Microbiol.* 4:225. doi: 10.3389/fmicb.2013.00225
- Basiliko, N., Henry, K., Gupta, V., Moore, T. R., Driscoll, B. T., and Dunfield, P. F. (2013). Controls on bacterial and archaeal community structure and greenhouse gas production in natural, mined, and restored Canadian peatlands. *Front. Microbiol.* 4:215. doi: 10.3389/fmicb.2013.00215
- Beier, S., and Bertilsson, S. (2013). Bacterial chitin degradation—mechanisms and ecophysiological strategies. *Front. Microbiol.* 4:149. doi: 10.3389/fmicb.2013.00149
- Bradford, M. A. (2013). Thermal adaptation of decomposer communities in warming soils. *Front. Microbiol.* 4:333. doi: 10.3389/fmicb.2013.00333
- Conant, R. T., Ryan, M. G., Ågren, G. I., Birge, H. E., Davidson, E. A., Eliasson, P. E., et al. (2011). Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. *Global Change Biol.* 17, 3392–3404. doi: 10.1111/j.1365-2486.2011.02496.x
- de Vries, F. T., and Shade, A. (2013). Controls on soil microbial community stability under climate change. *Front. Microbiol.* 4:265. doi: 10.3389/fmicb.2013.00265
- Dijkstra, F. A., Carrillo, Y., Pendall, E., and Morgan, J. A. (2013). Rhizosphere priming: a nutrient perspective. *Front. Microbiol.* 4:216. doi: 10.3389/fmicb.2013.00216
- Griggs, D., Stafford-Smith, M., Gaffney, O., Rockström, J., Öhman, M. C., Shyamsundar, P., et al. (2013). Sustainable development goals for people and planet. *Nature* 495, 305–307. doi: 10.1038/495305a
- Hobbie, J. E., and Hobbie, E. A. (2013). Microbes in nature are limited by carbon and energy: the starving-survival lifestyle in soil and consequences for estimating microbial rates. *Front. Microbiol.* 4:324. doi: 10.3389/fmicb.2013.00324
- Holden, S. R., and Treseder, K. K. (2013). A meta-analysis of soil microbial biomass responses to forest disturbances. *Front. Microbiol.* 4:163. doi: 10.3389/fmicb.2013.00163
- Kappler, U., and Nouwens, A. S. (2013). Metabolic adaptation and trophic strategies of soil bacteria—C1- metabolism and sulfur chemolithotrophy in *Starkeya novella*. *Front. Microbiol.* 4:304. doi: 10.3389/fmicb.2013.00304
- Moorhead, D. L., Rinkes, Z. L., Sinsabaugh, R. L., and Weintraub, M. N. (2013). Dynamic relationships between microbial biomass, respiration, inorganic nutrients and enzyme activities: informing enzyme-based decomposition models. *Front. Microbiol.* 4:223. doi: 10.3389/fmicb.2013.00223
- Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., and Richter, A. A. (2014). Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. *Front. Microbiol.* 5:22. doi: 10.3389/fmicb.2014.00022
- Rinkes, Z. L., Sinsabaugh, R. L., Moorhead, D. L., Grandy, A. S., and Weintraub, M. N. (2013). Field and lab conditions alter microbial enzyme and biomass dynamics driving decomposition of the same leaf litter. *Front. Microbiol.* 4:260. doi: 10.3389/fmicb.2013.00260
- Rinnan, R., Gierth, D., Bilde, M., Rosenørn, T., and Michelsen, A. (2013). Off-season biogenic volatile organic compound emissions from heath mesocosms: responses to vegetation cutting. *Front. Microbiol.* 4:224. doi: 10.3389/fmicb.2013.00224
- Rousk, J., and Bååth, E. (2011). Growth of saprotrophic fungi and bacteria in soil. *FEMS Microbiol. Ecol.* 78, 17–30. doi: 10.1111/j.1574-6941.2011.01106.x
- Rousk, K., Jones, D. L., and DeLuca, T. H. (2013). Moss-cyanobacteria associations as biogenic sources of nitrogen in boreal forest ecosystems. *Front. Microbiol.* 4:150. doi: 10.3389/fmicb.2013.00150
- Schimel, D. S. (1995). Terrestrial ecosystems and the carbon-cycle. *Global Change Biol.* 1, 77–91. doi: 10.1111/j.1365-2486.1995.tb00008.x
- Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., et al. (2011). Persistence of soil organic matter as an ecosystem property. *Nature* 478, 49–56. doi: 10.1038/nature10386
- Stein, L. Y., and Nicol, W. N. (2011). Grand challenges in terrestrial microbiology. *Front. Microbiol.* 2:6. doi: 10.3389/fmicb.2011.00006
- Steinweg, J. M., Dukes, J. S., Paul, E. A., and Wallenstein, M. D. (2013). Microbial responses to multi-factor climate change: effects on soil enzymes. *Front. Microbiol.* 4:146. doi: 10.3389/fmicb.2013.00146
- Thomson, B. C., Ostle, N. J., McNamara, N. P., Oakley, S., Whiteley, A. S., Bailey, M. J., et al. (2013). Plant soil interactions alter carbon cycling in an upland grassland soil. *Front. Microbiol.* 4:253. doi: 10.3389/fmicb.2013.00253
- Treseder, K. K., Balser, T. C., Bradford, M. A., Brodie, E. L., Dubinsky, E. A., Eviner, V. T., et al. (2012). Integrating microbial ecology into ecosystem models: challenges and priorities. *Biogeochemistry* 109, 7–18. doi: 10.1007/s10533-011-9636-5

- Waksman, S. A., and Gerretsen, F. C. (1931). Influence of temperature and moisture upon the nature and extent of decomposition of plant residues by microorganisms. *Ecology* 12, 33–60
- Zelege, J., Sheng, Q., Wang, J.-G., Huang, M.-Y., Xia, F., Wu J.-H., and Quan, Z. (2013). Effects of *Spartina alterniflora* invasion on the communities of methanogens and sulfate-reducing bacteria in estuarine marsh sediments. *Front. Microbiol.* 4:243. doi: 10.3389/fmicb.2013.00243

Received: 11 February 2014; accepted: 27 February 2014; published online: 14 March 2014.

Citation: Rousk J and Bengtson P (2014) Microbial regulation of global biogeochemical cycles. *Front. Microbiol.* 5:103. doi: 10.3389/fmicb.2014.00103

This article was submitted to Terrestrial Microbiology, a section of the journal *Frontiers in Microbiology*.

Copyright © 2014 Rousk and Bengtson. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Bacterial chitin degradation—mechanisms and ecophysiological strategies

Sara Beier^{1,2,3} and Stefan Bertilsson^{1*}

¹ Department of Ecology and Genetics, Limnology, Uppsala University, Uppsala, Sweden

² Laboratoire d'Océanographie Microbienne, Observatoire Océanologique, UPMC Paris 06, UMR 7621, Banyuls sur mer, France

³ Laboratoire d'Océanographie Microbienne, Observatoire Océanologique Centre National de la Recherche Scientifique, UMR 7621, Banyuls sur mer, France

Edited by:

Per Bengtson, Lund University, Sweden

Reviewed by:

Steffen Kolb, University of Bayreuth, Germany

Helmut Buergermann, Eawag: Swiss Federal Institute of Aquatic Science and Technology, Switzerland

*Correspondence:

Stefan Bertilsson, Department of Ecology and Genetics, Limnology, Uppsala University, Norbyvägen 18D, SE-75236 Uppsala, Sweden
e-mail: stebe@ebc.uu.se

Chitin is one of the most abundant polymers in nature and interacts with both carbon and nitrogen cycles. Processes controlling chitin degradation are summarized in reviews published some 20 years ago, but the recent use of culture-independent molecular methods has led to a revised understanding of the ecology and biochemistry of this process and the organisms involved. This review summarizes different mechanisms and the principal steps involved in chitin degradation at a molecular level while also discussing the coupling of community composition to measured chitin hydrolysis activities and substrate uptake. Ecological consequences are then highlighted and discussed with a focus on the cross feeding associated with the different habitats that arise because of the need for extracellular hydrolysis of the chitin polymer prior to metabolic use. Principal environmental drivers of chitin degradation are identified which are likely to influence both community composition of chitin-degrading bacteria and measured chitin hydrolysis activities.

Keywords: chitin, particles, organic matter, bacteria, interactions, cross-feeding, glycoside hydrolase

INTRODUCTION

The occurrence of chitin is widespread in nature and chitin serves as a structural element in many organisms, e.g., fungi, crustaceans, insects or algae (Gooday, 1990a,b). Chitin is composed of linked amino sugar subunits. Similar to cellulose and murein, it makes a shortlist of highly abundant biopolymers with enormous global production rates estimated at approximately 10^{10} – 10^{11} tons year⁻¹ (Gooday, 1990a; Whitman et al., 1998; Kaiser and Benner, 2008). There are no reports of quantitatively significant long-term accumulation of chitin in nature, implying efficient degradation and turnover (Tracey, 1957; Gooday, 1990a).

In accordance with the abundance and ubiquity of chitin, chitin-degrading enzymes are also detected in many types of organisms, such as fungi, bacteria (Gooday, 1990a), archaea (Huber et al., 1995; Tanaka et al., 1999; Gao et al., 2003), rotifers (Štrojsová and Vrba, 2005), some algae (Vrba et al., 1996; Štrojsová and Dyhrman, 2008), but also carnivorous plants or in digestive tracts of higher animals (Gooday, 1990a).

Bacteria are believed to be major mediators of chitin degradation in nature. In soil systems, chitin hydrolysis rates have been shown to correlate with bacterial abundance (Kielak et al., 2013), but depending on temperature, pH, or the successional stage of the degradation process, also fungi may be quantitatively important agents of chitin degradation (Gooday, 1990a; Hallmann et al., 1999; Manucharova et al., 2011). In aquatic systems, plating and *in situ* colonization experiments convincingly demonstrate that bacteria are the main mediators of chitin degradation (Aumen, 1980; Gooday, 1990a). However, occasionally, dense fungal colonization of chitinous zooplankton carapaces has been observed (Wurzbacher et al., 2010) and some diatoms have also been

shown to hydrolyze chitin oligomers (Vrba et al., 1996, 1997). A further source of chitin-modifying enzymes in aquatic systems are enzymes released during molting of planktonic crustaceans (Vrba and Machacek, 1994). Nevertheless, it is not yet clear whether the enzymes released by diatoms and molting zooplankton react with particulate chitin to any significant extent or if their hydrolytic activity is limited to dissolved chitin oligomers.

Chitin is the polymer of (1→4)- β -linked N-acetyl-D-glucosamine (GlcNAc). The single sugar units are rotated 180° to each other with the disaccharide N,N'-diacetylchitobiose [(GlcNAc)₂] as the structural subunit. In nature, chitin varies in the degree of deacetylation and therefore the distinction from chitosan, which is the completely deacetylated form of the polymer, is not strict. Chitin is classified into three different crystalline forms: the α -, β -, and γ -form, which differ in the orientation of chitin micro-fibrils. With few exceptions, natural chitin occurs associated to other structural polymers such as proteins or glucans, which often contribute more than 50% of the mass in chitin-containing tissue (Attwood and Zola, 1967; Schaefer et al., 1987; Merzendorfer and Zimoch, 2003). Chitin is a structural homologue of cellulose where the latter is composed of glucose instead of GlcNAc subunits. Also murein in bacterial cell walls can be considered a structural chitin homologue, as it is composed of alternating (1→4)- β -linked GlcNAc and N-acetylmuramic acid units.

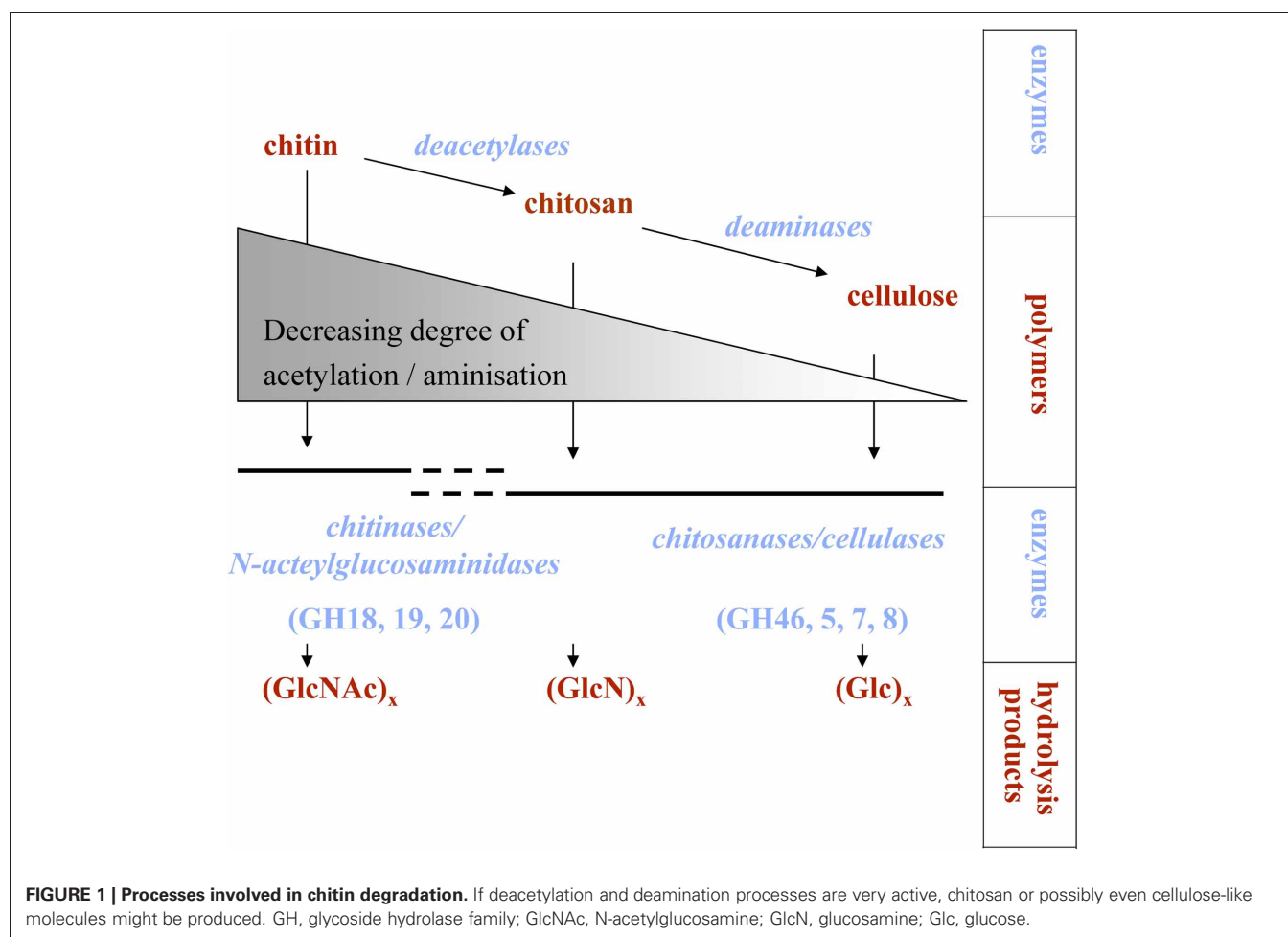
A process is called chitinoclastic if chitin is degraded. If this degradation involves the initial hydrolysis of the (1→4)- β -glycoside bond, as seen for chitinase-catalyzed chitin degradation, the process is called chitinolytic. Growth on chitin is not necessarily accompanied by the direct dissolution of its polymeric

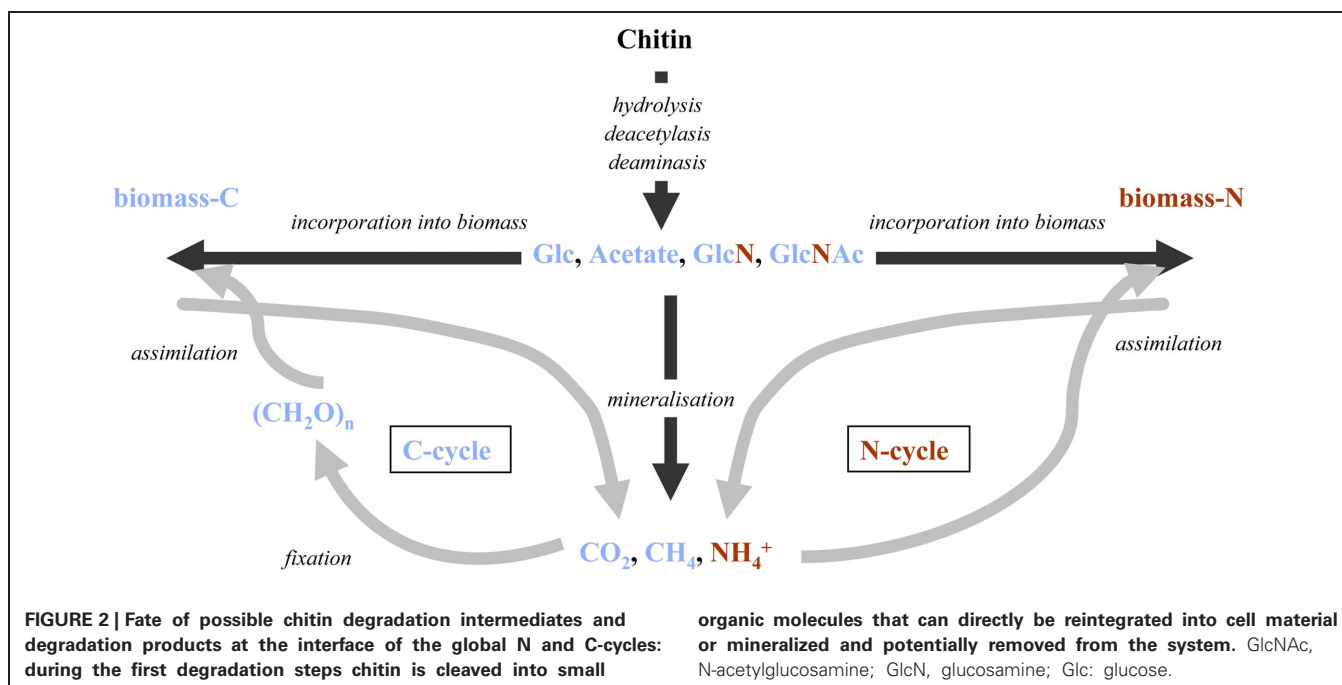
structure. Alternatively, chitin can be deacetylated to chitosan or possibly even cellulose-like forms, if it is further subjected to deamination (**Figure 1**). Such a degradation mechanism has been suggested in some early studies (ZoBell and Rittenberg, 1938; Campbell and Williams, 1951). Chitinases and chitosanases overlap in substrate specificity, while their respective efficiency is controlled by the degree of deacetylation of the polymeric substrate (Somashekar and Joseph, 1996) (**Figure 1**). Besides specific chitosanases, also cellulases can possess considerable chitosan-cleaving activity (Xia et al., 2008). Furthermore, lysozyme has also been shown to hydrolyze chitin, even if processivity is low when compared to true chitinases (Skujinš et al., 1973). Cellulases can also bind directly to chitin (Ekborg et al., 2007; Li and Wilson, 2008), but there are no reports of these enzymes actually hydrolyzing the polymers.

Few studies have compared the quantitative importance of different chitinoclastic pathways, and the studies available suggest that chitin degradation via initial deacetylation might be more important in soil and sediment compared to water environments (Hillman et al., 1989; Gooday, 1990a). The quantitative importance of different chitinoclastic pathways from a global perspective has, to the best of our knowledge, never been assessed. In the following sections, we will focus on the chitinolytic pathway.

The quantitative significance of chitin has been recognized for some time and there has been great interest in identifying processes and factors controlling its degradation. Accordingly, the biochemistry, molecular biology, and biogeochemistry of chitin degradation have been summarized in reviews published already some 20 years ago (Gooday, 1990a; Cohen-Kupiec and Chet, 1998; Keyhani and Roseman, 1999). More recently, the development and widespread use of culture-independent molecular methods in microbial ecology have enabled further dissection of microbial processes controlling chitin degradation in more complex natural environments and diverse microbial communities. These methodological advances combined with the significance of chitin as a critical link between the carbon and nitrogen cycles (**Figure 2**) has led to a revived interest in the quantitative importance of chitin turnover in marine systems (Souza et al., 2011).

There is clearly a need for an updated account of the diverse mechanisms involved in chitinolysis and the ecological consequences of this process for bacteria. A focus on bacteria rather than all other organisms involved in chitin degradation is warranted since bacterial chitin degradation takes place in all major ecosystems and because their metabolism and growth have such a central role in most ecosystem-scale biogeochemical





cycles. However, also non-bacterial or non-chitinolytic chitin-degraders will occasionally be mentioned and discussed where their activities would influence bacterial chitin degradation. In light of recent developments in molecular methods, a particular emphasis will be on how the participation and interactions of specific microbial populations and community composition influence the process. We further identify gaps in knowledge and needs for further research.

BIOCHEMISTRY OF CHITIN HYDROLYSIS

Chitin degradation is a highly regulated process, and the hydrolytic enzymes are induced by products of the chitin hydrolases, GlcNAc (Techkarnjanaruk et al., 1997), or soluble chitin oligomers $(GlcNAc)_2-6$ (Keyhani and Roseman, 1996; Miyashita et al., 2000; Li and Roseman, 2004; Meibom et al., 2004), depending on the organism under scrutiny. In contrast to $(GlcNAc)_2$, GlcNAc has also been reported to act as a suppressor of chitinase expression in a *Streptomyces* strain (Miyashita et al., 2000) and this may be because its main origin in natural systems could be from murein in cell walls rather than chitin (Benner and Kaiser, 2003). Other factors more generally regulating the expression of these and other hydrolytic enzymes are nutrient regime and availability of other, more readily available growth substrates (Techkarnjanaruk et al., 1997; Keyhani and Roseman, 1999; Delpin and Goodman, 2009a,b). The variety of regulating factors are likely to reflect the wide range of ecological niches occupied by chitin degraders.

Complete lysis of the insoluble chitin polymer typically consists of three principal steps (1) cleaving the polymer into water-soluble oligomers, (2) splitting of these oligomers into dimers, and (3) cleavage of the dimers into monomers. The first two steps are usually catalyzed by chitinases. The occurrence of chitinases in bacteria is widespread among phyla and the production

of multiple chitinolytic enzymes by individual bacterial strains appear to be a common trait (e.g., Fuchs et al., 1986; Romaguera et al., 1992; Saito et al., 1999; Shimosaka et al., 2001; Tsujibo et al., 2003). Chitinases are typically grouped into family 18 and 19 glycoside hydrolases. The latter are rare in bacteria except for some members of the genus *Streptomyces* (Ohno et al., 1996; Saito et al., 1999; Watanabe et al., 1999; Shimosaka et al., 2001; Tsujibo et al., 2003). It has been hypothesized that family 18 and 19 glycoside hydrolases have evolved separately, as genes belonging to these two analogous gene families show little or no sequence homology, nor share the same molecular-level catalytic mechanism (Perrakis et al., 1994; Davies and Henrissat, 1995; Hart et al., 1995). The occurrence of multiple genes in a single organism may be the result of gene duplication or acquisition of genes from other organisms via lateral gene transfer (Hunt et al., 2008). In support of the former mechanism, different chitinase gene sequences found within single organisms are often almost identical. However, there are examples where chitinase genes coexisting in a single organism are very different and cluster with chitinase sequences from rather distantly related organisms (Saito et al., 1999; Suzuki et al., 1999; Karlsson and Stenlid, 2009). This suggests lateral gene transfer also between distantly related organisms.

Multiple chitinases within a single organisms are believed to lead to a more efficient use of the respective substrate as a result of synergistic enzyme interactions or contrasting affinities to different substrate forms (Svitil et al., 1997). One example of this is the extensively studied chitinase system of *Serratia marcescens*, which is based on several chitinases with slightly different functions. *S. marcescens* produces four family 18 chitinases ChiA, ChiB, ChiC1, and ChiC2, all of which are released into the surrounding medium (Suzuki et al., 1998). ChiC2 results from a posttranslational modification of ChiC1 (Gal et al., 1998; Suzuki

et al., 1999) and hydrolytic activities of ChiC2 were lower on crystalline substrates compared to ChiC1 whereas no further differences were identified (Suzuki et al., 1999), leaving the function of ChiC2 unclear. By combining ChiA, ChiB, and ChiC1, synergistic effects on chitin degradation have been observed, implying differential action sites and/or molecular reaction mechanisms for the three enzymes (Suzuki et al., 2002). Indeed it was later shown that ChiC is a non-processive endoenzyme that cleaves the chitin polymer randomly, whereas both ChiA and ChiB are processive enzymes cleaving off disaccharides while sliding along the chitin polymer (Horn et al., 2006; Sikorski et al., 2006). Multiple action mechanisms are also implied for each of the latter two chitinases as it has been demonstrated that ChiA and ChiB degrade β -chitin microfibrils unidirectionally from opposite ends of the polymer (Hult et al., 2005). Still, the major end products from all three enzymes are disaccharides, whereas monosaccharides are produced as byproducts in substantially lower amounts (Horn et al., 2006). There are other examples where multiple enzymes within an organism catalyze the metabolism of a single substrate, with cellulose as a pertinent example (Rabinovich et al., 2002). It seems conceivable that enzyme multiplicity might be a general feature in polymer degrading processes caused by the structural complexity of the substrate. This would then allow parallelized or successive contrasting modes of action on the same polymer.

β -N-acetyl-hexosaminidases, usually affiliated with family 20 glycoside hydrolases, finally cleave GlcNAc from the non-reducing end of the water soluble chitin oligomers produced by chitinases (Scigelova and Croust, 1999). In bacteria, this last step typically takes place in the cytoplasm or the periplasmic space (Bassler et al., 1991; Keyhani and Roseman, 1996; Drouillard et al., 1997; Techkarnjanaruk and Goodman, 1999). In some bacteria, enzymes other than the family 20 glycoside hydrolases are involved in hydrolyzing GlcNAc from chitin oligomers (Tsuji et al., 1994; Chitlaru and Roseman, 1996; Park et al., 2000). Recent research also suggests that some family 20 glycoside hydrolases can cleave GlcNAc directly from chitin polymers and hence function as chitinases (LeClerc et al., 2007).

Chitin degradation is also influenced by more cryptic factors. For example, a chitin-binding protein without any catalytic domain has been shown to facilitate the degradation of β -chitin by disrupting the crystalline chitin polymer structure (Vaaje-Kolstad et al., 2005). The protein showed significant sequence similarity to a gene product in *Streptomyces olivaceoviridis* known to have high affinity to α -chitin (Schnellmann et al., 1994). It has been proposed that the ability to produce such proteins with high specific affinity to a certain crystalline chitin structure may be decisive for the ability of bacteria to differentiate and react to specific crystalline chitin structures (Svitil et al., 1997). Such chitin-binding domains may also influence chitin degradation indirectly by facilitating adhesion of cells to chitinous substrates, a trait that is of particular importance in aquatic environments (Montgomery and Kirchman, 1993, 1994; Pruzzo et al., 1996).

Since the insoluble chitin polymer has to be cleaved outside of the bacterial cell barrier, metabolic use of chitin also relies on efficient uptake systems for hydrolysis products. In some cultivated bacterial strains, PTS (phosphoenolpyruvat: glucose

phosphotransferase system) transporters are responsible for the main GlcNAc uptake. However, the uptake activity of other specific GlcNAc transporters as well as transporters with a broader substrate range (including sugar monomers like glucose, glucosamine, fructose and mannose) have also been described (Mobley et al., 1982; Postma et al., 1993; Bouma and Roseman, 1996). The quantitative importance of these two substrate uptake strategies, highly specific or more versatile, is not clear and culture independent assays based on inhibition experiments provide contrasting results concerning the specificity of GlcNAc-uptake systems. Whereas Riemann and Azam (2002) found a specific inhibition of the bacterial GlcNAc-uptake by glucose, this was not the case in an earlier study by Vrba et al. (1992). Reasons for such conflicting results could be a different set of organisms being present at the respective sampling sites, i.e., due to the different environment under scrutiny in the respective study (marine vs. freshwater) or seasonal differences in nutrient status of the system.

Radiotracer studies in lake water suggest differentiation in GlcNAc and (GlcNAc)₂ uptake among phylogenetic groups of bacteria with the (GlcNAc)₂ uptake being quantitatively more important (Beier and Bertilsson, 2011). This implies that the two hydrolysis products are taken up by different transporter systems in freshwater ecosystems. The earlier discussed role of (GlcNAc)₂ as main hydrolysis product of chitinases (Horn et al., 2006) and the quantitative importance of the (GlcNAc)₂ uptake mentioned above (Beier and Bertilsson, 2011) corroborates the observation that bacterial β -N-acetyl-hexosaminidases are often intracellular enzymes. Consequently, the relevance of (GlcNAc)₂ transport through the cell barrier during the process of chitin degradation is evident.

SPECIES INTERACTIONS DURING CHITIN DEGRADATION IN DIFFERENT HABITATS

Particles that contain chitin can act as a source of chitin degradation intermediates to the surrounding medium (Smith et al., 1992; Kirchman and White, 1999). This implies that chitinolytic bacteria sometimes process more chitin polymers than they are able to use themselves. For instance, only a minority of cells in a pure culture of *Pseudoalteromonas* S91 growing on chitin as a sole source of carbon and nitrogen hydrolyzed chitin (Baty et al., 2000a,b). It was assumed that cells with no apparent chitinase activity fed on hydrolysis products produced in excess by the chitinase-positive subpopulation. This type of multicellular cooperation is a strategy often observed in bacteria (Shapiro, 1998) and has been described for several chitinolytic strains (Gaffney et al., 1994; Chernin et al., 1998; DeAngelis et al., 2008). Considering the complexity of the chitinolytic cascade, with approximately 50 different proteins being induced (Keyhani and Roseman, 1999; Li and Roseman, 2004; Meibom et al., 2004), a partitioning of the clonal population into a chitinase up-regulated subpopulation that supply hydrolysis products to their kin could be a successful survival mechanism. Such intraspecific cross-feeding might also explain the excess enzymatic activity observed on particles in aquatic systems (Smith et al., 1992; Kirchman and White, 1999). However, in natural environments, the release of hydrolysis products would not only serve specific clonal populations, but also

open up the possibility for interspecific cross-feeding. The existence of interspecies cross-feeding therefore seems plausible and studies on bacterial pure cultures have indeed demonstrated that there are organisms that grow on GlcNAc (Kaneko and Colwell, 1978) or (GlcNAc)₂ (Keyhani and Roseman, 1997) without possession of the enzymes for chitinolytic activity.

The habitat structure in which polymer degradation takes place might have great consequences for this kind of interspecies interactions. The flux of dissolved substances as hydrolyses products is physically constrained in aerated soils. Accordingly hydrolyses products will remain in close spatial proximity to the place of enzymatic action. In terrestrial systems, interspecies metabolic interactions will therefore likely be limited to organisms growing directly adjacent to each other in biofilms. Besides commensal sharing of such hydrolysis products (Everuss et al., 2008) there is also a potential for specialized interactions between organisms such as synergistic coupling and the recently described parasitism that rely on bi-directional exchange of e.g., metabolic inhibitors and chitin degradation intermediates among specific bacterial populations (Jagmann et al., 2010). In contrast, released hydrolyses products in aquatic systems will be subject to transport by diffusion and hydrological flow away from the site where hydrolysis took place. Because of the facilitated transport of hydrolysis products away from the hydrolytic site, quantitatively significant cross-feeding events can occur over longer distances in this biome as observed previously (Cho and Azam, 1988; Beier and Bertilsson, 2011; Eckert et al., 2013). Thus, it seems likely that such long-distance cross-feeding relationships could favor rather unspecific and unidirectional commensal interactions, where the receiving organism is less likely to critically depend on the interaction. Sediments or waterlogged soils in wetlands may represent habitats with intermediate transport constraints, locally sharing transport characteristics with both environments outlined above.

To the best of our knowledge, no studies exist that target species interactions during chitin degradation in soil environments specifically, nor are we aware of studies that compare the above suggested general differences in cross-feeding between aquatic and terrestrial habitats. However, a number of culture-independent studies in aquatic environments that quantify the fraction of chitin degraders vs. chitin consumers in the total bacterial community support the existence of significant cross-feeding during chitin degradation (**Table 1**): chitinolytic organisms were estimated to represent 0.1–5.8% (average about 1%) of all prokaryotes in a variety of aquatic ecosystems (Cottrell et al., 1999; Beier et al., 2011). An even lower fraction of cells displayed active chitinolytic activity in natural aquatic habitats (0–1.9%) (Beier and Bertilsson, 2011; Beier et al., 2012). In contrast, between 4 and 40% of the bacteria, or one third of the DNA-replicating bacteria, were shown to incorporate chitin hydrolysis products (Nedoma et al., 1994; Riemann and Azam, 2002; Beier and Bertilsson, 2011; Eckert et al., 2013).

The assumption that the uptake of polymer-derived metabolites in aquatic system often occurs over longer distances is supported by the observation that typically free-living bacterial groups appear to be quantitatively important receivers of this hydrolyzed material (Cho and Azam, 1988; Beier and Bertilsson,

2011; Eckert et al., 2013). For such long-distance substrate acquisition, the free-living organisms receiving the hydrolysis products are likely to profit from the action of other hydrolytic bacteria that are in close proximity to the polymeric substrate: any hydrolytic enzymes produced by free living cells across such long distances would have a low probability of encountering the substrate and even in this case the majority of resulting hydrolysis products would not be encountered by the free-living cell. Model findings indicate that the area around a polymer-hydrolyzing bacterium, from which hydrolysate can be efficiently collected, is limited to approximately 10 µm distance from the polymeric source (Vetter et al., 1998). Free-living bacteria might occasionally be within this distance to a chitinous particle, but it is uncertain whether the gain from such occasional degradation product uptake can balance the costs for maintenance of the polymer hydrolyzing machinery. On the other hand, it has recently been demonstrated that a member of the typically free-living lineage *Actinobacteria* ac1 hosts genes to take up GlcNAc while also encoding a chitinase gene (Garcia et al., 2013). However, it still remains to be demonstrated, whether or not these gene products can solubilize polymeric chitin.

Because of the more pronounced dilution of the released hydrolyses products in aquatic systems, a successful receiving organism residing such a long distance from the polymer hydrolysis site would likely also feature high affinity uptake systems. In agreement with this idea, Boyer (1994) observed radiolabeled chitin degradation intermediates in sediment but not in water after incubating both type of samples with ¹⁴C labeled chitin. This suggests that organisms with higher substrate affinity are present in the water samples compared to organisms present in the sediment. It remains to be tested whether the remaining intermediates in sediments would be metabolized over longer timescales or become resistant to further degradation by diagenetic processes. It is also unknown, whether organisms with high substrate affinity influence the efficiency of polymer degradation or if they are irrelevant for the overall ecosystem functioning.

TAXONOMIC IDENTITY OF CHITINOLYTIC ORGANISMS

Qualitative characterization of the chitinolytic community by means of culture-independent molecular methods such as PCR amplification of chitinase genes or metagenomic approaches usually results in a rather rough level of identification. This is due to the supposedly extensive lateral gene-transfer and the limited taxonomic coverage of characterized reference organisms. One consequence of this is that a large number of chitinase gene sequences cannot be clearly affiliated to specific taxa. However, at a broader phylogenetic resolution recent studies in aquatic environments indicate that group A chitinases were by far the most abundant phylogenetic subgroup of family 18 glycoside hydrolases (Beier et al., 2011). More detailed information about the taxonomic identity of microorganisms that consume the chitin degradation products can be obtained by either cultivation approaches or by using radiotracer techniques. The bias inherent in studies that describe natural bacterial communities using exclusively cultivation-dependent approaches are well-known (Amann et al., 1995), but the bias appear to be of quantitative rather than qualitative concern.

Table 1 | Fraction of chitinolytic, chitinolytically active, and chitin hydrolysis products incorporating cells (no results of culture-dependent studies are listed here, since quantitative values are likely strongly biased).

Fraction of cells	System	Method	References
5.5%	Brackish water	Chitinase genes in metagenomes (fraction of chitinolytic cells)	Cottrell et al., 1999
0.1%	Marine water	Chitinase genes in metagenomes (fraction of chitinolytic cells)	Cottrell et al., 1999
3.1%	Freshwater	Chitinase genes in metagenomes (fraction of chitinolytic cells)	Beier et al., 2011
0.7–1.5%	Brackish water	Chitinase genes in metagenomes (fraction of chitinolytic cells)	Beier et al., 2011
0.2–5.8%	Marine water	Chitinase genes in metagenomes (fraction of chitinolytic cells)	Beier et al., 2011
1.3%	Hypersaline water	Chitinase genes in metagenomes (fraction of chitinolytic cells)	Beier et al., 2011
Not detectable	Freshwater	¹ ELF® 97 (fraction of chitinolytically active cells)	Beier and Bertilsson, 2011
up to 1.9%	Freshwater	ELF® 97 (fraction of chitinolytically active cells)	Beier et al., 2012
4.2–38.9%	Freshwater	² MAR-FISH (fraction of GlcNAc incorporating cells)	Nedoma et al., 1994
7%	Freshwater	MAR-FISH (fraction of (GlcNAc) ₂ incorporating cells)	Beier and Bertilsson, 2011
6–7%	Freshwater	MAR-FISH (fraction of GlcNAc incorporating cells)	Beier and Bertilsson, 2011
8%	Freshwater	MAR-FISH (fraction of GlcNAc incorporating cells)	Eckert et al., 2013
43% of DNA synthesizing bacteria	Marine water	Streptozotocin sensitivity (fraction of GlcNAc incorporating cells)	Riemann and Azam, 2002

¹ ELF® 97: ELF® 97 chitinase-N-acetylglucosaminidase substrat.

² MAR-FISH: microautoradiography—fluorescence in situ hybridization.

In aquatic systems, *Cytophaga-Flavobacteria* are known to profit from chitin addition and have been detected in dense cluster on chitinous particles where they also assimilate chitin hydrolysis products (Cottrell and Kirchman, 2000; Beier and Bertilsson, 2011). This suggests a central role of *Cytophaga-Flavobacteria* in aquatic chitin degradation where they also benefit from this material as a substrate. In contrast, in soil environments bacteria affiliated with *Actinomyces* are often identified as being active chitin degraders, as they display enhanced growth and activity upon chitin addition. Members of this phylum are also frequently recovered in cultivation dependent studies of chitin degraders (Metcalf et al., 2002; Manucharova et al., 2011). However, in both of these biomes, chitinoclastic bacteria from other phylogenetic groups, including *Proteobacteria* and *Firmicutes*, are also commonly observed (Cottrell et al., 2000; Brzezinska and Donderski, 2006; Yasir et al., 2009). The high phylogenetic diversity within the frequently isolated chitinolytic bacteria may therefore reflect a high ecological diversity of chitin degraders and could also explain why chitin does not accumulate in nature, but instead seems to be degraded under all possible environmental conditions (Tracey, 1957; Gooday, 1990a).

The composition of the chitin utilizing community—including active degraders and organisms profiting from cross-feeding events—might be decisive for the fate of chitin. It seems plausible that i.e., gram-positive chitin consumers use a higher percentage of GlcNAc in anabolic processes to synthesize the murein needed in abundance for production of their cell wall, while gram-negative bacteria might allocate more of these substrates to catabolic energy acquisition. Indeed, the fraction of hydrolyzed chitin respired to CO₂ in natural ecosystems varies considerably between 30 and 93% (Table 2). Whereas the presence of other substrates has been shown to influence mineralization rate of GlcNAc (Mobley et al., 1982), it remains to be determined if the species composition of chitin consumers, as speculated above, has any significant influence of the actual chitin mineralization rates.

Table 2 | Fraction of hydrolyzed chitin that is mineralized.

Chitin mineralization (% of hydrolyzed chitin)	System	Method	References
93%	Freshwater	¹ ¹⁴ C, 25°C, crab shells	Boyer, 1994
78%	Freshwater	¹⁴ C, 15°C, crab shells	Boyer, 1994
30%	Brackish water	¹⁴ C, purified fungal chitin	Kirchman and White, 1999
55–72%	Freshwater sediment	¹⁴ C, 25°C, crab shells	Boyer, 1994
50–75%	Freshwater sediment	¹⁴ C, 15°C, crab shells	Boyer, 1994

¹ ¹⁴C: chitin mineralization estimated based on ¹⁴C labeled tracer compounds.

Since the taxonomic identity of the chitin-degrading and chitin-utilizing organisms might be decisive to ecosystem functioning, i.e., as outlined above for mineralization rates of chitin, it seems important to learn more about key players involved in different environments. One feasible strategy might be to combine designed experiments with single-cell isotope tracer methods. Another option is the direct coupling of chitin degradation traits to other metabolic features and taxonomic affiliation via single cell genome sequencing of uncultured microorganisms (Stepanaukas and Sieracki, 2007).

DYNAMICS OF THE CHITINOLYTIC COMMUNITY STRUCTURE AND CHITIN DEGRADATION RATES

Chitin degradation is a regulated trait and chitin degraders will be able to also metabolize other substrates than chitin. Therefore, the coupling between the abundance and composition of the chitinolytic community and their collective hydrolytic activity might not always be strong. A number of different methods,

such as weight loss, ^{14}C labeled chitin tracer experiments or incubation experiments with colorimetric or fluorogenic substrate analogs, have been applied to measure hydrolytic activity during chitin degradation. Due to the variety of different methods applied, measuring i.e., potential or actual rates, individual values for chitin hydrolytic activity in different studies are difficult to compare directly (Tables 3, 4). Instead we will describe trends in environmental control of chitin degradation detected consistently across several studies and if possible compare these patterns to shifts in the chitinolytic community composition. All methods for activity measurements have in common that they do not differentiate between different organisms hydrolyzing the chitin. Depending on the method used, also enzymes other than chitinases, such as chitosanases, β -N-acetylhexosaminidases or lysozymes might contribute to the measured rates (Höltje, 1996; Vrba et al., 1996). Community shifts are in most cases detected by molecular analyses of group A chitinases of the family 18 glycoside hydrolase (Table 5). Bacterial as well as non-bacterial organisms capable of chitin hydrolysis, such as those that possess β -N-acetylhexosaminidases and lysozyme are also frequently carrying group A chitinase genes. The targeted group A chitinases can thus also include genes from fungi, algae and higher animals (Hobel et al., 2005; Beier et al., 2012). Therefore, most organisms that contribute to the measured chitinolytic process should be included in the community analyses.

Temperature is often considered as a critical factor controlling chitin degradation rates. There are several reports of variation in

chitin degradation rates with the highest activity during periods of high *in situ* temperature (Hood and Meyers, 1977; Rodríguez-Kábana et al., 1983; Hillman et al., 1989; Gooday et al., 1991; Ueno et al., 1991; Boyer, 1994; Metcalfe et al., 2002). Analogously, observations that different chitinoclastic strains were isolated during different seasons provided support that temperature could also affect the composition of the chitinoclastic community (Warnes and Rux, 1982). In some of these studies reporting temperature dependency for chitin hydrolysis rates, substrate availability might have been a cryptic underlying factor driving the observed correlation. In aquatic ecosystems for example, chitinous zooplankton can be dominant contributors to polymeric chitin and are known to increase seasonally in response to warmer temperature. In agreement with this, Beier et al. (2012) recently detected pronounced seasonal dynamics in the chitinolytic community using cultivation-independent molecular methods, but it was not evident from this study if temperature or alternate autocorrelated environmental factors such as chitin supply via crustacean zooplankton were the major environmental factors driving the community shifts.

There are also studies that revealed that temperature seems to play a minor role: in the York River, the correlation between chitin degradation and temperature was much less evident in the water column compared to the sediments (Boyer, 1994). Further exceptions are reported for the North Sea where higher chitin degradation rates were observed in October/November compared to the warmer period during July/August (Gooday et al., 1991). Also in these studies, however, chitin availability seemed

Table 3 | Chitin hydrolysis rates measured in natural habitats (values from experimental manipulations measured along with controls from natural habitats were excluded from the table).

Chitin hydrolysis rates	System	Method	References
0.00043–0.0005% d ⁻¹	Marine water	^{14}C , 1°C, synthesized chitin	Herwig et al., 1988
27% d ⁻¹	Freshwater	^{14}C , 15°C, crab shells	Boyer, 1994
30% d ⁻¹	Freshwater	^{14}C , 25°C, crab shells	Boyer, 1994
<1% d ⁻¹	Brackish water	^{14}C , <i>in situ</i> T, purified fungal chitin	Kirchman and White, 1999
8.1% d ⁻¹	Brackish water	<i>In situ</i> —weight loss on squid pen—yearly mean	Gooday et al., 1991
0.5–4.4% d ⁻¹	Freshwater-sediment interface	<i>In situ</i> —weight loss on purified chitin—different seasons	Warnes and Rux, 1982
0.1–4.5% d ⁻¹	Brackish water-sediment interface	<i>In situ</i> —weight loss on squid pen—yearly mean	Gooday et al., 1991
12–16% d ⁻¹	Freshwater sediment	^{14}C , 15°C, crab shells	Boyer, 1994
22–27% d ⁻¹	Freshwater sediment—sand	^{14}C , 25°C, crab shells	Boyer, 1994
0.0002 – 0.005% d ⁻¹	Marine sediment	^{14}C , 1°C, synthesized chitin	Herwig et al., 1988
2.6–2.8% d ⁻¹	Brackish sediment	<i>In situ</i> —weight loss on squid pen—yearly mean	Gooday et al., 1991
2.8% d ⁻¹	Brackish sediment	<i>In situ</i> —weight loss on squid pen—yearly mean	Gooday et al., 1991
1% d ⁻¹	Brackish sediment	Weight loss on squid pen	Hillman et al., 1989
*0.6–1.1% d ⁻¹	Soil	<i>In situ</i> —weight loss on crab shell chitin	Metcalfe et al., 2002

If possible, values given in different units in the original publications were transformed into a single unit.

¹ ^{14}C : degradation rates estimated based on ^{14}C labeled tracer compounds.

*Values derived from digitalized figures using the Engauge Digitizer Program (<http://digitizer.sourceforge.net/index.php?c=5>).

Table 4 | Chitinase and β -N-acetyl-hexosaminidase enzyme activities in natural habitats.

Enzyme activities	System	Method	References
$5.4 \times 10^{-5} - 3.1 \times 10^2 \text{ nmol d}^{-1} \text{ ml}^{-1}$	Freshwater	¹ MUF-NAG, <i>in situ</i> T, 100 μM	Vrba et al., 1992
$1.3 \times 10^{-5} - 1.3 \times 10^{-4} \text{ nmol d}^{-1} \text{ ml}^{-1}$	Freshwater	MUF-NAG, <i>in situ</i> T, 50 μM	Beier et al., 2012
$*2.8 \times 10^2 - 3.4 \times 10^2 \text{ nmol d}^{-1} \text{ g}^{-1}$ (wet)	Wetland sediment	² pNP-NAG, 25°C, 5 mM	Jackson and Vallaire, 2009
$*4.2 \times 10^{-1} - 1.4 \times 10^2 \text{ nmol d}^{-1} \text{ ml}^{-1}$ (wet)	Wetland sediment	MUF-NAG, respective annual mean T, 400 μM	Kang et al., 2005
$*1.7 \times 10^0 - 9.2 \times 10^0 \mu\text{g d}^{-1} \text{ g}^{-1}$ (dry)	Saltmarsh sediment	pNP-NAG, 30°C, 5 mM	Duarte et al., 2008
$*2.4 \times 10^3 - 7.1 \times 10^3 \text{ nmol d}^{-1} \text{ g}^{-1}$ (dry)	Soil	pNP-NAG, 25°C, 2 mM	Rietl and Jackson, 2012
Not detectable	Freshwater	³ MUF-DC, 4°C, 50 μM	Köllner et al., 2012
Up to $5.4 \times 10^1 \text{ nmol d}^{-1} \text{ ml}^{-1}$	Freshwater	MUF-DC, <i>in situ</i> T, 50 μM	Beier et al., 2012
$4.2 \times 10^{-3} - 2.1 \times 10^{-1} \text{ nmol d}^{-1} \text{ g}^{-1}$ (dry)	Freshwater sediment	MUF-DC, 4°C, 50 μM	Köllner et al., 2012
$2.5 \times 10^1 - 7.5 \times 10^3 \text{ nmol d}^{-1} \text{ g}^{-1}$ (dry)	Soil	MUF-DC, 37°C, 60 μM	Ueno et al., 1991
Up to $5.4 \times 10^3 \text{ nmol d}^{-1} \text{ g}^{-1}$ (dry)	Soil	⁴ MUF-TC, 37°C, 25 μM	Ueno et al., 1991
$*5.4 \times 10^3 - 6.3 \times 10^0 \mu\text{g d}^{-1} \text{ g}^{-1}$ (dry)	Brackish sediment	⁵ DNP, 15°C, 0.5 mg ml ⁻¹	Hillman et al., 1989

If possible, values given in different units in the original publications were transformed into a single unit (listed values do not provide a complete overview on all measurements performed but display examples, values from experimental manipulations measured along with controls from natural habitats were excluded from the table).

¹MUF-NAG: β -N-acetyl-hexosaminidase/chitinase hydrolysis rates estimated based on the fluorogenic substrate analog N-acetyl-b-D-glucosaminide

²pNP-NAG: N-acetyl-hexosaminidase/chitinase hydrolysis rates estimated based on the fluorogenic substrate analog pNP- β -N-acetylglucosaminide

³MUF-DC: β -N-acetyl-hexosaminidase/chitinase hydrolysis rates estimated based on the fluorogenic substrate analog methylumbelliferyl-diacetyl-chitobioside

⁴MUF-TC: β -N-acetyl-hexosaminidase/chitinase hydrolysis rates estimated based on the fluorogenic substrate analog methylumbelliferyl-diacetyl-chitotrioside

⁵DNP: N-acetyl-hexosaminidase/chitinase hydrolysis rates estimated based on the fluorogenic substrate analog 3,4-dinitrophenyl-tetra-N-acetyl chitotetraoside

*Values derived from digitalized figures using the Engauge Digitizer Program (<http://digitizer.sourceforge.net/index.php?c=5>).

Table 5 | Chitinase gene copies numbers in natural habitats.

Gene copies	System	Method	References
Up to $3.4 \times 10^2 \text{ ml}^{-1}$	Freshwater	¹ qPCR on ² GH18 genes	Köllner et al., 2012
$3.4 \times 10^4 - 4.2 \times 10^7 \text{ g}^{-1}$ (wet)	Freshwater sediment	qPCR on GH18 genes	Xiao et al., 2005
Up to $\sim 8.5 \times 10^4 \text{ g}^{-1}$ (dry)	Freshwater sediment	qPCR on GH18 genes	Köllner et al., 2012
$2.5 \times 10^3 \text{ g}^{-1}$ (wet)	Soil	qPCR on GH18 genes	Xiao et al., 2005
$2.3 \times 10^8 - 9.3 \times 10^9 \text{ g}^{-1}$ (dry)	Soil	qPCR on GH18 genes	Gschwendtner et al., 2010
$7 \times 10^5 - 9.3 \times 10^6 \text{ g}^{-1}$ (wet)	Soil	qPCR on GH18 genes	Brankatschk et al., 2011
$3 \times 10^7 \text{ g}^{-1}$ (wet)	Soil	qPCR on GH18 genes	Kielak et al., 2013
$*4.6 \times 10^6 - 1.1 \times 10^7 \text{ g}^{-1}$ (wet)	Soil	qPCR on GH18 genes	Cretoiu et al., 2012

¹qPCR: quantitative polymerase chain reaction.

²GH18: family 18 glycoside hydrolase.

*Values derived from digitalized figures using the Engauge Digitizer Program (<http://digitizer.sourceforge.net/index.php?c=5>).

to have influenced chitin degradation rates, as maximum chitinase activity coincided with high abundances of chitin-containing organisms (Kirchman and White, 1999; LeClerc and Hollibaugh, 2006). In aquatic systems, the water-sediment interface represents a habitat where chitin accumulates as a result of sedimentation of chitinous particles. This environment is usually also identified as a hotspot for chitin degradation when compared to the water column or the bulk sediment (Hood and Meyers, 1977; Warnes and Rux, 1982; Gooday et al., 1991). In soils, decreasing chitinase activity has been observed over depth and, this pattern has been attributed to the higher presence of chitin-containing organisms in the upper soil layers (Rodríguez-Kábana et al., 1983; Ueno et al., 1991). A direct coupling of chitin concentration and

the chitinolytic community has also been demonstrated in an experiment where chitin-amendment of a soil caused an increase in chitinase gene copy numbers (Xiao et al., 2005; Kielak et al., 2013).

Only a few studies have directly related measured hydrolysis rates to shifts on the chitinolytic community: It has for example been shown that high chitinase activity measured after a soil was amended with sludge or chitin was accompanied by a decrease in the diversity of chitinases (Metcalfe et al., 2002; Kielak et al., 2013). Two recent studies in a terrestrial and an aquatic environment also reported a significant correlation between chitinase gene copy numbers and measured chitin hydrolysis rates (Brankatschk et al., 2011; Köllner et al., 2012). A correlation

between changes in the composition of the chitinolytic community and chitin hydrolysis rates has also been observed in a temporal survey of lake bacterioplankton (Beier et al., 2012), which indicates that apart from environmental factors also the community composition *per se* could be decisive for measured rates.

In summary, the available data suggest that temperature and chitin supply are important environmental factors controlling both chitin hydrolysis rates and the chitinolytic community structure. This further implies the existence of a link between dynamic shifts in the chitinolytic community and measured chitin hydrolysis rates across spatially or temporally connected habitats. Based on these observations, we speculate that organisms that contribute in significant ways to chitin degradation may in fact be specialized on chitin substrate use even if they likely also are able to metabolize other substrates.

Environments with limited connectivity or gene flow, such as systems located in different climate zones or systems that vary in salinity, have been shown to host dramatically different chitinolytic communities (Terahara et al., 2009; Beier et al., 2011; Manucharova et al., 2011). Recent evidence suggests that such isolated communities are adapted to the local prevailing conditions, as it was shown that the temperature optimum for maximal chitin degradation in soil was strongly correlated to the climate zone where the samples originated from (Manucharova et al., 2011). There may, however, still be constraints on such local adaptation, as suggested by Kang et al. (2005) who demonstrated a significant positive correlation of β -N-acetylhexosaminidase activities in wetlands with the annual mean temperature of the respective system. Future molecular studies targeting expression patterns for chitinases coupled to the presence of chitinase genes and measured rates would no doubt greatly increase our ability to decipher the mechanisms and controls underlying the process of chitin degradation, not least by identifying key players and their sensitivity to environmental change.

REFERENCES

- Amann, R. I., Ludwig, W., and Schleifer, K. H. (1995). Phylogenetic identification and *in-situ* detection of individual microbial-cells without cultivation. *Microbiol. Rev.* 59, 143–169.
- Attwood, M. M., and Zola, H. (1967). The association between chitin and protein in some chitinous tissues. *Comp. Biochem. Physiol.* 20, 993–998. doi: 10.1016/0010-406X(67)90069-2
- Aumen, N. G. (1980). Microbial succession on a chitinous substrate in a woodland stream. *Microb. Ecol.* 6, 317–327. doi: 10.1007/BF02010494
- Bassler, B. L., Yu, C., Lee, Y. C., and Roseman, S. (1991). Chitin utilization by marine bacteria - degradation and catabolism of chitin oligosaccharides by *Vibrio furnissii*. *J. Biol. Chem.* 266, 24276–24286.
- Baty, A. M., Eastburn, C. C., Diwu, Z., Techkarnjanaruk, S., Goodman, A. E., and Geesey, G. G. (2000a). Differentiation of chitinase-active and non-chitinase-active subpopulations of a marine bacterium during chitin degradation. *Appl. Environ. Microbiol.* 66, 3566–3573. doi: 10.1128/AEM.66.8.3566-3573.2000
- Baty, A. M., Eastburn, C. C., Techkarnjanaruk, S., Goodman, A. E., and Geesey, G. G. (2000b). Spatial and temporal variations in chitinolytic gene expression and bacterial biomass production during chitin degradation. *Appl. Environ. Microbiol.* 66, 3574–3585. doi: 10.1128/AEM.66.8.3574-3585.2000
- Beier, S., and Bertilsson, S. (2011). Uncoupling of chitinase activity and uptake of hydrolyses products in freshwater bacterioplankton. *Limnol. Oceanogr.* 56, 1179–1188. doi: 10.4319/lo.2011.56.4.1179
- Beier, S., Jones, C. M., Mohit, V., Hallin, S., and Bertilsson, S. (2011). Global phylogeography of chitinase genes in aquatic metagenomes. *Appl. Environ. Microbiol.* 77, 1101–1106. doi: 10.1128/AEM.01481-10
- Beier, S., Mohit, V., Ettema, T. J. G., Östman, Ö., Tranvik, L. J., and Bertilsson, S. (2012). Pronounced seasonal dynamics of freshwater chitinase genes and chitin processing. *Environ. Microbiol.* 14, 2467–2479. doi: 10.1111/j.1462-2920.2012.02764.x
- Benner, R., and Kaiser, K. (2003). Abundance of amino sugars and peptidoglycan in marine particulate and dissolved organic matter. *Limnol. Oceanogr.* 48, 118–128. doi: 10.4319/lo.2003.48.1.0118
- Bouma, C. L., and Roseman, S. (1996). Sugar transport by the marine chitinolytic bacterium *Vibrio furnissii*. Molecular cloning and analysis of the glucose and N-acetylglucosamine permeases. *J. Biol. Chem.* 271, 33457–33467. doi: 10.1074/jbc.271.52.33457
- Boyer, J. N. (1994). Aerobic and anaerobic degradation and mineralization of ^{14}C chitin by water column and sedimentinocula of the York River estuary, Virginia. *Appl. Environ. Microbiol.* 60, 174–179.
- Brankatschk, R., Töwe, S., Kleinedam, K., Schlöter, M., and Zeyer, J. (2011). Abundances and potential activities of nitrogen cycling microbial communities along a

CONCLUDING REMARKS

In the previous sections, mechanisms and ecophysiological strategies of microbial chitin degradation and the role of parameters, such as temperature and chitin supply in determining chitin degradation rates have been discussed along with an account of compositional variation in chitinoclastic communities. The absence of long-term accumulation of chitin in natural systems implies that *de novo* production of chitin is the ultimate limiting factor controlling its degradation and turnover in nature. Still, the fate of this material with regards to production of new biomass or complete mineralization to inorganic constituents varies to a considerably and the underlying factors controlling this variation are only marginally understood. Besides the presence of other, more readily degraded substrates, also the composition of the bacterial community involved into chitin utilization could influence the fraction of chitin being mineralized, i.e., by the substrate affinity toward hydrolyses products. Habitat structure might determine such general characteristics of the inherent chitin utilizing community and therefore also dictate the fate of chitin in terms of its mineralization rates. This may have major implications for the cycling of carbon and nitrogen in food webs i.e., by carbon or nitrogen removal due to mineralization and volatilization. We therefore conclude that the interactive roles of habitat and the chitinolytic or chitin utilizing community and their taxonomic identification merits further investigation.

The process of chitin degradation is easier to target than degradation of many other polymers such as the structurally heterogeneous lignin and humic acids or even cellulose. This is because of its simple structure and the existence of primer-systems targeting the chitin modifying enzymes. Chitin degradation could therefore be explored as a general model for understanding microbial degradation of biopolymers in the biosphere.

ACKNOWLEDGMENTS

We thank anonymous reviewers for constructive comments on the manuscript. We acknowledge funding from the Swedish Research Council and the Swedish Research Council Formas.

- chronosequence of a glacier forefield. *ISME J.* 5, 1025–1037. doi: 10.1038/ismej.2010.184
- Brzezinska, M. S., and Donderski, W. (2006). Chitinolytic bacteria in two lakes of different trophic status. *Pol. J. Ecol.* 54, 295–301.
- Campbell, L. L., and Williams, O. B. (1951). A study of chitin-decomposing microorganisms of marine origin. *J. Gen. Microbiol.* 5, 894–905. doi: 10.1099/00221287-5-5-894
- Chernin, L. S., Winsom, M. K., Thompson, J. M., Haran, S., Bycroft, B. W., Chet, I., et al. (1998). Chitinolytic activity in *Chromobacterium violaceum*: substrate analysis and regulation by quorum sensing. *J. Bacteriol.* 180, 4435–4441.
- Chitlaru, E., and Roseman, S. (1996). Molecular cloning and characterization of a novel beta-N-acetyl-D-glucosaminidase from *Vibrio furnissii*. *J. Biol. Chem.* 271, 33433–33439. doi: 10.1074/jbc.271.52.33433
- Cho, B. C., and Azam, F. (1988). Major role of bacteria in biogeochemical fluxes in the oceans interior. *Nature* 332, 441–443. doi: 10.1038/332441a0
- Cohen-Kupiec, R., and Chet, I. (1998). The molecular biology of chitin digestion. *Curr. Opin. Biotechnol.* 9, 270–277. doi: 10.1016/S0958-1669(98)80058-X
- Cottrell, M. T., and Kirchman, D. L. (2000). Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. *Appl. Environ. Microbiol.* 66, 1692–1697. doi: 10.1128/AEM.66.4.1692-1697.2000
- Cottrell, M. T., Moore, J. A., and Kirchman, D. L. (1999). Chitinases from uncultured marine microorganisms. *Appl. Environ. Microbiol.* 65, 2553–2557.
- Cottrell, M. T., Wood, D. N., Yu, L. Y., and Kirchman, D. L. (2000). Selected chitinase genes in cultured and uncultured marine bacteria in the alpha- and gamma-subclasses of the proteobacteria. *Appl. Environ. Microbiol.* 66, 1195–1201. doi: 10.1128/AEM.66.3.1195-1201.2000
- Cretoiu, M. S., Kielak, A. M., Abu Al-Soud, W., Sorensen, S. J., and van Elsas, J. D. (2012). Mining of unexplored habitats for novel chitinases - *chiA* as a helper gene proxy in metagenomics. *Appl. Microbiol. Biotechnol.* 94, 1347–1358. doi: 10.1007/s00253-012-4057-5
- Davies, G., and Henrissat, B. (1995). Structures and mechanisms of glycosyl hydrolases. *Structure* 3, 853–859. doi: 10.1016/S0969-2126(01)00220-9
- DeAngelis, K. M., Lindow, S. E., and Firestone, M. K. (2008). Bacterial quorum sensing and nitrogen cycling in rhizosphere soil. *FEMS Microbiol. Ecol.* 66, 197–207. doi: 10.1111/j.1574-6941.2008.00550.x
- Delpin, M. W., and Goodman, A. E. (2009a). Nitrogen regulates chitinase gene expression in a marine bacterium. *ISME J.* 3, 1064–1069. doi: 10.1038/ismej.2009.49
- Delpin, M. W., and Goodman, A. E. (2009b). Nutrient regime regulates complex transcriptional start site usage within a *Pseudoalteromonas* chitinase gene cluster. *ISME J.* 3, 1053–1063. doi: 10.1038/ismej.2009.54
- Drouillard, S., Armand, S., Davies, G. J., Vorgias, C. E., and Henrissat, B. (1997). *Serratia marcescens* chitinase is a retaining glycosidase utilizing substrate acetamido group participation. *Biochem. J.* 328, 945–949.
- Duarte, B., Reboreda, R., and Cacador, I. (2008). Seasonal variation of extracellular enzymatic activity (EEA) and its influence on metal speciation in a polluted salt marsh. *Chemosphere* 73, 1056–1063. doi: 10.1016/j.chemosphere.2008.07.072
- Eckert, E. M., Baumgartner, M., Huber, I. M., and Penththal, J. (2013). Grazing resistant freshwater bacteria profit from chitin and cell-wall-derived organic carbon. *Environ. Microbiol.* doi: 10.1111/1462-2920.12083. [Epub ahead of print].
- Ekborg, N. A., Morrill, W., Burgoyne, A. M., Li, L., and Distel, D. L. (2007). CelAB, a multifunctional cellulase encoded by *Teredinibacter turnerae* T7902^T, a culturable symbiont isolated from the wood-boring marine bivalve *Lyrodus pedicellatus*. *Appl. Environ. Microbiol.* 73, 7785–7788. doi: 10.1128/AEM.00876-07
- Everuss, K. J., Delpin, M. W., and Goodman, A. E. (2008). Cooperative interactions within a marine bacterial dual species biofilm growing on a natural biodegradable substratum. *Aquat. Microb. Ecol.* 53, 191–199. doi: 10.3354/ame01235
- Fuchs, R. L., McPherson, S. A., and Drahos, D. J. (1986). Cloning of a *Serratia marcescens* gene encoding chitinase. *Appl. Environ. Microbiol.* 51, 504–509.
- Gaffney, T. D., Lam, S. T., Ligon, J., Gates, K., Frazelle, A., Di Maio, J., et al. (1994). Global regulation of expression of antifungal factors by a *Pseudomonas fluorescens* biological control strain. *Mol. Plant-Microbe Interact.* 7, 455–463. doi: 10.1094/MPMI-7-0455
- Gal, S. W., Choi, J. Y., Kim, C. Y., Cheong, Y. H., Choi, Y. J., Lee, S. Y., et al. (1998). Cloning of the 52-kDa chitinase gene from *Serratia marcescens* KCTC2172 and its proteolytic cleavage into an active 35-kDa enzyme. *FEMS Microbiol. Lett.* 160, 151–158. doi: 10.1111/j.1574-6968.1998.tb12905.x
- Gao, J., Bauer, M. W., Shockley, K. R., Pysz, M. A., and Kelly, R. M. (2003). Growth of hyperthermophilic archaeon *Pyrococcus furiosus* on chitin involves two family 18 chitinases. *Appl. Environ. Microbiol.* 69, 3119–3128. doi: 10.1128/AEM.69.6.3119-3128.2003
- Garcia, S. L., McMahon, K. D., Martinez-Garcia, M., Srivastava, A., Sczyrba, A., Stepanauskas, R., et al. (2013). Metabolic potential of a single cell belonging to one of the most abundant lineages in freshwater bacterioplankton. *ISME J.* 7, 137–147. doi: 10.1038/ismej.2012.86
- Gooday, G. W. (1990a). The ecology of chitin degradation. *Adv. Microb. Ecol.* 11, 387–430. doi: 10.1007/978-1-4684-7612-5_10
- Gooday, G. W. (1990b). Physiology of microbial degradation of chitin and chitosan. *Biodegradation* 1, 177–190. doi: 10.1007/BF00058835
- Gooday, G. W., Prosser, J. I., Hillman, K., and Cross, M. G. (1991). Mineralization of chitin in an estuarine sediment - the importance of the chitosan pathway. *Biochem. Syst. Ecol.* 19, 395–400. doi: 10.1016/0305-1978(91)90056-6
- Gschwendtner, S., Reichmann, M., Müller, M., Radl, V., Munch, J., and Schlöter, M. (2010). Abundance of bacterial genes encoding for proteases and chitinases in the rhizosphere of three different potato cultivars. *Biol. Fertil. Soils* 46, 649–652. doi: 10.1007/s00374-010-0460-1
- Hallmann, J., Rodríguez-Kábana, R., and Kloepper, J. W. (1999). Chitin-mediated changes in bacterial communities of the soil, rhizosphere and within roots of cotton in relation to nematode control. *Soil Biol. Biochem.* 31, 551–560. doi: 10.1016/S0038-0717(98)00146-1
- Hart, P. J., Pflüger, H. D., Monzingo, A. F., Hollis, T., and Robertus, J. D. (1995). The refined crystal structure of an endochitinase from *Hordeum vulgare* L. seeds at 1.8 Å resolution. *J. Mol. Biol.* 248, 402–413. doi: 10.1016/S0022-2836(95)80059-X
- Herwig, R. P., Pellerin, N. B., Irgens, R. L., Maki, J. S., and Staley, J. T. (1988). Chitinolytic bacteria and chitin mineralization in the marine waters and sediments along the antarctic peninsula. *FEMS Microbiol. Ecol.* 53, 101–111. doi: 10.1111/j.1574-6968.1988.tb02653.x
- Hillman, K., Gooday, G. W., and Prosser, J. I. (1989). The mineralization of chitin in the sediments of the Ythan estuary, aberdeenshire, Scotland. *Estuar. Coast. Shelf Sci.* 29, 601–612. doi: 10.1016/0272-7714(89)90013-9
- Hobel, C. F. V., Marteinsson, V. T., Hreggvidsson, G. O., and Kristjánsson, J. K. (2005). Investigation of the microbial ecology of intertidal hot springs by using diversity analysis of 16S rRNA and chitinase genes. *Appl. Environ. Microbiol.* 71, 2771–2776. doi: 10.1128/AEM.71.5.2771-2776.2005
- Höltje, J. V. (1996). “Lysozyme substrates,” in *Lysozymes: Model Enzymes in Biochemistry and Biology*, ed P. Jollès (Basel: Birkhäuser Verlag), 105–111. doi: 10.1007/978-3-0348-9225-4_7
- Hood, M. A., and Meyers, S. P. (1977). Rates of chitin degradation in an estuarine environment. *J. Oceanogr. Soc. Jpn.* 33, 328–334. doi: 10.1007/BF02109578
- Horn, S. J., Sørbotten, A., Synstad, B., Sikorski, P., Sørli, M., Vårum, K. M., et al. (2006). Endo/exo mechanism and processivity of family 18 chitinases produced by *Serratia marcescens*. *FEBS J.* 273, 491–503. doi: 10.1111/j.1742-4658.2005.05079.x
- Huber, R., Stöhr, J., Hohenhaus, S., Rachel, R., Burggraf, S., Jannasch, H., et al. (1995). *Thermococcus chitonophagus* sp. nov., a novel, chitin-degrading, hyperthermophilic archaeum from a deep-sea hydrothermal vent environment. *Arch. Microbiol.* 164, 255–264. doi: 10.1007/BF02529959
- Hult, E. L., Katouno, F., Uchiyama, T., Watanabe, T., and Sugiyama, J. (2005). Molecular directionality in crystalline β-chitin: hydrolysis by chitinases A and B from *Serratia marcescens* 2170. *Biochem. J.* 388, 851–856. doi: 10.1042/BJ20050090
- Hunt, D. E., Gevers, D., Vahora, N. M., and Polz, M. F. (2008). Conservation of the chitin utilization pathway in the *Vibrionaceae*. *Appl. Environ. Microbiol.* 74, 44–51. doi: 10.1128/AEM.01412-07

- Jackson, C., and Vallaire, S. (2009). Effects of salinity and nutrients on microbial assemblages in Louisiana wetland sediments. *Wetlands* 29, 277–287. doi: 10.1672/08-86.1
- Jagmann, N., Brachvogel, H. P., and Philipp, B. (2010). Parasitic growth of *Pseudomonas aeruginosa* in co-culture with the chitinolytic bacterium *Aeromonas hydrophila*. *Environ. Microbiol.* 12, 1787–1802. doi: 10.1111/j.1462-2920.2010.02271.x
- Kaiser, K., and Benner, R. (2008). Major bacterial contribution to the ocean reservoir of detrital organic carbon and nitrogen. *Limnol. Oceanogr.* 53, 99–112. doi: 10.4319/lo.2008.53.1.0099
- Kaneko, T., and Colwell, R. R. (1978). Annual cycle of *Vibrio Parahaemolyticus* in Chesapeake Bay. *Microb. Ecol.* 4, 135–155. doi: 10.1007/BF02014284
- Kang, H. J., Freeman, C., Park, S. S., and Chun, J. (2005). N-Acetylglucosaminidase activities in wetlands: a global survey. *Hydrobiologia* 532, 103–110. doi: 10.1007/s10750-004-9450-3
- Karlsson, M., and Stenlid, J. (2009). Evolution of family 18 glycoside hydrolases: diversity, domain structures and phylogenetic relationships. *J. Mol. Microbiol. Biotechnol.* 16, 208–223. doi: 10.1159/000151220
- Keyhani, N. O., and Roseman, S. (1996). The chitin catabolic cascade in the marine bacterium *Vibrio furnissii*. Molecular cloning, isolation, and characterization of a periplasmic beta-N-acetylglucosaminidase. *J. Biol. Chem.* 271, 33425–33432. doi: 10.1074/jbc.271.52.33425
- Keyhani, N. O., and Roseman, S. (1997). Wild-type *Escherichia coli* grows on the chitin disaccharide, N, N'-diacetylchitobiose, by expressing the *cel* operon. *Proc. Natl. Acad. Sci. U.S.A.* 94, 14367–14371. doi: 10.1073/pnas.94.26.14367
- Keyhani, N. O., and Roseman, S. (1999). Physiological aspects of chitin catabolism in marine bacteria. *Biochim. Biophys. Acta* 1473, 108–122. doi: 10.1016/S0304-4165(99)00172-5
- Kielak, A. M., Cretoui, M. S., Semenov, A. V., Sorensen, S. J., and van Elsas, J. D. (2013). Bacterial chitinolytic communities respond to chitin and pH alteration in soil. *Appl. Environ. Microbiol.* 79, 263–272. doi: 10.1128/AEM.02546-12
- Kirchman, D. L., and White, J. (1999). Hydrolysis and mineralization of chitin in the Delaware estuary. *Aquat. Microb. Ecol.* 18, 187–196. doi: 10.3354/ame018187
- Köllner, K. E., Carstens, D., Keller, E., Vazquez, F., Schubert, C. J., Zeyer, J., et al. (2012). Bacterial chitin hydrolysis in two lakes with contrasting trophic statuses. *Appl. Environ. Microbiol.* 78, 695–704. doi: 10.1128/AEM.06330-11
- LeCleir, G. R., Buchan, A., Maurer, J., Moran, M. A., and Hollibaugh, J. T. (2007). Comparison of chitinolytic enzymes from an alkaline, hypersaline lake and an estuary. *Environ. Microbiol.* 9, 197–205. doi: 10.1111/j.1462-2920.2006.01128.x
- LeCleir, G. R., and Hollibaugh, J. T. (2006). Chitinolytic bacteria from alkaline hypersaline Mono Lake, California, USA. *Aquat. Microb. Ecol.* 42, 255–264. doi: 10.3354/ame042255
- Li, X. B., and Roseman, S. (2004). The chitinolytic cascade in *Vibrios* is regulated by chitin oligosaccharides and a two-component chitin catabolic sensor/kinase. *Proc. Natl. Acad. Sci. U.S.A.* 101, 627–631. doi: 10.1073/pnas.0307645100
- Li, Y., and Wilson, D. B. (2008). Chitin binding by *Thermobifida fusca* cellulase catalytic domains. *Biotechnol. Bioeng.* 100, 644–652. doi: 10.1002/bit.21808
- Manucharova, N. A., Vlasenko, A. N., Men'ko, E. V., and Zvyagintsev, D. G. (2011). Specificity of the chitinolytic microbial complex of soils incubated at different temperatures. *Microbiology* 80, 205–215. doi: 10.1134/S002626171102010X
- Meibom, K. L., Li, X. B. B., Nielsen, A. T., Wu, C. Y., Roseman, S., and Schoolnik, G. K. (2004). The *Vibrio cholerae* chitin utilization program. *Proc. Natl. Acad. Sci. U.S.A.* 101, 2524–2529. doi: 10.1073/pnas.0308707101
- Merzendorfer, H., and Zimoch, L. (2003). Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. *J. Exp. Biol.* 206, 4393–4412. doi: 10.1242/jeb.00709
- Metcalf, A. C., Krsek, M., Gooday, G. W., Prosser, J. I., and Wellington, E. M. H. (2002). Molecular analysis of a bacterial chitinolytic community in an upland pasture. *Appl. Environ. Microbiol.* 68, 5042–5050. doi: 10.1128/AEM.68.10.5042-5050.2002
- Miyashita, K., Fujii, T., and Saito, A. (2000). Induction and repression of a *Streptomyces lividans* chitinase gene promoter in response to various carbon sources. *Biosci. Biotechnol. Biochem.* 64, 39–43. doi: 10.1271/bbb.64.39
- Mobley, H. L. T., Doyle, R. J., Streips, U. N., and Langemeier, S. O. (1982). Transport and incorporation of N-acetyl-D-glucosamine in *Bacillus subtilis*. *J. Bacteriol.* 150, 8–15.
- Montgomery, M. T., and Kirchman, D. L. (1993). Role of chitin-binding proteins in the specific attachment of the marine bacterium *Vibrio harveyi* to chitin. *Appl. Environ. Microbiol.* 59, 373–379.
- Montgomery, M. T., and Kirchman, D. L. (1994). Induction of chitin-binding proteins during the specific attachment of the marine bacterium *Vibrio harveyi* to chitin. *Appl. Environ. Microbiol.* 60, 4284–4288.
- Nedoma, J., Vrba, J., Hejzlar, J., Šimek, K., and Straškrabová, V. (1994). N-acetylglucosamine dynamics in freshwater environments: concentration of amino sugars, extracellular enzyme activities, and microbial uptake. *Limnol. Oceanogr.* 39, 1088–1100. doi: 10.4319/lo.1994.39.5.1088
- Ohno, T., Armand, S., Hata, T., Nikaidou, N., Henrissat, B., Mitsutomi, M., et al. (1996). A modular family 19 chitinase found in the prokaryotic organism *Streptomyces griseus* HUT (6037). *J. Bacteriol.* 178, 5065–5070.
- Park, J. K., Keyhani, N. O., and Roseman, S. (2000). Chitin catabolism in the marine bacterium *Vibrio furnissii*. Identification, molecular cloning, and characterization of a N-N'-diacetylchitobiose phosphorylase. *J. Biol. Chem.* 275, 33077–33083. doi: 10.1074/jbc.M001042200
- Perrakis, A., Tews, I., Dauter, Z., Oppenheim, A. B., Chet, I., Wilson, K. S., et al. (1994). Crystal structure of a bacterial chitinase at 2.3 Å resolution. *Structure* 2, 1169–1180. doi: 10.1016/S0969-2126(94)00119-7
- Postma, P. W., Lengeler, J. W., and Jacobson, G. R. (1993). Phosphoenolpyruvate: carbohydrate phosphotransferase systems of bacteria. *Microbiol. Rev.* 57, 543–594.
- Pruzzo, C., Crippa, A., Bertone, S., Pane, L., and Carli, A. (1996). Attachment of *Vibrio alginolyticus* to chitin mediated by chitin-binding proteins. *Microbiology* 142, 2181–2186. doi: 10.1099/13500872-142-8-2181
- Rabinovich, M. L., Melnik, M. S., and Boloboba, A. V. (2002). Microbial cellulases (Review). *Appl. Biochem. Microbiol.* 38, 305–321. doi: 10.1023/A:1016264219885
- Riemann, L., and Azam, F. (2002). Widespread N-acetyl-D-glucosamine uptake among pelagic marine bacteria and its ecological implications. *Appl. Environ. Microbiol.* 68, 5554–5562. doi: 10.1128/AEM.68.11.5554-5562.2002
- Rietl, A. J., and Jackson, C. R. (2012). Effects of the ecological restoration practices of prescribed burning and mechanical thinning on soil microbial enzyme activities and leaf litter decomposition. *Soil Biol. Biochem.* 50, 47–57. doi: 10.1016/j.soilbio.2012.03.008
- Rodríguez-Kábana, R., Godoy, G., Morganjones, G., and Shelby, R. A. (1983). The determination of soil chitinase activity - conditions for assay and ecological studies. *Plant Soil* 75, 95–106. doi: 10.1007/BF02178617
- Romaguera, A., Menge, U., Breves, R., and Diekmann, H. (1992). Chitinases of *Streptomyces olivaceoviridis* and significance of processing for multiplicity. *J. Bacteriol.* 174, 3450–3454.
- Saito, A., Fujii, T., Yoneyama, T., Redenbach, M., Ohno, T., Watanabe, T., et al. (1999). High-multiplicity of chitinase genes in *Streptomyces coelicolor* A3. *Biosci. Biotechnol. Biochem.* 63, 710–718. doi: 10.1271/bbb.63.710
- Schaefer, J., Kramer, K. J., Garbow, J. R., Jacob, G. S., Stejskal, E. O., Hopkins, T. L., et al. (1987). Aromatic cross-links in insect cuticle: detection by solid-state ¹³C and ¹⁵N NMR. *Science* 235, 1200–1204. doi: 10.1126/science.3823880
- Schnellmann, J., Zeltins, A., Blaak, H., and Schrepf, H. (1994). The novel lectin-like protein Chb1 is encoded by a chitin-inducible *Streptomyces olivaceoviridis* gene and binds specifically to crystalline α -chitin of fungi and other organisms. *Mol. Microbiol.* 13, 807–819. doi: 10.1111/j.1365-2958.1994.tb00473.x
- Scigelova, M., and Crout, D. H. G. (1999). Microbial beta-N-acetylhexosaminidases and their biotechnological applications. *Enzyme Microb. Technol.* 25, 3–14. doi: 10.1016/S0141-0229(98)00171-9
- Shapiro, J. A. (1998). Thinking about bacterial populations as multicellular organisms. *Annu. Rev. Microbiol.* 52, 81–104. doi: 10.1146/annurev.micro.52.1.81
- Shimosaka, M., Fukumori, Y., Narita, T., Zhang, X. Y., Kodaira, R., Nogawa, M., et al. (2001). The

- bacterium *Burkholderia gladioli* strain CHB101 produces two different kinds of chitinases belonging to families 18 and 19 of the glycosyl hydrolases. *J. Biosci. Bioeng.* 91, 103–105. doi: 10.1016/S1389-1723(01)80123-7
- Sikorski, P., Sørbotten, A., Horn, S. J., Eijssink, V. G. H., and Vårum, K. M. (2006). *Serratia marcescens* chitinases with tunnel-shaped substrate-binding grooves show endo activity and different degrees of processivity during enzymatic hydrolysis of chitosan. *Biochemistry* 45, 9566–9574. doi: 10.1021/bi060370l
- Skujiņš, J., Puķīte, A., and McLaren, A. D. (1973). Adsorption and reactions of chitinase and lysozyme on chitin. *Mol. Cell. Biochem.* 2, 221–228. doi: 10.1007/BF01795475
- Smith, D. C., Simon, M., Alldredge, A. L., and Azam, F. (1992). Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature* 359, 139–142. doi: 10.1038/359139a0
- Somashekar, D., and Joseph, R. (1996). Chitosanases - properties and applications: a review. *Bioresour. Technol.* 55, 35–45. doi: 10.1016/0960-8524(95)00144-1
- Souza, C. P., Almeida, B. C., Colwell, R. R., and Rivera, I. N. (2011). The importance of chitin in the marine environment. *Mar. Biotechnol.* 13, 823–830. doi: 10.1007/s10126-011-9388-1
- Stepanaukas, R., and Sieracki, M. E. (2007). Matching phylogeny and metabolism in the uncultured marine bacteria, one cell at a time. *Proc. Natl. Acad. Sci. U.S.A.* 110, 9052–9057. doi: 10.1073/pnas.0700496104
- Štrojsová, A., and Dyhrman, S. T. (2008). Cell-specific beta-N-acetylglucosaminidase activity in cultures and field populations of eukaryotic marine phytoplankton. *FEMS Microbiol. Ecol.* 64, 351–361. doi: 10.1111/j.1574-6941.2008.00479.x
- Štrojsová, M., and Vrba, J. (2005). Direct detection of digestive enzymes in planktonic rotifers using enzyme-labelled fluorescence (ELF). *Mar. Freshwater Res.* 56, 189–195. doi: 10.1071/MF04280
- Suzuki, K., Sugawara, N., Suzuki, M., Uchiyama, T., Katouno, E., Nikaidou, N., et al. (2002). Chitinases, A, B, and C1 of *Serratia marcescens* 2170 produced by recombinant *Escherichia coli*: enzymatic properties and synergism on chitin degradation. *Biosci. Biotechnol. Biochem.* 66, 1075–1083. doi: 10.1271/bbb.66.1075
- Suzuki, K., Suzuki, M., Taiyoji, M., Nikaidou, N., and Watanabe, T. (1998). Chitin binding protein (CBP21) in the culture supernatant of *Serratia marcescens* 2170. *Biosci. Biotechnol. Biochem.* 62, 128–135. doi: 10.1271/bbb.62.128
- Suzuki, K., Taiyoji, M., Sugawara, N., Nikaidou, N., Henrissat, B., and Watanabe, T. (1999). The third chitinase gene (chiC) of *Serratia marcescens* 2170 and the relationship of its product to other bacterial chitinases. *Biochem. J.* 343, 587–596. doi: 10.1042/0264-6021:3430587
- Svitil, A. L., Chadhain, S. M. N., Moore, J. A., and Kirchman, D. L. (1997). Chitin degradation proteins produced by the marine bacterium *Vibrio harveyi* growing on different forms of chitin. *Appl. Environ. Microbiol.* 63, 408–413.
- Tanaka, T., Fujiwara, S., Nishikori, S., Fukui, T., Takagi, M., and Imanaka, T. (1999). A unique chitinase with dual active sites and triple substrate binding sites from the hyperthermophilic archaeon *Pyrococcus kodakaraensis* KOD1. *Appl. Environ. Microbiol.* 65, 5338–5344.
- Techkarnjanaruk, S., and Goodman, A. E. (1999). Multiple genes involved in chitin degradation from the marine bacterium *Pseudoalteromonas* sp. strain S91. *Microbiology* 145, 925–934. doi: 10.1099/13500872-145-4-925
- Techkarnjanaruk, S., Pongpattanakitsote, S., and Goodman, A. E. (1997). Use of a promoterless lacZ gene insertion to investigate chitinase gene expression in the marine bacterium *Pseudoalteromonas* sp. strain S9. *Appl. Environ. Microbiol.* 63, 2989–2996.
- Terahara, T., Ikeda, S., Noritake, C., Minamisawa, K., Ando, K., Tsuneda, S., et al. (2009). Molecular diversity of bacterial chitinases in arable soils and the effects of environmental factors on the chitinolytic bacterial community. *Soil Biol. Biochem.* 41, 473–480. doi: 10.1016/j.soilbio.2008.11.024
- Tracey, M. A. (1957). Chitin. *Rev. Pure Appl. Chem.* 7, 1–13.
- Tsujibo, H., Fujimoto, K., Tanno, H., Miyamoto, K., Imada, C., Okami, Y., et al. (1994). Gene sequence, purification and characterization of N-acetyl-β-glucosaminidase from a marine bacterium, *Alteromonas* sp. Strain O-7. *Gene* 146, 111–115. doi: 10.1016/0378-1119(94)90843-5
- Tsujibo, H., Kubota, T., Yamamoto, M., Miyamoto, K., and Inamori, Y. (2003). Characterization of chitinase genes from an alkaliphilic actinomycete, *Nocardiaopsis prasina* OPC-131. *Appl. Environ. Microbiol.* 69, 894–900. doi: 10.1128/AEM.69.2.894-900.2003
- Ueno, H., Miyashita, K., Sawada, Y., and Oba, Y. (1991). Assay of chitinase and N-acetylglucosaminidase activity in forest soils with 4-methylumbelliferyl derivatives. *Zeitschrift für Pflanzenernährung und Bodenkunde* 154, 171–175. doi: 10.1002/jpln.19911540304
- Vaae-Kolstad, G., Horn, S. J., van Aalten, D. M. F., Synstad, B., and Eijssink, V. G. H. (2005). The non-catalytic chitin-binding protein CBP21 from *Serratia marcescens* is essential for chitin degradation. *J. Biol. Chem.* 280, 28492–28497. doi: 10.1074/jbc.M504468200
- Vetter, Y. A., Deming, J. W., Jumars, P. A., and Krieger-Brockett, B. B. (1998). A predictive model of bacterial foraging by means of freely released extracellular enzymes. *Microb. Ecol.* 36, 75–92. doi: 10.1007/s002489900095
- Vrba, J., Filandr, P., Nedoma, J., and Simek, K. (1996). “Different sources of extracellular β-N-acetylhexosaminidases-like activities in freshwaters,” in *Chitin Enzymology*, ed R. A. A. Muzzarelli (Senigallia: Atec Edizioni), 293–301.
- Vrba, J., Kofroňová-Bobková, J., Pernthaler, J., Simek, K., Macek, M., and Psenner, R. (1997). Extracellular, low-affinity beta-N-acetylglucosaminidases linked to the dynamics of diatoms and crustaceans in freshwater systems of different trophic degree. *Internationale Revue der Gesamten Hydrobiologie* 82, 277–286. doi: 10.1002/iroh.19970820213
- Vrba, J., and Machacek, J. (1994). Release of dissolved extracellular Beta-N-Acetylglucosaminidase during crustacean molting. *Limnol. Oceanogr.* 39, 712–716. doi: 10.4319/lo.1994.39.3.0712
- Vrba, J., Nedoma, J., Simek, K., and Seda, J. (1992). Microbial decomposition of polymer organic-matter related to plankton development in a reservoir - activity of α-Glucosidase, β-Glucosidase, and β-N-acetylglucosaminidase and uptake of N-acetylglucosamine. *Arch. Hydrobiol.* 126, 193–211.
- Warnes, C. E., and Rux, T. P. (1982). “Chitin mineralization in a freshwater habitat,” in *International Conference on Chitin and Chitosan*, eds S. Hirano and S. Tokura (Sapporo: Japanese Society of Chitin and Chitosan), 191–195.
- Watanabe, T., Kanai, R., Kawase, T., Tanabe, T., Mitsutomi, M., Sakuda, S., et al. (1999). Family 19 chitinases of *Streptomyces* species: characterization and distribution. *Microbiology* 145, 3353–3363.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J. (1998). Prokaryotes: the unseen majority. *Proc. Natl. Acad. Sci. U.S.A.* 95, 6578–6583. doi: 10.1073/pnas.95.12.6578
- Wurzbacher, C. M., Barlocher, F., and Grossart, H. P. (2010). Fungi in lake ecosystems. *Aquat. Microb. Ecol.* 59, 125–149. doi: 10.3354/ame01385
- Xia, W., Liu, P., and Liu, J. (2008). Advance in chitosan hydrolysis by non-specific cellulases. *Bioresour. Technol.* 99, 6751–6762. doi: 10.1016/j.biortech.2008.01.011
- Xiao, X., Yin, X. B., Lin, H., Sun, L. G., You, Z. Y., Wang, P., et al. (2005). Chitinase genes in lake sediments of Ardley Island, Antarctica. *Appl. Environ. Microbiol.* 71, 7904–7909. doi: 10.1128/AEM.71.12.7904-7909.2005
- Yasir, M., Aslam, Z., Kim, S. W., Lee, S. W., Jeon, C. O., and Chung, Y. R. (2009). Bacterial community composition and chitinase gene diversity of vermicompost with antifungal activity. *Bioresour. Technol.* 100, 4396–4403. doi: 10.1016/j.biortech.2009.04.015
- ZoBell, C. E., and Rittenberg, S. C. (1938). The occurrence and characteristics of chitinolytic bacteria in the sea. *J. Bacteriol.* 35, 275–287.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 10 April 2013; accepted: 28 May 2013; published online: 14 June 2013.

Citation: Beier S and Bertilsson S (2013) Bacterial chitin degradation—mechanisms and ecophysiological strategies. *Front. Microbiol.* 4:149. doi: 10.3389/fmicb.2013.00149

This article was submitted to *Frontiers in Terrestrial Microbiology*, a specialty of *Frontiers in Microbiology*.

Copyright © 2013 Beier and Bertilsson. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.



Field and lab conditions alter microbial enzyme and biomass dynamics driving decomposition of the same leaf litter

Zachary L. Rinkes^{1*}, Robert L. Sinsabaugh², Daryl L. Moorhead¹, A. Stuart Grandy³ and Michael N. Weintraub¹

¹ Department of Environmental Sciences, University of Toledo, Toledo, OH, USA

² Department of Biology, University of New Mexico, Albuquerque, NM, USA

³ Department of Natural Resources and the Environment, University of New Hampshire, Durham, NH, USA

Edited by:

Johannes Rousk, Lund University, Sweden

Reviewed by:

Richard S. Winder, Natural Resources Canada, Canada

Nathan Basiliko, Laurentian University, Canada

*Correspondence:

Zachary L. Rinkes, Department of Environmental Sciences, University of Toledo, 2801 West Bancroft Street, Mail Stop 604, Toledo, OH 43606, USA
e-mail: zachary.rinkes@rockets.utoledo.edu

Fluctuations in climate and edaphic factors influence field decomposition rates and preclude a complete understanding of how microbial communities respond to plant litter quality. In contrast, laboratory microcosms isolate the intrinsic effects of litter chemistry and microbial community from extrinsic effects of environmental variation. Used together, these paired approaches provide mechanistic insights to decomposition processes. In order to elucidate the microbial mechanisms underlying how environmental conditions alter the trajectory of decay, we characterized microbial biomass, respiration, enzyme activities, and nutrient dynamics during early (<10% mass loss), mid- (10–40% mass loss), and late (>40% mass loss) decay in parallel field and laboratory litter bag incubations for deciduous tree litters with varying recalcitrance (dogwood < maple < maple-oak mixture < oak). In the field, mass loss was minimal (<10%) over the first 50 days (January–February), even for labile litter types, despite above-freezing soil temperatures and adequate moisture during these winter months. In contrast, microcosms displayed high C mineralization rates in the first week. During mid-decay, the labile dogwood and maple litters in the field had higher mass loss per unit enzyme activity than the lab, possibly due to leaching of soluble compounds. Microbial biomass to litter mass (B:C) ratios peaked in the field during late decay, but B:C ratios declined between mid- and late decay in the lab. Thus, microbial biomass did not have a consistent relationship with litter quality between studies. Higher oxidative enzyme activities in oak litters in the field, and higher nitrogen (N) accumulation in the lab microcosms occurred in late decay. We speculate that elevated N suppressed fungal activity and/or biomass in microcosms. Our results suggest that differences in microbial biomass and enzyme dynamics alter the decay trajectory of the same leaf litter under field and lab conditions.

Keywords: microbial biomass, extracellular enzyme, litter bag, decomposition, decomposer community, nutrients, turnover activity

INTRODUCTION

The mineralization of newly senescent leaf litter contributes approximately half of the annual carbon dioxide (CO₂) efflux from soils in temperate deciduous forests (Schlesinger and Andrews, 2000). Complex interactions between litter quality and microbial communities regulate the magnitude of this carbon (C) flux and determine the trajectory of decay (Berg and McClaugherty, 2008). For instance, the same litter exposed to different microbial communities frequently displays pronounced differences in chemistry, even after substantial mass loss (Wallenstein et al., 2011; Wickings et al., 2011, 2012). Additionally, the complexity and diversity of litter chemical composition and its impact on microbial community function may explain why diverse plant litter mixtures often follow different decay trajectories than the average of the component species alone (Meier and Bowman, 2010). However, we lack detailed data on how microbial communities respond to labile

and recalcitrant litter types at progressive stages of decomposition under field and laboratory conditions.

Decomposition rates of the same litter vary widely across terrestrial ecosystems. For instance, *Cornus* (dogwood) and *Quercus* (oak) mass loss can range from 50–75% to 25–55%, respectively, between field studies after one year of decomposition (Blair, 1988; Blair et al., 1992; Carreiro et al., 2000; Knoepp et al., 2005; Piatek et al., 2010). *Pinus* (pine) litter displayed highly variable decay rates over time after 5 years of field decomposition over 28 sites throughout North America (Gholz et al., 2000). Thus, it appears that site-specific factors influence microbial-substrate interactions and C flux patterns. We need to explain this underlying variability between field studies, especially how microbial behavior and decay rates of different litter types change in response to variations in climate and edaphic factors, to enhance the accuracy of decomposition models.

The influence of environmental factors on microbial dynamics and decomposition patterns is difficult to predict. For instance, soil temperature and moisture fluctuate seasonally in the field (Aerts, 1997; Liski et al., 2003), which influences microbial growth, as well as extracellular enzyme pools and activities (Baldrian et al., 2013). Variations in quality of plant litter, soil organic matter content and pH, and even wind velocity can alter microbial activity and decomposition rates (Berg and McClaugherty, 2008). Filamentous decomposers (i.e., actinomycetes and fungi) influence decomposition by physically integrating substrates that differ in C and nitrogen (N) availability, thus overcoming local nutrient limitation through translocation (Boberg et al., 2010). Therefore, the magnitude of decomposer responses to litter quality under field conditions is not consistent across sites due to the variable effects of biotic and abiotic factors on microbial-substrate interactions and C flux rates (Carreiro et al., 2000; Treseder, 2008; Snajdr et al., 2011).

In contrast to the influences of environmental conditions on decomposition in the field, laboratory microcosms isolate the intrinsic effects of litter and soil chemistry and microbial community from extrinsic effects of climatic variation. For instance, constant temperature and moisture conditions in the laboratory optimize CO₂ production and decay rates (Risk et al., 2008). Laboratory microcosms also eliminate variable C and nutrient subsidies to microbial activity (Salamanca et al., 1998). For instance, microcosms prevent decomposers from using adjacent litter resources, exclude new microbial colonizers (i.e., fungi) from translocating nutrients from external sources during later stages of decay, and disregard interactions between plant roots and nutrients (Teuben and Verhoef, 1992). In addition, microcosms disrupt *in-situ* microbial consortia and networks, and homogenize microbial functional behavior that otherwise is spatially compartmentalized or heterogeneous under field conditions (Kampichler et al., 2001). Thus, laboratory microcosm studies describe interactions between microbial communities and leaf litter with much lower variability than in the field, and provide mechanistic information describing underlying processes of decomposition needed to refine decomposition models (McGuire and Treseder, 2009).

Traditional predictive models of litter decay assume that microbial communities are black boxes functioning in the same way in different environments (Bradford and Fierer, 2012). While useful in stable environments, these models do not sufficiently describe C flow or microbial dynamics under variable conditions (Schimel and Weintraub, 2003; Manzoni and Porporato, 2009). For instance, conventional models fail to capture how changes in microbial function impact C gains and losses throughout decay (Treseder et al., 2011). In contrast, mechanistic decomposition models that incorporate both decomposers and their enzymes as explicit drivers of decay predict C dynamics under variable conditions better than earlier models that simulated litter decay as a first-order process without microbial decomposers (Moorhead and Sinsabaugh, 2006; Moorhead et al., 2012). However, parameterizing mechanistic models is difficult due to the highly interactive and variable factors that influence microbial activity.

Our goal was to elucidate the microbial mechanisms underlying the variability associated with field litter bag studies by monitoring the decomposition dynamics of contrasting litter species in both

field and lab settings. To accomplish this goal, we conducted parallel field and laboratory experiments using three litter species that varied in initial recalcitrance (dogwood < sugar maple < white oak) and an equal maple-oak mixture. We monitored mass loss, microbial biomass, and enzyme activity, and inorganic nutrient dynamics during decomposition. We established separate hypotheses for early (<10% mass loss), mid- (10–40% mass loss), and late (>40% mass loss) stages of decay:

Early Decay: We hypothesized that decomposition in early decay is regulated by the availability of water-soluble labile compounds. For instance, soluble substrates are preferentially consumed in fresh litter, primarily by decomposers with limited enzymatic capabilities. Thus, we expected that differences in mass loss between litter types in both the field and lab would increase with initial litter soluble content (dogwood > maple > mixture > oak). However, we predicted decay rates, microbial biomass to litter mass (B:C) ratios, and microbial demand for N and phosphorus (P) relative to C for all litter types to be greater in the lab than field, because of high water-soluble C availability in fresh litter combined with optimal temperature and moisture conditions for biological activity.

Mid Decay: We hypothesized that decomposition in mid-decay is regulated by the depletion of water-soluble C and labile nutrients, which would be reflected by increased extracellular enzyme activities targeting organic polymeric substrates. We expected microbial N and P demand to be greater in the lab than field, due to rapid depletion of nutrients from the limited amount of low-nutrient content soil used in the incubation. We predicted that nutrient limitation would decrease decay rates and B:C ratios for all litter types in the lab relative to the field, as decomposers cannot use external resources or translocate nutrients from adjacent litter in microcosms.

Late Decay: We hypothesized that the increasing relative proportion of lignin regulates decomposition in late decay. For instance, we used oxidative enzyme activity as a proxy for microbial breakdown of lignin. We expected that lab decomposition rates, B:C ratios, and microbial N and P demand would decrease in comparison to field incubations in response to the accumulation of nutrients in the lab microcosms. We predicted that lignolytic activity and biomass would be greater in the field, especially in the high lignin oak litter, due to increased fungal activity and the limited potential for excess nutrients to inhibit lignolytic activity.

Thus, we established separate hypotheses for early, mid-, and late decay to determine how environmental conditions and edaphic factors alter microbial-substrate interactions during decay.

MATERIALS AND METHODS

STUDY SITE

The study site was an oak-maple forest within the Oak Openings Region (N 41°33', W 83°50') of Northwest Ohio. Our study area was in the 1,500 ha Oak Openings Preserve Metropark. The mean annual temperature is 9.2°C and annual precipitation is 840 mm (DeForest et al., 2009). Soils have a low pH (~4.5) and are sandy, mixed, mesic Spodic Udipsamments (Noormets et al., 2008). Within the study area, we continually measured 30-min mean soil temperature [5 cm depth; CS107, Campbell Scientific

Inc. (CSI), Logan, UT, USA] and soil water content [SWC (%); CS616, CSI] in the top 20 cm from probes buried within 500 m of a micrometeorological tower operated by the University of Toledo.

LITTER COLLECTION

Cornus florida (dogwood), *Acer saccharum* (sugar maple) and *Quercus alba* (white oak) leaves were collected weekly in litter traps during October of 2009 within the study area. Litter was placed in paper bags, air dried, and maintained at room temperature at the University of Toledo. Using the same litter in both experiments minimized the potential for variation in litter chemistry to influence decomposition dynamics. Leaves (including petioles) were cut into 1 cm² pieces to control how much litter mass went into each litter bag and to facilitate use in lab microcosms.

FIELD LITTER BAG STUDY

Eight replicate 30 m² plots were randomly selected (i.e., randomized block design) within the study area at least 150 m apart and within 500 m of the micrometeorological tower. Litter bags were constructed from nylon mesh measuring 15 × 15 cm (1 mm² mesh size) and were filled with 5 g of either dogwood, sugar maple, white oak, or a 50% maple-oak mixture. Thirty litter bags (6 dogwood, 8 maple, 8 oak, and 8 of the mixture) were deployed in January 2010, into each of the 8 plots for a total of 240 litter bags. Litter bags were placed 3 m apart in direct contact with the soil surface and secured at their corners with 15 cm ground staples. Litter bags were collected during January 2010 (0 days), February 2010 (50 days), May 2010 (120 days), August 2010 (220 days), December 2010 (337 days), June 2011 (512 days), October 2011 (641 days), and May 2012 (849 days). Dogwood litter bags were harvested only six times (June 2011 and May 2012 were excluded) due to its rapid decomposition. Destructive harvests included analyses for mass loss, extracellular enzyme activities, microbial biomass-C (MB-C), dissolved organic C (DOC), ammonium (NH₄⁺), nitrate (NO₃⁻), and phosphate (PO₄³⁻), described below.

LABORATORY INCUBATION

The soil used in the laboratory incubation was a sandy soil low in C (0.6 ± 0.01%) and nutrient content, collected from a 10 m² area where the field litter bag study was conducted. Soil cores were taken from the top 5 cm (the depth with the highest biological activity), sieved (2 mm mesh) to remove coarse debris and organic matter, thoroughly mixed, and pre-incubated for 1 month in a dark 20°C incubator at 45% water-holding capacity (WHC). This is the water content that maximizes microbial respiration (Rinkes et al., 2013). The pre-incubation allowed microorganisms to acclimate to the conditions of the experiment and to metabolize labile soil C.

A 376-day laboratory incubation was established in 473-ml canning jars using the same litter as the field study (see above). Nylon mesh 6 cm × 6 cm litter bags (1 mm² mesh size) were constructed to lay flat inside each canning jar. Each litter bag contained 1 g litter and 1 g dry soil, which was used as a microbial inoculum to enhance colonization. Treatment jars included a litter bag placed in the middle of 99 g dry soil adjusted to 45% WHC. Soil-only control jars contained 100 g dry soil adjusted to 45% WHC. Eight sets of 32 litter bag + soil jars (each set with four jars each of dogwood, maple, oak, and the mixture) and four sets of soil-only control jars

(each set with four replicate jars), were incubated together. Jars were kept in a dark 20°C incubator with lids left loosely covered, which minimized water loss but allowed gas exchange. Jars were weighed initially and deionized water was added gravimetrically on a weekly basis to replace water lost to evaporation.

Litter bags were destructively harvested after 0, 2, 34, 99, 161, 230, 312, and 376 days of decomposition. Adhering soil particles were removed from the litter with a 2-mm brush before analyses for mass loss, enzyme activities, MB-C, DOC, NH₄⁺, NO₃⁻, and PO₄³⁻. Soil-only controls were destructively harvested less frequently than litter treatments, but respiration rates were monitored frequently in both litter bag + soil treatments and soil-only controls.

MICROBIAL RESPIRATION AND MASS LOSS

Respiration was quantified in the lab by measuring jar headspace CO₂ concentrations with a Li-820 infra-red gas analyzer (LI-COR Biosciences, Lincoln, NE, USA) according to the manufacturer's protocol. Jars were vented, sealed in canning jars with septae (No.:224100-181 Wheaton grey butyl stoppers) installed in the lids, and incubated at 20°C for minutes (early decay) to hours (late decay). Respiration was measured at 0, 1, 2, 3, 5, 7, 8, 25, 43, 53, 78, 99, 139, 161, 230, 259, and 376 days of incubation. Cumulative C mineralization was calculated for the laboratory experiment by determining mean rates between measurements and interpolating over time. The initial C content ranged from 40 to 45% for all litter types and was used to calculate overall C losses. A 2400 Series II CHNS/O Analyzer (PerkinElmer, Waltham, MA, USA) was used to obtain the litter C content. In addition, litter mass loss was calculated as the difference in dry weight before and after incubation in both the field and lab.

MICROBIAL BIOMASS AND NUTRIENTS

To extract litter samples for DOC, dissolved inorganic N (DIN), and dissolved inorganic P (DIP), 15-ml aliquots of an aqueous 0.5 M solution of potassium sulfate were added to each homogenized sample and agitated at 120 rpm on an orbital shaker for 1 h. Samples were vacuum filtered through Pall A/E glass fiber filters and frozen until analysis. Replicate samples were also fumigated with chloroform to quantify MB-C using a modification of the chloroform fumigation-extraction method (Brookes et al., 1985) described by Scott-Denton et al. (2006). Ethanol-free chloroform (2 ml) was added to 0.25 g (wet weight) of litter (including 3-litter free blank flasks) and incubated at room temperature for 24 h in a stoppered 250-ml Erlenmeyer flask. Following incubation, flasks were vented in a fume hood for 30 min and extracted as described above. Fumigated extracts were analyzed for total DOC on a Shimadzu total organic carbon (TOC-VCPN) analyzer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA) using the non-purgeable organic C manufacturer's protocol, which eliminates inorganic C prior to analysis. MB-C was calculated as the difference between DOC extracted from fumigated and non-fumigated samples. No extraction efficiency constant (k_{EC}) was applied because it is unknown for these samples.

Colorimetric microplate assays of the unfumigated sample extracts were used to analyze NH₄⁺, NO₃⁻ and PO₄³⁻ concentrations. NH₄⁺ concentrations were measured using a modified Berthelot reaction (Rhine et al., 1998). NO₃⁻ was determined using

a modification of the Griess reaction (Doane and Horwath, 2003), which involves the reduction of nitrate to nitrite for colorimetric determination. PO_4^{3-} was analyzed following the malachite green microplate analysis described by D'Angelo et al. (2001). Absorbance values were determined on a Bio-Tek Synergy HT microplate reader (Bio-Tek Inc., Winooski, VT, USA) according to the manufacturer's protocol.

ENZYME ASSAYS

Fluorimetric enzyme assays were conducted using procedures defined by Saiya-Cork et al. (2002). We measured β -1,4-glucosidase (BG), β -1,4-*N*-acetyl-glucosaminidase (NAG), leucine amino peptidase (LAP), and acid phosphatase (Phos) activities in 96-well microplates using methyl umbelliferyl linked fluorimetric substrates. BG hydrolyzes glucose from cellulose oligomers, especially cellobiose; NAG (a.k.a. chitinase) hydrolyzes *N*-acetyl glucosamine from chitin and peptidoglycan-derived oligomers; LAP hydrolyzes leucine and other amino acids from peptides; and Phos hydrolyzes phosphate from phosphate monoesters such as sugar phosphates. These enzymes were selected because they catalyze terminal reactions that release assimilable nutrients from organic C, N, and P sources (Sinsabaugh and Follstad Shah, 2012).

Slurries were made using 0.25 g of wet litter homogenized with 50 mM sodium acetate buffer (pH 4.5) using a Biospec Tissue Tearer (BioSpec Products, Bartlesville, OK, USA) according to the manufacturer's protocol. For the lab litter bags, adhering soil particles were removed from the litter using a 2-mm brush before addition to the slurry. We used a 200 μM substrate solution (4-MUB- β -D-glucoside for BG; 4-MUB-*N*-acetyl- β -D-glucosaminide for NAG; L-leucine-7-amino-4-methylcoumarin for LAP; and 4-MUB-phosphate for Phos) to ensure saturating substrate concentrations (German et al., 2011). After substrate addition, microplates were incubated at 20°C in darkness for at least 2 h.

High-throughput colorimetric assays were conducted in 96-well microplates for phenol oxidase (Phenox), which is a lignin-degrading enzyme, using 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) as a substrate (Floch et al., 2007). Assay wells included 200 μl aliquots of soil slurry followed by the addition of 100 μl of 1 mM ABTS. Negative controls received 200 μl of buffer and 100 μl ABTS and blank wells received 200 μl of soil slurry and 100 μl of buffer. Plates were incubated at 20°C in darkness for at least 2 h.

DATA ANALYSIS

The relationship between the remaining leaf litter mass and time (days) was fit to a simple negative exponential model of decay (Olson, 1963). Decay rate constants were determined from the equation $X_t = X_0 e^{-kt}$ where X_t is the mass remaining at time = t , X_0 is the initial mass, t is time in days and k is a decay rate constant in days^{-1} . Decay rate coefficients (k values) were calculated at different stages of decay for both the field and lab studies and k values for each stage were compared with a one-way analysis of variance (ANOVA) including litter type as the fixed effect.

Ratios of microbial biomass to litter mass (B:C) were compared at different stages of decay in both the field and lab studies using a two-way ANOVA with stage and litter type as fixed effects. B:C

ratios were examined in early decay (January and February 2010 field harvests and days 0–2 in lab), mid-decay (May 2010 through December 2010 field harvests, and days 34–161 in lab), and late decay (June 2011 through May 2012 field harvests, and days 230 through 367 in the lab).

Enzyme activities were expressed as μmol reaction product $\text{hour}^{-1} \text{g dry litter}^{-1}$. These values were subsequently integrated over all sample dates according to Sinsabaugh et al. (2002) to derive cumulative enzyme activities and calculate turnover activities. Turnover activity, or the cumulative amount of enzyme activity per unit mass loss, provides a basis for comparing microbial allocation toward extracellular enzymes and the efficiency of decomposition (higher turnover activity = lower mass loss per unit enzyme activity) across different litter types. In brief, cumulative enzyme activity was calculated by multiplying the average activity of an enzyme between harvest dates over a specific time period. Turnover activity for BG, NAG, LAP, Phos, and Phenox was calculated by dividing cumulative enzyme activities by total mass loss for each sample replicate over time. Mean turnover activity (mol) was calculated from the turnover activities for all replicates ($n = 8$ in field, $n = 4$ in lab). Separate two-way ANOVA's were performed at each stage of decay with litter type and enzyme as fixed factors. Turnover activities were examined in early decay (January through February 2010 field harvests and days 0–2 in lab), mid-decay (May 2010 through December 2010 field harvests, and days 34–161 in lab), and late decay (June 2011 through May 2012 field harvests, and days 230 through 367 in the lab) and overall.

We used BG:(NAG + LAP) and BG:Phos ratios as indicators of microbial demand for C relative to nutrients, and (NAG + LAP):Phos ratios as an indicator of microbial demand for N relative to P in both studies at each decay stage. Ratios of these C:N:P acquiring enzyme activities broadly converge on a 1:1:1 ratio across a wide range of sample types and ecosystems, and deviations from this ratio indicate relatively more or less decomposer demand for C, N, or P (Sinsabaugh et al., 2008). C:N:P ratios were examined in early decay (January and February 2010 field harvests and days 0–2 in lab), mid-decay (May 2010 through December 2010 field harvests, and days 34–161 in lab), and late decay (June 2011 through May 2012 field harvests, and days 230 through 367 in the lab) and overall.

In addition, data from the field litter bag study were analyzed using a mixed-model two-way multivariate ANOVA (MANOVA) with day and litter type as fixed effects and subplot (block) as a random effect. Mass loss, NH_4^+ , NO_3^- , PO_4^{3-} , BG, NAG, Phos, and Phenox means were compared for the six harvest dates for which we have data for all litter types (including dogwood). The block effect was not significant overall (Wilk's $\lambda = 0.89$, $P = 0.73$) or for each individual response variable, therefore the MANOVA was rerun without block as a factor (Scheiner and Gurevitch, 1993) and results from this final analysis were reported. For the laboratory incubation, mass loss, NH_4^+ , NO_3^- , PO_4^{3-} , BG, NAG, and Phos means were compared using a two-way MANOVA with day and litter type as fixed effects.

Differences among groups were considered significant if $P \leq 0.05$. Differences between groups were compared using Tukey multiple comparison post-hoc tests. Enzyme activities were log-transformed to meet assumptions for normality and

homogeneity of variance (Levene's test). Data were analyzed using SPSS Statistics version 17.0.

RESULTS

FIELD STUDY – CLIMATE AND MASS LOSS RELATIONSHIPS

Average weekly soil temperatures ranged from 1 to 24°C, while mean weekly SWC ranged from 2 to 10% over the course of the field study (**Figure 1A**). Over the initial 2 months of decomposition, litter mass loss was low for dogwood ($5.8 \pm 3.7\%$), maple ($4.7 \pm 3.2\%$), oak ($5.4 \pm 3.6\%$), and the maple-oak mixture ($1.7 \pm 1.3\%$; **Figure 1B**). During this time, soil temperatures averaged $5.6 \pm 0.13^\circ\text{C}$ and SWC averaged $6.7 \pm 0.16\%$. Dogwood, maple, oak, and the litter mixture lost $37 \pm 6.6\%$, $22.9 \pm 6.5\%$, $23.4 \pm 4.5\%$, and $24.6 \pm 6.3\%$ of their original masses, respectively, during May through August 2010 (**Figure 1B**). Mass loss then decreased for dogwood ($27.3 \pm 6.1\%$), maple ($19.1 \pm 5.5\%$), oak ($20.4 \pm 4.6\%$), and the mixture ($22.9 \pm 3.5\%$) between August 2010 and October 2011 (**Figure 1B**). SWC was highly variable but generally increased during the winter and decreased during the summer (**Figure 1A**). Mass loss was $<7\%$ for maple, oak, and the mixture between October 2011 and May 2012.

Decay rate coefficients were higher for dogwood than the other litter types during mid- and late decay and overall in the field (**Table 1**). In addition, decay rate coefficients were higher for dogwood and maple in the field than lab during mid-decay (**Table 1**). Overall, mass loss was greatest for dogwood ($76.5 \pm 2.7\%$) followed by maple ($60.9 \pm 1.9\%$), the maple-oak mixture ($58 \pm 2.6\%$), and oak ($56.1 \pm 7.5\%$). This resulted in a significant litter type effect on mass loss ($P < 0.01$; $F = 5.69$).

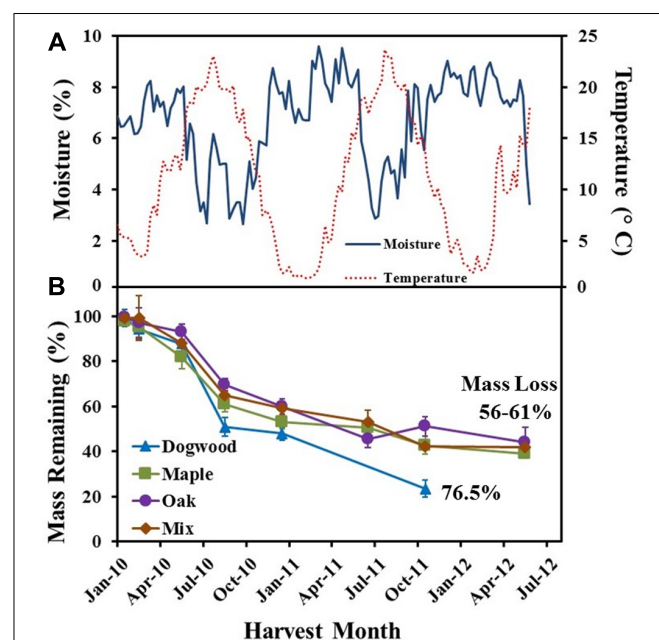


FIGURE 1 | (A) Soil temperature ($^\circ\text{C}$) and soil water content (%) weekly averages and **(B)** % mass remaining for dogwood, maple, oak, and the maple-oak mixture over a 2 1/2 year field litter bag study. Error bars show the standard error of the mean ($n = 8$).

LAB INCUBATION-MASS LOSS

In the lab, decay rate coefficients were significantly higher for dogwood than the other litter types during early decay, but similar among all litter types over the rest of the study and overall (**Table 1**). In addition, C mineralization rates for all litter types peaked within the first week and were higher on day 2 in dogwood than the other litter types ($P < 0.01$ for all; data not shown). Dogwood, maple, oak, and the litter mixture lost $20.7 \pm 0.6\%$, $15.9 \pm 0.3\%$, $16.8 \pm 0.5\%$, and $14.8 \pm 0.5\%$ of their original masses, respectively, between days 0 and 34. Over the next 127 days, mass loss was $15.6 \pm 0.9\%$ for dogwood, $18.3 \pm 1.5\%$ for maple, $21.3 \pm 1.5\%$ for oak, and $20.7 \pm 1.2\%$ for the mixture. Over the remainder of the incubation, dogwood, maple, and the mixture lost approximately 9% of the original mass, while oak lost $12.9 \pm 3.2\%$.

BIOMASS DYNAMICS

In the field, B:C ratios for all litter types in late decay were significantly higher than values in early or mid-decay ($P < 0.02$ for all; **Figure 2**). Maximum B:C ratios in the field ranged from 2 to 3% (**Figure 2**). B:C ratios in the lab were significantly higher in mid-decay than late-decay for all litter types ($P < 0.05$ for all; **Figure 2**). Maximum B:C values ranged from 1 to 3.5% in the lab (**Figure 2**).

INORGANIC NUTRIENTS

NH_4^+ significantly changed over time in both the field ($P < 0.01$; $F = 4.57$) and lab ($P < 0.01$; $F = 10.29$). NH_4^+ decreased during early decay for all litter types and remained low ($<20 \mu\text{g N g dry litter}^{-1}$) through mid-decay in the field (**Figure 3A**), but increased during late decay for maple, oak, and the maple-oak mixture. In the lab, NH_4^+ decreased during early decay and then increased during mid-decay for all litter types, but then decreased during late-decay and remained low ($<20.0 \mu\text{g N g dry litter}^{-1}$) between days 230 and 376 (**Figure 3B**).

Extractable NO_3^- was low ($<25.0 \mu\text{g N g dry litter}^{-1}$) for all litter types throughout the field study (**Figure 3C**). In contrast, NO_3^- increased quickly during mid- and late decay in the lab for all litter types (**Figure 3D**) and was significantly higher in dogwood compared to most other litters on days 161 and 230, which resulted in a significant litter type by day interaction for NO_3^- ($P < 0.01$; $F = 3.22$).

PO_4^{3-} significantly decreased for all litter types during early decay in the field (**Figure 3E**). PO_4^{3-} remained low for all litter types throughout mid-decay, but increased during late decay. PO_4^{3-} was significantly higher in the mixture during the initial harvest and in dogwood during the October 2011 harvest than other litter types ($P < 0.01$ for all; **Figure 3E**). In the lab, PO_4^{3-} concentrations increased for most litter types during mid- and late decay and were significantly higher in dogwood on day 230 than other litter types ($P < 0.01$ for all; **Figure 3F**). Overall, there were significant litter type by day interactions on PO_4^{3-} in the field ($P < 0.01$; $F = 7.49$) and lab ($P < 0.01$; $F = 4.37$).

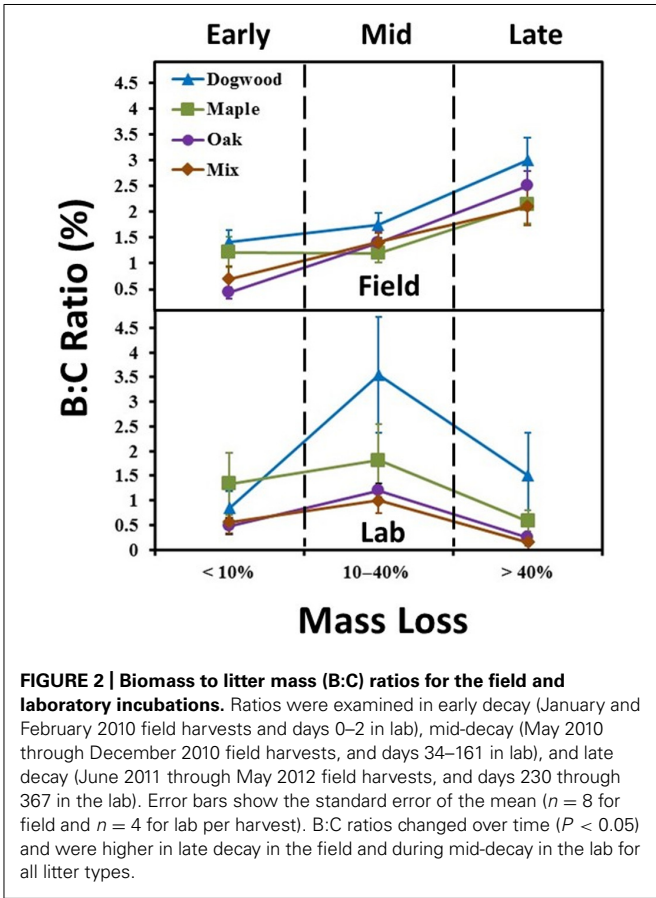
ENZYME ACTIVITY

In the field, BG activities peaked during late decay for maple, oak, and their mixture (**Figure 4A**). BG activity increased following

Table 1 | Decay rate coefficients (*k*) for dogwood, maple, oak, and the maple-oak mixture during early, mid-, and late decay, and over the entire duration of the 849-day (641-day for dogwood) field and 376-day laboratory litter bag studies.

	Early		Mid		Late		Overall			
	Field	Lab	Field	Lab	Field	Lab	Field		Lab	
	0–50 days	0–34 days	50–337 days	34–161 days	337 days - end	161–376 days				
	<i>k</i> (day ^{−1})		<i>k</i> (day ^{−1})		<i>k</i> (day ^{−1})		<i>k</i> (day ^{−1})	<i>R</i> ²	<i>k</i> (day ^{−1})	<i>R</i> ²
Dogwood	0.0012 a	0.0068 b	0.0026 d	0.0017 d	0.0020 e	0.0007 g	0.0023 h	0.97	0.0016 i	0.85
Maple	0.0010 a	0.0048 c	0.0020 e	0.0019 d	0.0006 f	0.0007 g	0.0011 g	0.91	0.0015 i	0.89
Oak	0.0011 a	0.0049 c	0.0016 e	0.0023 d	0.0006 f	0.0010 g	0.0009 g	0.88	0.0018 i	0.92
Mix	0.0005 a	0.0047 c	0.0018 e	0.0022 d	0.0007 f	0.0007 g	0.0010 g	0.93	0.0016 i	0.89

Decay coefficients for each decay stage in the field and lab were compared among the different litter types with 1-way ANOVAs and differences between litter types were compared with Tukey's post-hoc tests, when necessary. Lowercase letters designate significant differences between litter types within each decay stage and study. *R*² values represent the variance explained by the regression model and all *P* values are <0.05.



the initial harvest in the lab (Figure 4B) and was significantly higher in dogwood compared to other litter types on day 230 (not shown), which resulted in a significant litter type by day interaction (*P* < 0.01; *F* = 5.45). NAG activity increased following the initial harvest in the field and lab, but did not differ between litter types in the field (Figures 4C,D). NAG activity was higher

in dogwood than other litter types on day 230 (not shown) in the lab, which resulted in a significant litter type by day interaction (*P* < 0.01; *F* = 2.46). Phos activities peaked for maple, oak, and the mixture during late decay and were significantly higher in oak than dogwood during the December 2010 harvest and the mixture compared to maple during the October 2011 field harvest (Figure 4E). Phos also increased rapidly following the initial harvest in the lab and was significantly higher in oak than other litter types on day 2 (Figure 4F). Overall, there were significant litter type by day interactions on Phos in the field (*P* = 0.01; *F* = 2.73) and lab (*P* < 0.01; *F* = 2.30). LAP activity remained relatively low (<0.3 μmol h^{−1} g dry litter^{−1}) for all litters throughout both studies (data not shown).

Phenox activities were low in dogwood and maple throughout the field study and were significantly higher in mixed litter compared to other litter types during the October 2011 harvest (*P* < 0.01 for all; Figure 4G). Phenox activity was also elevated in oak during late decay (June 2011 and May 2012) (Figure 4G). Due to increasing Phenox activity in oak and the maple-oak mixture, there was a significant litter type by day interaction (*P* < 0.01; *F* = 4.90) on Phenox activity in the field. Phenox was not detectable at any time during the lab incubation.

TURNOVER ACTIVITY

Turnover activities were low (<15 mol) and did not differ between litter types or enzymes in the field during early decay (Figure 5A). In the lab, turnover activities were also low (<25 mol), but were significantly higher in oak and the mixture than other litters for Phos (*P* < 0.01; Figure 5B). BG and NAG turnover activities were >60 mol in the lab, but <50 mol in the field for all litter types during mid-decay (Figures 5C,D). Turnover activity differed between enzymes (*P* = 0.01; *F* = 3.44) and litter types (*P* = 0.04; *F* = 2.87) during late decay in the field due to higher Phenox and lower dogwood turnover activities compared to most other enzymes and litter types (Figure 5E). However, turnover activity for dogwood >maple, oak, and their

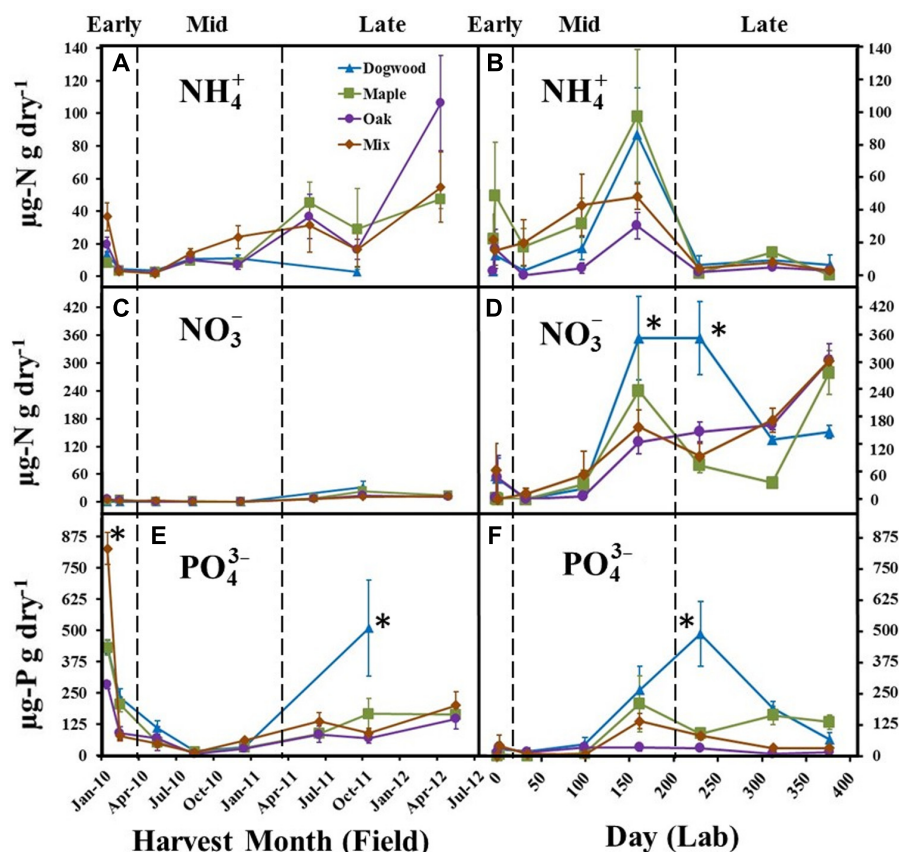


FIGURE 3 | NH_4^+ , NO_3^- , and PO_4^{3-} concentrations for dogwood, maple, oak, and the maple-oak mixture in the field litter bag study (A, C, E) and laboratory incubation (B, D, F) during early (<10% mass loss), mid-(10–40%

mass loss), and late (>40% mass loss) decay. Error bars show the standard error of the mean ($n = 8$ for field and $n = 4$ for lab). Asterisks (*) denote significant differences ($P < 0.05$) between litter types on a harvest date.

mixture in the lab (Figure 5F), which produced a significant litter type effect ($P = 0.03$; $F = 3.20$). Turnover activities were as much as an order of magnitude higher in the lab than field during late decay (Figures 5E,F). Overall, turnover activity in dogwood < maple < oak for BG, NAG, and Phos in the field ($P < 0.04$ for all; Figure 5G). Maple decomposition required $2.2\times$ more BG per unit mass loss, $1.8\times$ more NAG, and $2.1\times$ more Phos than dogwood, while oak and the maple-oak mixture required approximately $1.5\times$ more BG, NAG, and Phos than maple. In the lab, turnover activity in dogwood > maple and oak for BG and NAG ($P < 0.02$ across all enzymes), but did not differ between litter types for Phos (Figure 5H). Dogwood decomposition required approximately $2\times$ more BG and NAG than other litter types.

Phenox turnover activity was undetectable in early decay and significantly higher than most other enzymes during mid-and late decay and overall in the field ($P < 0.01$ across all enzymes; Figures 5A,C,E,G). Overall, Phenox was $2\text{--}4\times$ higher in oak compared to dogwood, maple, and the maple-oak mixture. LAP turnover activity was significantly lower than most other enzymes and did not differ between litter types in both the field and lab (Figure 5). Differences in enzyme turnover activities between litter types resulted in significant enzyme type by litter type interactions

during mid-decay ($P < 0.01$; $F = 2.39$) and overall in the field ($P < 0.01$; $F = 6.63$) and during early decay ($P < 0.01$; $F = 8.75$) and overall in the lab ($P < 0.01$; $F = 3.56$).

ENZYME ACTIVITY RATIOS

In early decay, C:N acquisition ratios were >1 for all litter types in both studies. C:P acquisition ratios were also >1 for all litter types in the field, but in the lab, C:P acquisition ratios were <0.5 for maple, oak, and their mixture and >1 for dogwood (Figure 6A). Additionally, N:P ratios were <1 (data not shown) for all litter types in the lab during early decay, but ratios increased to >1 during mid- and late decay. Field C:N and C:P ratios in mid-decay were >1. In the lab, C:N acquisition ratios were <1 for dogwood, maple, and oak, although the maple-oak mixture C:N and C:P ratios were >1 (Figure 6B). During late-decay, all litter types in the field had C:N and C:P ratios <1, but these patterns were reversed in the lab (Figure 6C). Overall, C:N and C:P ratios were >1 for all litter types in both studies (Figure 6D).

DISCUSSION

TEMPERATURE CONTROL OF MICROBIAL ACTIVITY

In contrast to expectations, mass loss during the initial 2 months of field decomposition (January–February) was <6%, even in the

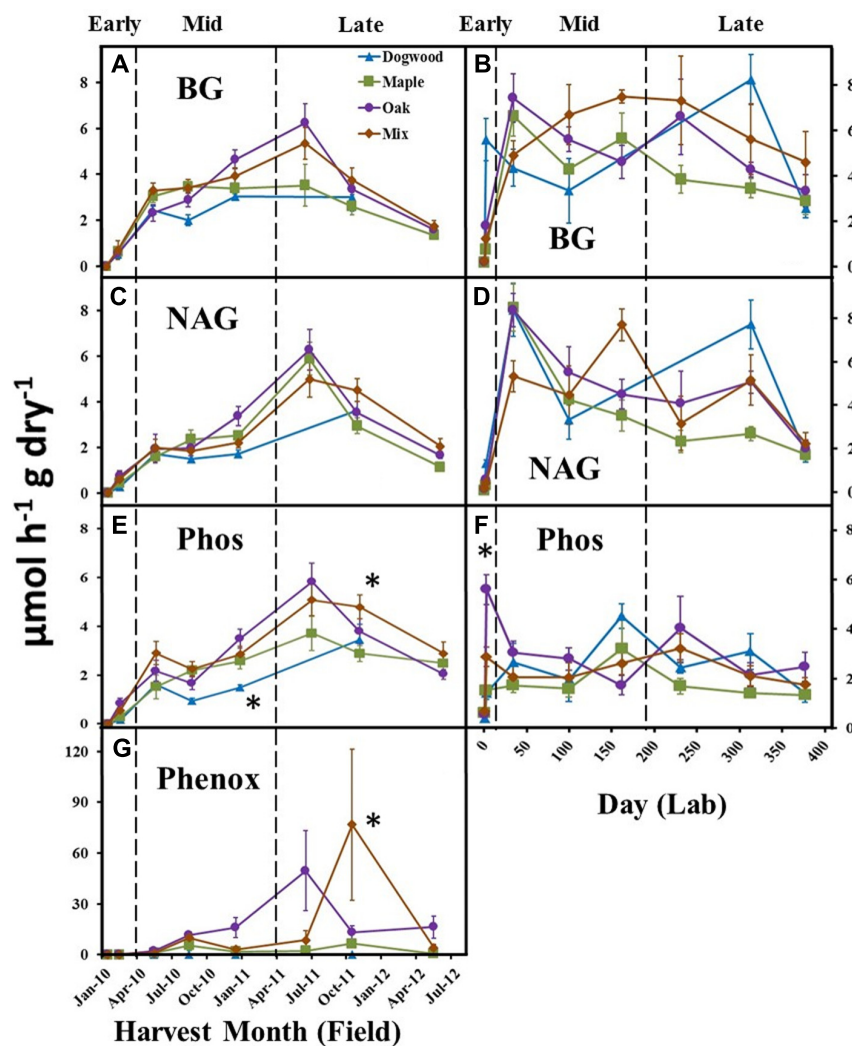


FIGURE 4 | BG, NAG, Phos, and Phenox activities over time for dogwood, maple, oak, and mixed litter in the field litterbag study (A, C, E, G) and laboratory incubation (B, D, F) during early (<10% mass loss), mid- (10–40% mass loss), and late (>40% mass loss)

decay. Phenox activity was not detected in the lab. Error bars show the standard error of the mean ($n = 8$ for field and $n = 4$ for lab). An * designates differences between litter species at each harvest date.

highly labile dogwood litter. We also found that enzyme activities and B:C ratios were lower than those found in later decay stages. Although temperature and moisture are strong controls on heterotrophic respiration (Schimel et al., 1994; Ise and Moorcroft, 2006; Suseela et al., 2012), field conditions during early decay included above-freezing soil temperatures (5.6°C), adequate moisture (32% WHC), and a substantial snowpack during most of February that minimized soil freezing. There was likely abundant labile C in fresh litter, and lower microbial demand for nutrients than C in all litters except oak (co-limited by C and P) suggests that either the high abundance of cellulose in fresh litter stimulated BG activity or N and P limitations were not acute. Additionally, greater mass loss for all litters under much lower moisture conditions during May through August 2010 suggests that field litter decomposition was not water-limited early in decay. Thus, we conclude that low temperatures suppressed

decay rates during January–February. Temperature sensitivities are commonly reported to be inversely proportional to litter quality (Mikan et al., 2002; Fierer et al., 2006), with low temperatures inhibiting the degradation of easily available C polymers (Koch et al., 2007). For instance, Prescott (2010) proposed that microbial activity is uniformly low at temperatures below 10°C regardless of other factors, which is consistent with our findings, even with abundant labile C. In addition, it is known that the Q_{10} for respiration increases toward low temperatures and is about 4.5 at 10°C and 2.5 at 20°C (Kirschbaum, 1995, 2006). Thus, our finding that field decay rates were approximately 11 times lower than the lab during early decay is compatible with progressively higher Q_{10} toward lower temperatures, as suggested by developments in the understanding of how temperature affects microbial rates during decomposition (Kirschbaum, 2013).

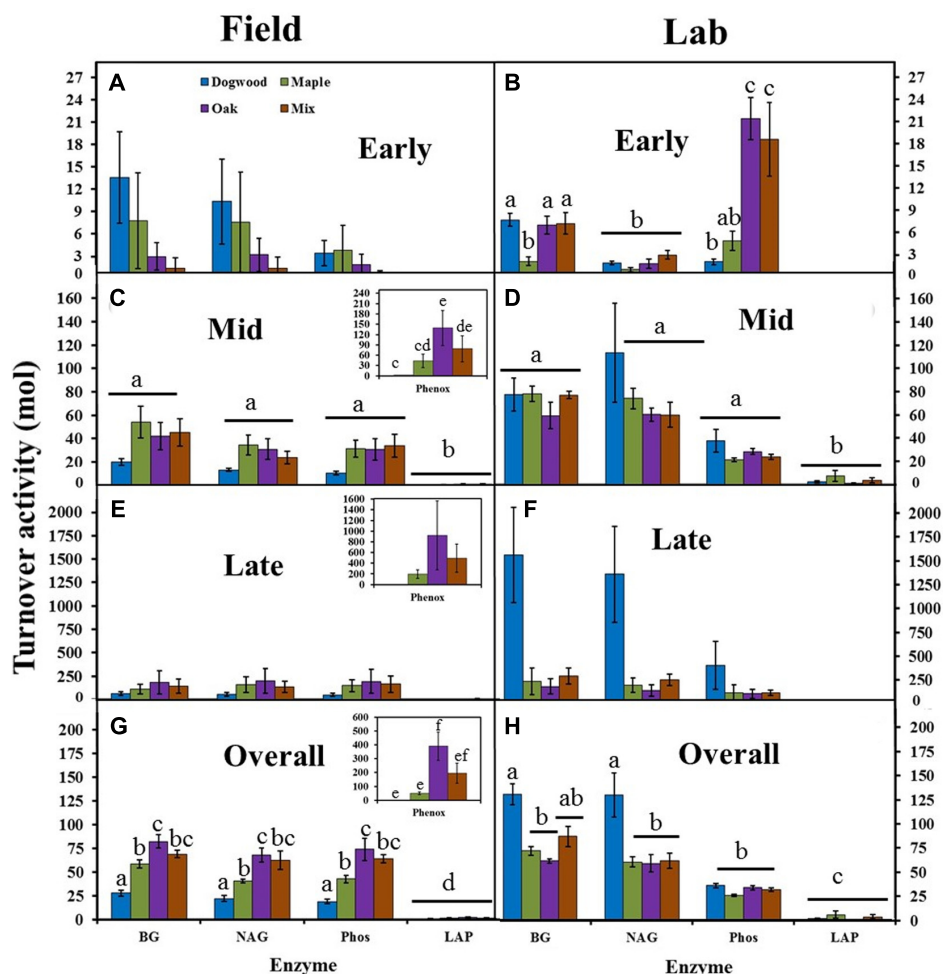


FIGURE 5 | BG, NAG, LAP, Phos, and Phenox turnover activities (mol) for dogwood, maple, oak, and the maple-oak mixture in the field litter bag study and laboratory incubation during early (A, B), mid- (C, D), and late (E, F) decay, and overall (G, H). No Phenox activity was detected in the lab or during early decay in the field. Turnover activities (high turnover activity = high enzyme activity per unit mass loss) were compared with separate two-way ANOVAs (field and lab) during each decay stage and

lowercase letters designate significant differences within and across enzymes for each study. Error bars show the standard error of the mean ($n = 8$ for field and $n = 4$ for lab). No significant differences between enzymes or litter types occurred during early decay in the field. Phenox turnover activity was significantly higher than most other enzymes during late decay in the field, while dogwood turnover activity was significantly higher than other litter types during late decay in the lab.

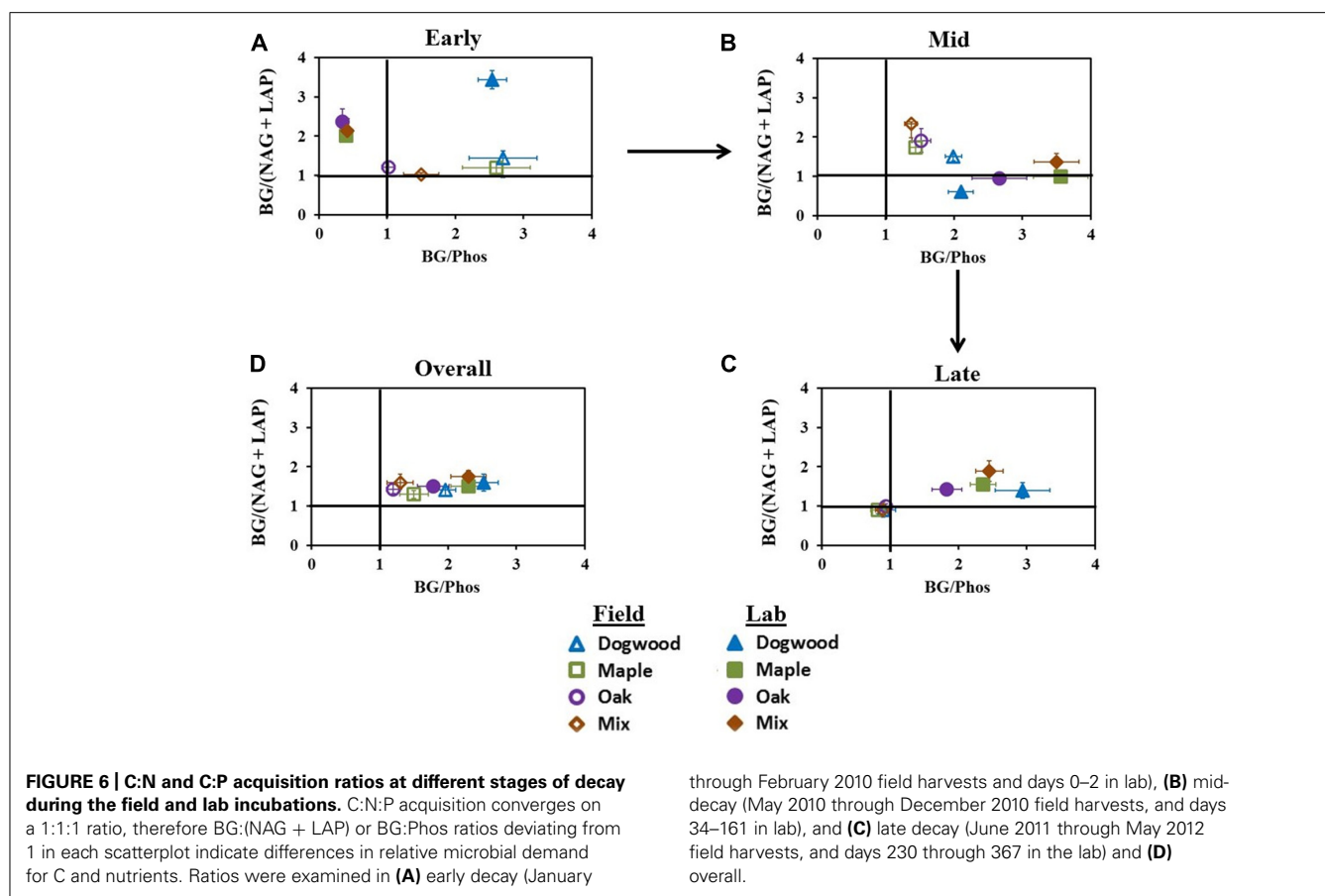
CARBON AND PHOSPHORUS EFFECTS ON MICROBIAL ACTIVITY

Under lab conditions, respiration peaked within the first week for all litter types, with the highest peak in dogwood. Dogwood has high concentrations of water-soluble compounds, cellulose, and nutrients (Moorhead and Sinsabaugh, 2000), and was the only litter type where microbial demand for C was greater than P during early decay (Figure 6A). It is possible that greater labile C availability in dogwood stimulated BG activity, as cellulose degradation increased more rapidly in dogwood than other litters during the first few days of decomposition (Figure 4B). However, litter types with high labile C concentrations often decompose rapidly when microorganisms are not P-limited because fast growing decomposers have proportionately higher P requirements (Gusewell and Freeman, 2005). Microbes were less efficient at hydrolyzing phosphate during early decay in our maple and oak litters (Figure 5B). Thus it is possible that higher dogwood P

availability increased C mineralization rates and mass loss relative to more recalcitrant litters. These contrasts in demand for C and P between litter types suggest that litter quality and/or P availability influenced decomposer responses to leaf litter under temperature controlled conditions.

ENVIRONMENTAL INFLUENCES ON MICROBIAL BIOMASS AND ENZYME DYNAMICS

Mass loss per unit enzyme activity was greater in the field than lab during mid-decay (Figures 5C,D). During this time, higher decay rates occurred in dogwood and maple under field than lab conditions (Table 1). Blair (1988) found that the total soluble content of dogwood and maple litter (>30% higher than oak) was the most important factor in first-year mass loss. This is likely due in part to the acceleration of mass loss by precipitation and leaching of soluble substrates. Decomposers also preferentially metabolize



compounds in the soluble pool, as highly labile low-molecular weight substrates (i.e., sugars, phenolics, amino acids) are taken up with no enzymatic breakdown (Rinkes et al., 2011; Glanville et al., 2012). Thus, we speculate that the combination of leaching and microbial uptake of soluble compounds increased decay rates and mass loss per unit enzyme activity in the more labile litters under field conditions. It is unlikely leaching was as significant in microcosms because litter bags were placed in the middle of pre-wetted soil and only small amounts of water (1–2 ml) were added to the soil on a weekly basis to maintain moisture conditions. Thus, our findings suggest that environmental factors and litter soluble content influenced decay rates and mass loss per unit enzyme activity between studies during mid-decay.

It is also possible that greater faunal colonization and resulting fragmentation in the field increased the proportion of litter accessible to microbial attack and decreased the enzymatic effort needed to degrade dogwood and maple compared to more recalcitrant litters in the field (Cornelissen et al., 1999; Yang et al., 2012). For instance, lignified litters have high structural integrity that reduces soil faunal activity and fragmentation rates (Holdsworth et al., 2008). However, the exclusion of key soil macrofauna (e.g., non-native earthworms) due to the 1 mm² mesh size of litter bags likely limited overall fragmentation in both studies compared to natural conditions. For example, litter decomposition rates are often more than eight-times greater in earthworm-accessible large mesh litter bags than in fine mesh bags that exclude them in

temperate deciduous forests (Holdsworth et al., 2008; Fox et al., 2010). Therefore, we likely underestimated decay rates in both studies compared to natural conditions by excluding various soil faunal fragmenters.

Decomposer demand for N was greater than C in the lab for most litters during mid-decay (Figure 6B). Consistent with our mid-decay hypothesis, isolation from external nutrient subsidies and the limited amount of low nutrient soil used in the incubation likely reduced N availability and increased decomposer N limitation (Boberg et al., 2010). We also found higher BG activities (Figures 4A,B), but lower cellulose degradation per unit enzyme activity during mid-decay and overall in the lab than field for most litter types (Figure 5). It is possible that decomposers degraded soluble compounds and hemicellulose more rapidly under optimal lab conditions during early decay, especially in dogwood. If so, cellulose was a more important C source to decomposers in the lab after early decay. This likely stimulated BG activity, but decreased mass loss relative to the field. These differences in mass loss per unit enzyme activity and enzymatic effort directed toward C- and N- acquisition between decomposition studies suggest that both C and N availability constrain decomposer responses to litter quality.

Strong contrasts in mass loss per unit enzyme activity occurred between studies during late decay (Figures 5E,F). In addition, biomass to litter mass (B:C) ratios peaked in all litters during late decay in the field. However, B:C ratios declined between mid- and

late decay in the lab (**Figure 2**). Coupled field and microcosm experiments demonstrate that field soils behave differently following disturbance (Teuben and Verhoef, 1992; Salamanca et al., 1998), primarily due to alterations in microbial community abundance, activity and composition. It is possible that soil collection decreased saprophytic fungal abundance (Coleman et al., 1988) and/or disrupted the N- and P-transporting mycelial network of mycorrhizae, likely increasing enzymatic effort to obtain nutrients from organic sources (Hart and Reader, 2004; Sheng et al., 2012). For instance, there is often an inverse relationship between total enzyme pool size and fungal biomass (Sinsabaugh et al., 2002). However, it is likely that hyphal transfer of nutrients by actinomycetes between soil and litter bags still occurred in both studies. Another potential explanation is that NO_3^- accumulation (**Figure 3D**) suppressed ectomycorrhizal and saprotrophic fungal abundance (Carfrae et al., 2006; Wallenstein et al., 2006) and/or decreased overall microbial biomass in the lab (Ramirez et al., 2012). However, it is possible that high N throughputs occurred in the field as well, as our finding of consistently low field NO_3^- concentrations are based on *in-situ* snap-shot measurements. Thus, our findings suggest relationships between microbial biomass and litter quality were not constant between the field and lab during late decay, likely due to differences in microbial community composition and/or N availability.

THE INFLUENCE OF EDAPHIC FACTORS ON MICROBIAL FUNCTION

Oxidative enzyme activities only increased substantially in the high lignin oak litter and the maple-oak mixture in the field during late decay. Lignin is an aromatic polymer that is highly resistant to biological degradation, surrounds holocellulose in plant cell walls, and blocks microbial access to cell membrane proteins (Berg and McClaugherty, 2008). Thus, it is possible that a higher abundance of fungi specializing in lignin degradation in the field increased oxidative enzyme production in oak and the mixture to obtain protected labile N compounds (Talbot and Treseder, 2012). This is supported by our observation that microbial demand for N relative to C was greater for all litter types in the field after 40% mass loss (**Figure 6C**). Because the C return on investing in lignin degrading enzymes is low (Kirk and Farrell, 1987; Dashtban et al., 2010), our findings also suggest that decomposers likely degraded lignin to acquire shielded cellulose. For instance, the peak in BG activity was concomitant with the peak in oxidative enzyme activity in oak (**Figure 4**). Given that specialized fungal groups produce potent oxidative enzymes driving lignin degradation during later decay stages, an increase in their abundance and activity under field conditions likely influenced lignin degradation in our study (Bending and Read, 1997; Baldrian and Valaskova, 2008).

Oxidative enzyme activities never increased for any litter type in the lab. Consistent with our late-decay hypothesis, NO_3^- accumulated in the lab (**Figure 3D**) and possibly decreased microbial demand for N relative to C across all litter types (**Figure 6C**). Although N can positively influence fungal colonization in fresh litter (Rousk and Bååth, 2007), we speculate that excess N suppressed ectomycorrhizal and saprotrophic fungal activity during late-decay (Waldrop and Zak, 2006), which decreased lignin decomposition rates (Grandy et al., 2008). Additionally, the

inability of lignin degrading fungi to colonize from adjacent litter in the lab probably limited lignin degradation, as spatial and temporal variations in fungal abundance and activity in field settings are common (Lindahl et al., 2007; Osono, 2007; Feinstein and Blackwood, 2013). It is also possible that cellulose was a more important resource than lignin to decomposers during late decay in the lab, which increased C:N acquisition ratios. For instance, mass loss was never greater than 50% for any litter type in microcosms, suggesting that unshielded cellulose may not have been completely depleted. Overall, however, our results suggest that decomposer isolation from external resources, and/or N inhibition of microbial activity decreased litter mass loss rates in the lab during late decay.

We found that mass loss of the maple-oak mixture was the average rate of the two individual litters, and hypothesize that this additive effect may be explained by N availability influences on microbial function. For instance, it is possible that the relative changes in microbial activity and decomposition rates of maple and oak due to changes in N availability were equal and the decomposition rate of the mixture was the average of the individual rates (Berglund and Ågren, 2012). Furthermore, unequal proportions of litter commonly result in non-additive effects (Mao and Zeng, 2012). Thus, the observed additive effect in our study was likely a result of N movement between the equal mixture of oak and maple litter used in our experiment (Schimel and Hattenschwiler, 2007).

IMPLICATIONS FOR DECOMPOSITION MODELS

Our findings suggest that variable influences of climate and edaphic factors on microbial biomass, enzyme dynamics, and decomposition rates alter the trajectory of decay of varying leaf litter types. Although different microorganisms respond to temperature increases differently, our findings imply that microbial activity is predictably low across all decomposer groups below a temperature $>5.6^\circ\text{C}$ regardless of litter composition. Although not explicitly measured, we speculate that leaching and fragmentation increase access to soluble C compounds that do not require enzymatic hydrolysis prior to uptake, resulting in greater mass loss per unit enzyme activity in labile litter types. Additionally, our study demonstrates that N availability alters microbial responses to litter composition, with potentially strong effects on microbial abundance, activity, and enzymatic effort per unit mass loss in mid- and late decay. Different functional groups of decomposers may respond differently to N availability and target different substrates depending on their relative demand for C and N at different decay stages. Therefore, linking the N demands of different functional groups of decomposers to microbial behavior and decay rates of different litter substrates is likely to enhance the predictive capabilities of decomposition models.

CONCLUSION

This study demonstrates that decomposition of the same leaf litter under field and lab conditions can result in strikingly different decay patterns due to the variable influences of climate and edaphic factors on microbial-substrate interactions. For instance, low temperatures decreased microbial activity early in decay, even in highly labile litters. These results in combination with previous findings of low microbial activity below

10°C and a higher Q_{10} at lower temperatures imply that even a small increase at lower temperatures may cause a substantial increase in CO_2 flux. Furthermore, sharp contrasts in enzymatic effort directed toward C-, N-, and P-acquisition between litter types and studies indicate the importance of nutrient constraints on decomposer responses to litter quality. Finally, microbial biomass did not have a constant relationship to litter chemistry between the field and lab, which was likely caused by differences in nutrient availability and/or microbial community composition and function. Therefore, linking the C and N demands of different decomposer groups to decay rates of varying C substrates is likely to enhance mechanistic decomposition models.

REFERENCES

- Aerts, R. (1997). The freezer defrosting: global warming and litter decomposition rates in cold biomes. *J. Ecol.* 94, 713–724. doi: 10.1111/j.1365-2745.2006.01142.x
- Baldrian, P., Snajdr, J., Merhautova, V., Dobiasova, P., Cajthaml, T., and Valaskova, V. (2013). Responses of the extracellular enzyme activities in hardwood forests to soil temperature and seasonality and the potential effects of climate change. *Soil Biol. Biochem.* 56, 60–68. doi: 10.1016/j.soilbio.2012.01.020
- Baldrian, P., and Valaskova, V. (2008). Degradation of cellulose by basidiomycetous fungi. *FEMS Microbiol. Rev.* 32, 501–521. doi: 10.1111/j.1574-6976.2008.00106.x
- Bending, G. D., and Read, D. J. (1997). Lignin and soluble phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. *Mycol. Res.* 11, 1348–1354. doi: 10.1017/S0953756297004140
- Berg, B., and McClaugherty, C. (2008). *Plant Litter: Decomposition, Humus Formation, Carbon Sequestration*. Berlin: Springer-Verlag.
- Berglund, S. L., and Ågren, G. I. (2012). When will litter mixtures decompose faster or slower than individual litters? A model for two litters. *Oikos* 121, 1112–1120. doi: 10.1111/j.1600-0706.2011.19787.x
- Blair, J. M. (1988). Nutrient release from decomposing foliar litter of three tree species with special reference to calcium, magnesium, and potassium dynamics. *Plant Soil* 110, 49–55. doi: 10.1007/BF02143538
- Blair, J. M., Crossley, D. A., and Callahan, L. C. (1992). Effects of litter quality and microarthropods on N dynamics and retention of exogenous N in decomposing litter. *Biol. Fertil. Soils* 12, 241–252. doi: 10.1007/BF00336039
- Boberg, J. E., Finlay, R. D., Stenlid, J., and Lindahl, B. D. (2010). Fungal C translocation restricts N-mineralization in heterogeneous environments. *Funct. Ecol.* 24, 454–459. doi: 10.1111/j.1365-2435.2009.01616.x
- Bradford, M. A., and Fierer, N. (2012). “The biogeography of microbial communities and ecosystem processes: implications for soil and ecosystem models,” *Soil Ecology and Ecosystem Services*, ed. D. H. Wall (Oxford, UK: Oxford University Press), 189–200.
- Brookes, P. C., Landman, A., Pruden, G., and Jenkinson, D. S. (1985). Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.* 17, 837–842. doi: 10.1016/0038-0717(85)90144-0
- Carfrae, J. A., Skene, K. R., Sheppard, L. J., Ingleby, K., and Crossley, A. (2006). Effects of nitrogen with and without acidified sulphur on an ectomycorrhizal community in Sitka spruce (*Picea sitchensis* Bong. Carr) forest. *Environ. Pollut.* 141, 131–138. doi: 10.1016/j.envpol.2005.08.020
- Carreiro, M. M., Sinsabaugh, R. L., Repert, D. A., and Parkhurst, P. F. (2000). Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology* 81, 2359–2365. doi: 10.1890/0012-9658(2000)081[2359:MESELD]2.0.CO;2
- Coleman, D. C., Crossley, D. A., Beare, M. H., and Hendrix, P. F. (1988). Interactions of organisms at root/soil and litter/soil interfaces in terrestrial ecosystems. *Agric. Ecosyst. Environ.* 24, 177–134. doi: 10.1016/0167-8809(88)90060-6
- Cornelissen, J. H. C., Perez-Harguindeguy, N., Diaz, S., Grime, J. P., Marzano, B., Cabido, M., et al. (1999). Leaf structure and defense control litter decomposition rate across species and life forms in regional floras on two continents. *New Phytol.* 143, 191–200. doi: 10.1046/j.1469-8137.1999.00430.x
- D’Angelo, E., Crutchfield, J., and Vandierviere, M. (2001). Rapid, sensitive, microscale determination of phosphate in water and soil. *J. Environ. Qual.* 30, 2206–2209. doi: 10.2134/jeq2001.2206
- Dashtban, N., Schraft, H., Syed, T. A., and Qin, W. (2010). Fungal biodegradation and enzymatic modification of lignin. *Int. J. Biochem. Mol. Biol.* 1, 36–50.
- DeForest, J. L., Chen, J., and McNulty, S. G. (2009). Leaf litter is an important mediator of soil respiration in an oak-dominated forest. *Int. J. Biometeorol.* 53, 127–134. doi: 10.1007/s00484-008-0195-y
- Doane, T. A., and Horwath, W. R. (2003). Spectrophotometric determination of nitrate with a single reagent. *Anal. Lett.* 36, 2713–2722. doi: 10.1081/AL-120024647
- Feinstein, L. M., and Blackwood, C. B. (2013). The spatial scaling of saprotrophic fungal beta diversity in decomposing leaves. *Mol. Ecol.* 22, 1171–1184. doi: 10.1111/mec.12160
- Fierer, N., Colman, B. P., Schimel, J. P., and Jackson, R. B. (2006). Predicting the temperature dependence of microbial respiration in soil: a continental-scale analysis. *Glob. Biogeochem. Cycles* 20, GB2036. doi: 10.1029/2005GB002644
- Floch, C., Alarcon-Gutierrez, E., and Criquet, S. (2007). ABTS assay of phenol oxidase activity in soil. *J. Microbiol. Methods* 71, 319–324. doi: 10.1016/j.mimet.2007.09.020
- Fox, V. L., Buehler, C. P., Byers, C. M., and Drake, S. E. (2010). Forest composition, leaf litter, and songbird communities in oak- vs. maple-dominated forests in the eastern U.S. *For. Ecol. Manage.* 259, 2426–2432. doi: 10.1016/j.foreco.2010.03.019
- German, D. P., Weintraub, M. N., Grandy, A. S., Lauber, C. L., Rinkes, Z. L., and Allison, S. D. (2011). Optimization of hydrolytic and oxidative methods for ecosystem studies. *Soil Biol. Biochem.* 43, 1387–1397. doi: 10.1016/j.soilbio.2011.03.017
- Gholz, H. L., Wedin, D. A., Smitherman, S. M., Harmon, M. E., and Patron, W. J. (2000). Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. *Glob. Change Biol.* 6, 751–765. doi: 10.1046/j.1365-2486.2000.00349.x
- Glanville, H., Rousk, J., Golyshev, P., and Jones, D. L. (2012). Mineralization of low molecular weight carbon substrates in soil solution under laboratory and field conditions. *Soil Biol. Biochem.* 48, 88–95. doi: 10.1016/j.soilbio.2012.01.015
- Grandy, A. S., Sinsabaugh, R. L., Neff, J. C., Stursova, M., and Zak, D. R. (2008). Nitrogen deposition effects on soil organic chemistry are linked to variation in enzymes, ecosystems and size fractions. *Biogeochemistry* 91, 37–49. doi: 10.1007/s10533-008-9257-9
- Gusewell, S., and Freeman, C. (2005). Nutrient limitation and enzyme activities during litter decomposition of nine wetland species in relation to litter N:P ratios. *Funct. Ecol.* 19, 582–593. doi: 10.1111/j.1365-2435.2005.01002.x
- Hart, M., and Reader, R. J. (2004). Do arbuscular mycorrhizal fungi recover from disturbance differently? *Trop. Ecol.* 45, 97–111.
- Holdsworth, A. R., Frelich, L. E., and Reich, P. B. (2008). Litter decomposition in earthworm-invaded northern hardwood forests: role of invasion degree and litter chemistry. *Ecoscience* 15, 536–544. doi: 10.2980/154-3151
- Ise, T., and Moorcroft, P. R. (2006). The global-scale temperature and moisture dependencies of soil organic carbon decomposition: an analysis using a mechanistic model. *Biogeochemistry* 80, 217–231. doi: 10.1007/s10533-006-9019-5
- Kampichler, C., Bruckner, A., and Kandeler, E. (2001). Use of enclosed

- model ecosystems in soil ecology: a bias towards laboratory research. *Soil Biol. Biochem.* 33, 269–275. doi: 10.1016/S0038-0717(00)00140-1
- Kirk, T. K., and Farrell, R. L. (1987). Enzymatic “combustion”: the microbial degradation of lignin. *Annu. Rev. Microbiol.* 41, 465–505. doi: 10.1146/annurev.mi.41.100187.002341
- Kirschbaum, M. U. F. (1995). The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. *Soil Biol. Biochem.* 27, 753–760. doi: 10.1016/0038-0717(94)00242-S
- Kirschbaum, M. U. F. (2006). The temperature dependence of organic matter decomposition—still a topic of debate. *Soil Biol. Biochem.* 38, 2510–2518. doi: 10.1016/j.soilbio.2006.01.030
- Kirschbaum, M. U. F. (2013). Seasonal variations in the availability of labile substrate confound the temperature dependence of organic matter decomposition. *Soil Biol. Biochem.* 57, 568–576. doi: 10.1016/j.soilbio.2012.10.012
- Koch, O., Tschirko, D., and Kandeler, E. (2007). Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils. *Glob. Biogeochem. Cycles* 21, GB4017. doi: 10.1029/2007GB002983
- Knoepf, J. D., Reynolds, B. C., Crossley, D. A., and Swank, W. T. (2005). Long-term changes in forest floor processes in southern Appalachian forests. *For. Ecol. Manage.* 220, 300–312. doi: 10.1016/j.foreco.2005.08.019
- Lindahl, B. D., Ihrmark, K., Boberg, J., Trumbore, S. E., Hogberg, P., Stenlid, J., et al. (2007). Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytol.* 173, 611–620. doi: 10.1111/j.1469-8137.2006.01936.x
- Liski, J., Nissinen, A., Erhard, M., and Taskinen, O. (2003). Climatic effects on litter decomposition from arctic tundra to tropical rainforest. *Glob. Change Biol.* 9, 575–584. doi: 10.1046/j.1365-2486.2003.00605.x
- Manzoni, S., and Porporato, A. (2009). Soil carbon and nitrogen mineralization: theory and models across scales. *Soil Biol. Biochem.* 41, 1355–1379. doi: 10.1016/j.soilbio.2009.02.031
- Mao, R., and Zeng, D. (2012). Non-additive effects vary with the number of component residues and their mixing proportions during residue mixture decomposition: a microcosm study. *Geoderma* 170, 112–117. doi: 10.1016/j.geoderma.2011.11.008
- McGuire, K. L., and Treseder, K. K. (2009). Microbial communities and their relevance for ecosystem models: decomposition as a case study. *Soil Biol. Biochem.* 42, 529–535. doi: 10.1016/j.soilbio.2009.11.016
- Meier, C. L., and Bowman, W. D. (2010). Chemical composition and diversity influence non-additive soil responses to litter mixtures: implications for effects of species loss. *Soil Biol. Biochem.* 42, 1447–1454. doi: 10.1016/j.soilbio.2010.05.005
- Mikan, C., Schimel, J. P., and Doyle, A. P. (2002). Temperature controls of microbial respiration in arctic tundra soils above and below freezing. *Soil Biol. Biochem.* 34, 1785–1795. doi: 10.1016/S0038-0717(02)00168-2
- Moorhead, D. L., Lashermes, G., and Sinsabaugh, R. L. (2012). A theoretical model of C- and N-acquiring exoenzyme activities, which balances microbial demands during decomposition. *Soil Biol. Biochem.* 53, 133–141. doi: 10.1016/j.soilbio.2012.05.011
- Moorhead, D. L., and Sinsabaugh, R. L. (2000). Simulated patterns of litter decay predict patterns of extracellular activities. *Appl. Soil Ecol.* 14, 71–79. doi: 10.1016/S0929-1393(99)00043-8
- Moorhead, D. L., and Sinsabaugh, R. L. (2006). A theoretical model of litter decay and microbial interaction. *Ecol. Monogr.* 76, 151–174. doi: 10.1890/0012-9615(2006)076[0151:ATMOLD]2.0.CO;2
- Noormets, A., McNulty, S. G., DeForest, J. L., Sun, G., Li, Q., and Chen, J. (2008). Drought during canopy development has lasting effect on annual carbon balance in a deciduous temperate forest. *New Phytol.* 179, 818–828. doi: 10.1111/j.1469-8137.2008.02501.x
- Olson, J. S. (1963). Energy storage and the balance of producers and decomposers in ecological systems. *Ecology* 44, 322–331. doi: 10.2307/1932179
- Osono, T. (2007). Ecology of ligninolytic fungi associated with leaf litter decomposition. *Ecol. Res.* 22, 955–974. doi: 10.1007/s11284-007-0390-z
- Piatek, K. B., Munasinghe, P., Peterjohn, W. T., Adams, W. B., and Cumming, J. R. (2010). A decrease in oak litter mass changes nutrient dynamics in the litter layer of a central hardwood forest. *North. J. Appl. For.* 27, 97–104.
- Prescott, C. (2010). Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? *Biogeochemistry* 101, 133–149. doi: 10.1007/s10533-010-9439-0
- Ramirez, K. S., Craine, J. M., and Fierer, N. (2012). Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Glob. Change Biol.* 18, 1918–1927. doi: 10.1111/j.1365-2486.2012.02639.x
- Rhine, E. D., Sims, G. K., Mulvaney, R. L., and Pratt, E. J. (1998). Improving the berthelot reaction for determining ammonium in soil extracts and water. *Soil Sci. Soc. Am. J.* 62, 473–480. doi: 10.2136/sssaj1998.03615995006200020026x
- Rinkes, Z. L., DeForest, J. L., Grandy, A. S., Moorhead, D. L., and Weintraub, M. N. (2013). Interactions between leaf litter quality, particle size, and microbial community during the earliest stage of decay. *Biogeochemistry*. doi: 10.1007/s10533-013-9872-y [Epub ahead of print].
- Rinkes, Z. L., Weintraub, M. N., DeForest, J. L., and Moorhead, D. L. (2011). Microbial substrate preference and community dynamics during the decomposition of *Acer saccharum*. *Fungal Ecol.* 4, 396–407. doi: 10.1016/j.funeco.2011.01.004
- Risk, D., Kellman, L., Beltrami, H., and Diochon, A. (2008). In-situ incubations highlight the environmental constraints on soil organic carbon decomposition. *Environ. Res. Lett.* 3, 04400. doi: 10.1088/1748-9326/3/4/044004
- Rousk, J., and Bååth, E. (2007). Fungal and bacterial growth in soil with plant materials of different C/N ratios. *FEMS Microbiol. Ecol.* 62, 258–267. doi: 10.1111/j.1574-6941.2007.00398.x
- Saiya-Cork, K. R., Sinsabaugh, R. L., and Zak, D. R. (2002). The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biol. Biochem.* 34, 1309–1315. doi: 10.1016/S0038-0717(02)00074-3
- Salamanca, E. F., Kaneko, N., and Katagiri, S. (1998). Effects of leaf litter mixtures on the decomposition of *Quercus serrata* and *Pinus densiflora* using field and laboratory microcosm methods. *Ecol. Eng.* 10, 53–73. doi: 10.1016/S0925-8574(97)10020-9
- Scheiner, S. M., and Gurevitch, J. (1993). *Design and Analysis of Ecological Experiments*. USA: CRC Press, 445 p.
- Schimel, J. P., Braswell, B. H., Holland, E. A., McKeown, E., Ojima, D. S., Painter, T. H., et al. (1994). Climatic, edaphic, and biotic controls over storage and turnover of carbon in soils. *Glob. Biogeochem. Cycles* 8, 279–293. doi: 10.1029/94GB00993
- Schimel, J. P., and Hattenschwiler, S. (2007). Nitrogen transfer between decomposing leaves of different N status. *Soil Biol. Biochem.* 39, 1428–1436. doi: 10.1016/j.soilbio.2006.12.037
- Schimel, J. P., and Weintraub, M. N. (2003). The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biol. Biochem.* 35, 549–563. doi: 10.1016/S0038-0717(03)00015-4
- Schlesinger, W. H., and Andrews, J. A. (2000). Soil respiration and the global carbon cycle. *Biogeochemistry* 48, 7–20. doi: 10.1023/A:1006247623877
- Scott-Denton, L. E., Rosenstiel, T. N., and Monson, R. K. (2006). Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Glob. Change Biol.* 12, 205–216. doi: 10.1111/j.1365-2486.2005.01064.x
- Sheng, M., Lalande, R., Hamel, C., Ziadi, N., and Shi, Y. (2012). Growth of corn roots and associated arbuscular mycorrhizae are affected by long-term tillage and phosphorus fertilization. *Agron. J.* 104, 1672–1678. doi: 10.2134/agronj2012.0153
- Sinsabaugh, R. L., Carreiro, M. M., and Repert, D. A. (2002). Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochemistry* 60, 1–24. doi: 10.1023/A:1016541114786
- Sinsabaugh, R. L., and Follstad Shah, J. (2012). Eoenzymatic stoichiometry and ecological theory. *Annu. Rev. Ecol. Evol. Syst.* 43, 313–333. doi: 10.1146/annurev-ecolsys-071112-124414
- Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., Allison, S. D., Crenshaw, C., et al. (2008). Stoichiometry of soil enzyme activity at global scale. *Ecol. Lett.* 11, 1252–1264. doi: 10.1111/j.1461-0248.2008.01245.x
- Snajdr, J., Cajthaml, T., Valaskova, V., Merhautova, P., Petrankova, M., Spetz, P., et al. (2011). Transformation of *Quercus petraea* litter: successive changes in litter chemistry are reflected in differential enzyme activity and changes in the microbial community composition. *FEMS Microbiol. Ecol.* 75, 291–303. doi: 10.1111/j.1574-6941.2010.00999.x
- Susela, V., Conant, R. D., Wallenstein, M. D., and Dukes, J. S. (2012). Effects of soil moisture on the temperature

- sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment. *Glob. Change Biol.* 18, 336–348. doi: 10.1111/j.1365-2486.2011.02516.x
- Talbot, J. M., and Treseder, K. K. (2012). Interactions among lignin, cellulose, and nitrogen drive litter-chemistry-decay relationships. *Ecology* 93, 345–354. doi: 10.1890/11-0843.1
- Treseder, K. K. (2008). Nitrogen additions and microbial biomass: a metaanalysis of ecosystem studies. *Ecol. Lett.* 11, 1111–1120. doi: 10.1111/j.1461-0248.2008.01230.x
- Treseder, K. K., Balser, T. C., Bradford, M. A., Brodie, E. L., Dubinsky, E. A., Eviner, V. T., et al. (2011). Integrating microbial ecology into ecosystem models: challenges and priorities. *Biogeochemistry* 109, 7–18. doi: 10.1007/s10533-011-9636-5
- Teuben, A., and Verhoef, H. A. (1992). Relevance of microcosm and mesocosm experiments for studying soil ecosystem processes. *Soil Biol. Biochem.* 24, 1179–1183. doi: 10.1016/0038-0717(92)90069-A
- Waldrop, M. P., and Zak, D. R. (2006). Response of oxidative enzyme activities to nitrogen deposition affects soil concentrations of dissolved organic carbon. *Ecosystems* 9, 921–933. doi: 10.1007/s10021-004-0149-0
- Wallenstein, M. D., Hess, A. M., Lewis, M. R., Steltzer, H., and Ayres, E. (2011). Decomposition of aspen leaf litter results in unique metabolomes when decomposed under different tree species. *Soil Biol. Biochem.* 42, 484–490. doi: 10.1016/j.soilbio.2009.12.001
- Wallenstein, M. D., McNulty, S., Fernandez, I. J., Boggs, J., and Schlesinger, W. H. (2006). Nitrogen fertilization decreases forest fungal and bacterial biomass in three long-term experiments. *For. Ecol. Manage.* 222, 459–468. doi: 10.1016/j.foreco.2005.11.002
- Wickings, K., Grandy, A. S., Reed, S., and Cleveland, C. (2011). Management intensity alters decomposition via biological pathways. *Biogeochemistry* 104, 365–379. doi: 10.1007/s10533-010-9510-x
- Wickings, K., Grandy, A. S., Reed, S., and Cleveland, C. (2012). The origin of litter chemical complexity during decomposition. *Ecol. Lett.* 15, 1180–1188. doi: 10.1111/j.1461-0248.2012.01837.x
- Yang, X., Yang, Z., Warren, M. W., and Chen, J. (2012). Mechanical fragmentation enhances the contribution of Collembola to leaf litter decomposition. *Eur. J. Soil Biol.* 53, 23–31. doi: 10.1016/j.ejsobi.2012.07.006
- Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
- Received: 15 May 2013; accepted: 14 August 2013; published online: 03 September 2013.
- Citation:** Rinkes ZL, Sinsabaugh RL, Moorhead DL, Grandy AS and Weintraub MN (2013) Field and lab conditions alter microbial enzyme and biomass dynamics driving decomposition of the same leaf litter. *Front. Microbiol.* 4:260. doi: 10.3389/fmicb.2013.00260
- This article was submitted to *Terrestrial Microbiology*, a section of the journal *Frontiers in Microbiology*.
- Copyright © 2013 Rinkes, Sinsabaugh, Moorhead, Grandy and Weintraub. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution and reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Dynamic relationships between microbial biomass, respiration, inorganic nutrients and enzyme activities: informing enzyme-based decomposition models

D. L. Moorhead^{1*}, Z. L. Rinkes¹, R. L. Sinsabaugh² and M. N. Weintraub¹

¹ Department of Environmental Sciences, University of Toledo, Toledo, OH, USA

² Department of Biology, University of New Mexico, Albuquerque, NM, USA

Edited by:

Johannes Rousk, Lund University, Sweden

Reviewed by:

Kirsten Hofmockel, Iowa State University, USA

Peter Bottomley, Oregon State University, USA

*Correspondence:

D. L. Moorhead, Department of Environmental Science, University of Toledo, 2801 W. Bancroft St., Toledo, OH 43606, USA
e-mail: daryl.moorhead@utoledo.edu

We re-examined data from a recent litter decay study to determine if additional insights could be gained to inform decomposition modeling. Rinkes et al. (2013) conducted 14-day laboratory incubations of sugar maple (*Acer saccharum*) or white oak (*Quercus alba*) leaves, mixed with sand (0.4% organic C content) or loam (4.1% organic C). They measured microbial biomass C, carbon dioxide efflux, soil ammonium, nitrate, and phosphate concentrations, and β -glucosidase (BG), β -N-acetyl-glucosaminidase (NAG), and acid phosphatase (AP) activities on days 1, 3, and 14. Analyses of relationships among variables yielded different insights than original analyses of individual variables. For example, although respiration rates per g soil were higher for loam than sand, rates per g soil C were actually higher for sand than loam, and rates per g microbial C showed little difference between treatments. Microbial biomass C peaked on day 3 when biomass-specific activities of enzymes were lowest, suggesting uptake of litter C without extracellular hydrolysis. This result refuted a common model assumption that all enzyme production is constitutive and thus proportional to biomass, and/or indicated that part of litter decay is independent of enzyme activity. The length and angle of vectors defined by ratios of enzyme activities (BG/NAG vs. BG/AP) represent relative microbial investments in C (length), and N and P (angle) acquiring enzymes. Shorter lengths on day 3 suggested low C limitation, whereas greater lengths on day 14 suggested an increase in C limitation with decay. The soils and litter in this study generally had stronger P limitation (angles $>45^\circ$). Reductions in vector angles to $<45^\circ$ for sand by day 14 suggested a shift to N limitation. These relational variables inform enzyme-based models, and are usually much less ambiguous when obtained from a single study in which measurements were made on the same samples than when extrapolated from separate studies.

Keywords: extracellular enzymes, models, decomposition, soil microorganisms, enzyme efficiency

INTRODUCTION

Decomposition occupies a central position in global biogeochemical cycles and mathematical models play a central role in efforts to understand them and predict future changes. Decomposition models span a wide range of temporal, spatial, and hierarchical scales of resolution (Manzoni and Porporato, 2009), from physiologically based simulations of microbial activity in laboratory cultures (Resat et al., 2012) to empirical models that estimate gas flux dynamics over regional landscapes (Niu et al., 2012). The scale of interest necessarily defines the resolution of the appropriate model (Reynolds and Leadley, 1992). In any case, decomposition of the most common structural polymers comprising dead organic matter, i.e., cellulose, hemicellulose, and lignin, is largely accomplished at the biochemical level by the activities of extracellular enzymes produced by microorganisms (Burns, 1983; Sinsabaugh, 1994). Thus representative models minimally require detailed information about interactions between microorganisms, their extracellular enzymes, and substrates they degrade. Fortunately, studies of enzyme activity in the environment have

expanded rapidly over the last few years [see review by Burns et al. (2013)] and enzyme-based models are beginning to emerge (Schimel and Weintraub, 2003; Allison, 2005; Moorhead et al., 2012; Resat et al., 2012; Wang et al., 2013). Unfortunately, the data needed to develop and test these models are incomplete and often gleaned piecemeal from disparate studies which raises questions about cross study comparisons. Herein we briefly review the development of enzyme-based decomposition models, highlight common information gaps, and demonstrate the contribution to modeling objectives obtained from closely integrated studies of the substrate-enzyme-microbe (SEM) system during decomposition.

BACKGROUND ENZYME MODELS

The first models to link enzyme activity to decomposition were the statistically based enzyme decay models (EDMs) that regressed litter mass loss against cumulative measures of enzyme activities (Sinsabaugh, 1994; Jackson et al., 1995). These models

demonstrated that the activities of enzymes that hydrolyze related groups of compounds, like cellulose and hemicellulose, correlate with each other. Thus a single indicator enzyme could be used as a proxy for the combined activities of a suite of enzymes that degrade a particular substrate, such as β -glucosidase (BG) for holocellulose, β -N-acetyl-glucosaminidase (NAG) for chitin and peptidoglycan, leucine amino-peptidase (LAP) for proteins, and acid or alkaline phosphatase (AP) for organic P. More recently, a synthesis of collected measurements from soils, sediments, and freshwater plankton (Sinsabaugh and Follstad Shah, 2012) showed that the patterns of activities for key indicator enzymes (i.e., BG, NAG, LAP, and AP) integrated the metabolic and stoichiometric requirements of decomposer organisms with the relative availabilities of C, N, and P from environmental sources. This enzymatic stoichiometry theory (EST) provides a rationale for mechanistic models linking enzyme activity to decomposition, but is based on observations usually including key enzyme activities and total N, P, and organic C pool sizes, and seldom includes microbial biomass or metabolic activity, such as respiration. For this reason, EST describes the overall phenomenon but not the mechanisms of SEM interactions (Reynolds and Leadley, 1992).

Dynamic enzyme-based models typically include explicit pools of enzymes, microbial biomass, and substrate (**Figure 1**) with substrate decomposition providing resources supporting biomass and enzyme production, as well as respiration (Sinsabaugh and Moorhead, 1997; Vetter et al., 1998; Schimel and Weintraub, 2003; Allison, 2005, 2012; Allison et al., 2010, 2011; Folse and Allison, 2012; Moorhead et al., 2012; Resat et al., 2012; Wang et al., 2013). Turnover of microbial biomass and enzymes is commonly needed to balance these pools with substrate input. However, data on turnover rates are rarely published (but see Allison, 2006). Even the simple model in **Figure 1** requires information that is seldom available, and incorporates hypothetical relationships that are controversial. For example few experimental studies have simultaneously examined the dynamics of microbial biomass, enzymes, and substrate, so instead, feedback controls necessary to balance the relative SEM relationships, such as production and turnover rates, are often “fit” to maintain model stability (e.g., Sinsabaugh and Moorhead, 1997; Schimel and Weintraub, 2003; Lawrence et al., 2009). Even the relative flows

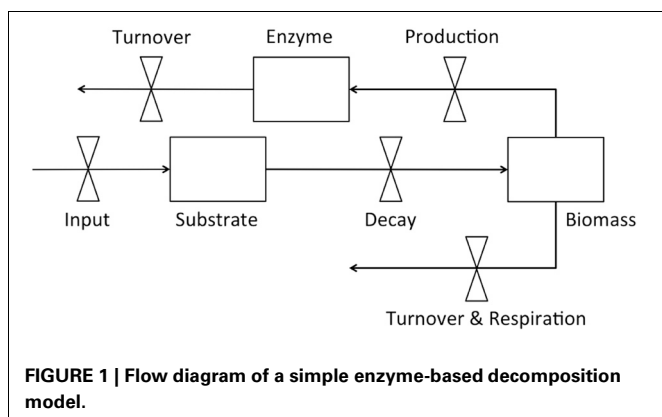
of resources to enzymes, biomass, and respiration are uncertain, depending on which model of carbon use efficiency is employed (Wang and Post, 2012; Sinsabaugh et al., 2013; Wang et al., 2013), and whether enzyme production is constitutive, inducible, or both (Schimel and Weintraub, 2003; Allison, 2005). Finally, the enzyme pool size is never measured directly, and instead potential activity is assumed to be proportional to the concentration of enzyme.

More complex models simulate the degradation of different substrates by different types of enzymes, but retain all the limitations of simpler models while adding more pools and associated uncertainties about multiple SEM interactions (Allison, 2005; Folse and Allison, 2012; Moorhead et al., 2012). Sinsabaugh and Follstad Shah (2012) argued that patterns of enzyme activity reflect microbial metabolic and stoichiometric needs, limited by patterns of resource availability. This general rationale integrated energy and nutrient controls in decomposition models long before enzymes were explicitly included (e.g., Parnas, 1975; Parton et al., 1987; Skjemstad et al., 2004). More recently, Moorhead et al. (2012) used this rationale to derive an analytical solution for the optimum allocation of C and N acquiring enzyme activities by decomposer microorganisms. Similarly, Allison (2005) assumed that enzyme production was induced by resource deficits and allocated this production among C, N, and P acquiring enzymes to balance microbial requirements. Both of the latter models also retained the uncertainties of simpler enzyme-based models despite generating relative patterns of different enzyme activities that could be compared to experimental studies. Thus, many of the underlying assumptions built into enzyme-based models remain untested.

INFORMATION GAPS

In general, enzyme-based models simulate the flow of carbon and nutrients between pools that are often difficult to empirically quantify or test. Nonetheless, any effort to incorporate first-principle mechanisms driving soil organic matter dynamics must address the fundamental relationships within the SEM system, including the basic physiology of decomposer microorganisms and the dynamics of their extracellular enzymes. Most experimental studies to date have limited power to support or test key assumptions of these models. For example, microbial biomass is rarely monitored during decomposition, and the dynamic interaction between biomass and system carbon (**Figure 1**) is unclear. Microbial biomass is usually reported to be low, seldom exceeding 2–3% of the total organic C pool in soils (Anderson and Domsch, 1989; Wardle, 1998), but the mechanisms controlling biomass are poorly defined. Without measures of microbial biomass, we cannot directly relate enzyme activities and CO₂ efflux to the microbial pool responsible for their production.

Even when microbial biomass is monitored closely, other aspects of decomposition are often omitted. For example, Kuehn et al. (2000), Gessner (2001), and Suberkropp (2001) all monitored microbial biomass dynamics on decomposing litter in aquatic ecosystems. However, none of these studies measured extracellular enzyme activities. In contrast, a suite of detailed studies examining litter decay in a Mediterranean ecosystem closely monitored microbial biomass, enzyme activities, litter



mass and chemistry, and respiration (Fioretto et al., 2000, 2001, 2003, 2005, 2007). However, their focus was primarily on C dynamics and they measured neither N nor P acquiring enzyme activities. Also, Fioretto et al. (2007) found that high seasonal variation in moisture stress produced high seasonal variation in enzyme activities that decoupled apparent activities from litter mass loss. Thus physical variations can obscure biological relationships. In short, few studies have obtained the basic measurements needed to develop or test enzyme-based decomposition models.

Here we examine observations made during a detailed study of litter decomposition in laboratory microcosms, including simultaneous measures of enzyme activities, microbial biomass, and CO₂ efflux. Our goals are to (1) analyze these data from the perspective of model requirements, i.e., for relationships between variables in comparison to separate analyses of independent variables, and thus (2) illustrate how simultaneous measures of these metrics during decomposition can provide insights to relationships needed to inform enzyme-based models.

CASE STUDY WITH ALTERNATIVE ANALYSES

We use new analyses of data reported by Rinkes et al. (2013) as a case study of how relationships among simultaneous measures of enzyme activities, microbial biomass, and CO₂ efflux can be useful for decomposition modeling (Figure 1). In brief, they conducted a short-term (14 days) laboratory study of litter decomposition to evaluate the relationships between CO₂ efflux, microbial C, extracellular enzyme activities, and changes in soil mineral N and P concentrations during the initial onset of litter decay. Their explicit goals were to examine (1) the effects of soil type, and both litter quality and surface area; and (2) the interactions between litter decay and the organic matter “priming effect” at the start of decomposition. To these ends, 1 g of sugar maple (*Acer saccharum*) or white oak (*Quercus alba*) leaves were cut to one of three sizes (ground, 0.25 cm², and 1.0 cm²), and mixed with 50 g of either a sandy soil with low soil organic C (SOC) content (0.4%) or a loam with moderate SOC content (4.1%), wetted to 45% water-holding capacity (WHC) and incubated at 20°C. Soils were first pre-incubated for 5 months in a dark 20°C incubator at 45% WHC. The pre-incubation allowed for microorganisms to acclimate to experimental conditions and to metabolize as much extant labile C as possible, in order to better isolate the specific response of litter additions. Microbial biomass C, soil NH₄⁺, NO₃⁻, and PO₄³⁻ concentrations, and β-glucosidase (BG), β-N-acetyl-glucosaminidase (NAG), and acid phosphatase (AP) activities were measured on days 0 (initial values), 3 and 14; CO₂ efflux was measured on days 1, 2, 3, 4, 6, 7, and 14.

Rinkes et al. (2013) discussed the primary results of their experiment in detail, but models require specific types of related information not reported in their study, such as CO₂ efflux and enzyme activity per unit microbial biomass. Thus we analyzed their data differently to achieve a separate set of goals. Rinkes et al. (2013) were interested in the effects of litter surface area on decomposition and had 4 replicates of each litter size class (3 sizes) for each combination of litter (21) and soil type (2 soils). This provided three means ($N = 4$) for each measured system parameter for each combination of soil and litter type by date. However,

Rinkes et al. (2013) did not link the CO₂ efflux measurements to the same microcosm replicates as other system measurements. As a result, CO₂ efflux could not be paired to enzyme activity or soil nutrient content by replicate. Instead, mean C fluxes from a particular combination of litter type, litter size and soil type for a date were compared to the means of the other system measures that is there were three means per date for each system parameter, defined by litter particle size class, and litter and soil type. We limited our attention to CO₂ efflux rates on days 1, 3, and 14, which corresponded to the timing of measures taken for other system parameters. Finally, we did not subtract values of CO₂ efflux and enzyme activities observed in soil-only controls from litter addition treatments as Rinkes et al. (2013) did, because we were interested in total system behaviors and subtracting control values from treatment values emphasizes litter dynamics, alone.

Our primary interest in using these data was to explore direct relationships between microbial dynamics, enzyme activities, and litter decay, and how they varied with soil and litter characteristics. Thus we calculated total organic carbon (TOC = SOC + litter C) through time. All incubations began with 1 g litter (44% C for both oak and maple) and 50 g soil, but sand had 0.4% SOC content whereas loam had 4.1% SOC, resulting in 0.64 and 2.49 g TOC, respectively. CO₂ efflux rates were subtracted from TOC over time to estimate remaining C per unique replicate ($N = 4$ by day, litter size, soil, and litter type). We then calculated three mean values (one for each litter particle size) of CO₂ efflux and TOC for each combination of day, litter, and soil type that could then be compared to the mean values of other system characteristics, e.g., enzyme activities, biomass, and soil nutrient concentrations. We also assumed that CO₂ efflux measures on day 1 corresponded to initial biomass, nutrient, and enzyme measures on day 0, although they were taken over 24 h from the start of the experiment, and thus likely overestimate CO₂ efflux rate per unit biomass because the biomass was growing (reported below).

The statistical analyses performed by Rinkes et al. (2013) usually examined the effects of litter particle size, litter type, and soil type on system characteristics, e.g., CO₂ efflux, enzyme activity, and mineral nutrients. However, our focus was not on litter particle size, so we analyzed the three litter particle size classes together, which reduced most of our analyses to Two-Way ANOVAs (litter and soil), with separate analyses conducted for each day. These differences in statistical design often resulted in differences between studies. In particular, significant effects of litter particle size, or interactions between particle size and other main effects were not apparent in our analyses. This often led to different conclusions about the contributions of the other independent factors (below) and illustrates the potential impact empirical studies could have on decomposition models by including additional analyses that explicitly address modeling needs.

CARBON FLUX RATES

C flux rates can be expressed in several ways. For example, Rinkes et al. (2013) reported CO₂ efflux per g soil but subtracted soil-only control values from treatments. We did not subtract control values and also estimated rates per g TOC and per g microbial biomass because activities per unit microbial biomass are

necessary to develop and test relationships in mechanistic models (**Figure 1**). For this reason we examined microbial biomass ($\mu\text{g C}\cdot\text{g soil}^{-1}$) by day, soil, and litter type, and found that it was initially greater (day 0) for loam than sand (**Table 1**). However, by day 3 there were no differences between treatments, but on day 14, biomass was again higher for loam than sand. Rinkes et al. (2013) noted that microbial biomass was initially greater for loam than sand controls, but there were no differences between treatments at any time after soil-only control values were subtracted. They also reported that total (uncorrected) biomass increased from day 0 to day 3 (values between 140–300 $\mu\text{g C}\cdot\text{g soil}^{-1}$) and generally decreased from day 3 to day 14. Thus our results are consistent with the few observations they reported.

Rinkes et al. (2013) reported significant effects of litter type, litter particle size, and soil type on CO_2 efflux rates, but values of- and differences between litter and soil types were presented by litter particle size, making it difficult to compare their values to our estimates. Also, their rates were adjusted for soil-only control values and thus were slightly lower than we report. We found that although the total rate of CO_2 efflux per g soil ($\mu\text{g C}\cdot\text{g soil}^{-1}\cdot\text{d}^{-1}$) was higher in loam than sand on all days, differences between litter types only occurred on day 3 when efflux was higher for maple than oak litter (**Table 1**). In contrast to rates per g soil, we found that rates of CO_2 efflux per g TOC ($\text{mg C}\cdot\text{g C}^{-1}\cdot\text{d}^{-1}$) were not different between treatments on day 1, and rates were higher for sand than loam on both day 3 and day 14 (**Table 1**). On day 3 the CO_2 efflux rate was also higher for maple than oak litter.

These differences in CO_2 efflux rates per g soil and per g TOC can be explained by the large differences in SOC content between sand (ca. 0.4%) and loam (ca. 4.1%). Rinkes et al. (2013) found evidence of a priming effect for loam, suggesting that a portion of the C loss from loam was from the SOC pool rather than litter. However, SOC is likely more recalcitrant than litter, and because litter was a larger fraction of the TOC in sand than loam it likely supported a higher rate of respiration per g TOC. Apparently, differences per g soil between treatments were due to greater amounts of SOC and associated microbial biomass (see below) for loam than sand. Indeed, the higher CO_2 efflux rate per g TOC on day 3 for sand may have resulted from the higher biomass: TOC ratio in sand on this same day (see below).

The biomass-specific respiration rates ($\text{g C}\cdot\text{g C}^{-1}\cdot\text{d}^{-1}$) were calculated for day 1 by dividing CO_2 efflux rates on day 1 ($\mu\text{g C}\cdot\text{g soil}^{-1}\cdot\text{d}^{-1}$) by the microbial biomass on day 0 ($\mu\text{g C}\cdot\text{g soil}$), which may have slightly overestimated rates because biomass was increasing during the first 24 h of the study (**Table 1**). Regardless, biomass-specific rates were higher for oak than maple on day 1. On other days there were no differences between treatments. This result also suggested a rapid convergence in metabolic characteristics of the microbial community driving C flux regardless of soil or litter type. In addition, CO_2 efflux rates were higher for maple than oak litter on day 3, whether calculated per g soil or g TOC (**Table 1**), suggesting more rapid decay of the less recalcitrant maple litter even though it did not support a higher biomass: TOC value.

TOTAL CARBON LOSSES

Rinkes et al. (2013) subtracted the C lost from soil-only controls from their estimates of total C losses from treatments. They

found consistent differences between soil and litter types, and occasionally differences between litter particle sizes. We did not subtract control values from treatments and found that cumulative CO_2 efflux (mg C) was higher for loam than sand on all days (**Table 1**). On day 14, our mean values fell within the range of values reported by Rinkes et al. (2013) for the three particle sizes. We also found that C loss was higher for maple than oak on days 3 and 14 (**Table 1**). Not surprisingly, regressions showed that incubation time (day) explained most of the C loss in both loam ($N = 14$, $R^2 = 0.896$; $P = 0.01$) and sand ($N = 14$, $R^2 = 0.860$; $P < 0.01$). The overall loss rate in loam (0.00357 d^{-1}) was 1.6 times greater than sand (0.00228 d^{-1}). However, due to higher SOC content, loam lost <3% of its initial TOC by day 14 whereas sand lost about 5% (**Table 1**). There was also a slightly higher C loss (ca. 0.5%) from maple than oak litter in both soil types (both $P \leq 0.05$).

Rinkes et al. (2013) found that the litter pool (ca. 0.44 g C) contributed most of the C to CO_2 efflux because it was probably more labile than SOC, given a 5-month soil pre-incubation at optimal temperature and moisture conditions. Thus, the cumulative CO_2 effluxes (calculated above) suggest that loam lost the equivalent of about 10% of its initial litter C by day 14 whereas sand lost only 7%. If the difference in C losses between sand and loam was due largely to the priming effect of litter addition on SOC turnover (Blagodatskaya and Kuzyakov, 2008), then the C loss from loam SOC (ca. 13 mg) approximated 0.6% of the initial SOC pool size.

Separate analyses by day revealed that the amount of estimated litter C remaining (mg C) on day 14 was greater for sand than loam, and greater for oak than maple (**Table 1**). This was consistent with larger cumulative C losses from maple than oak on days 3 and 14 for both soil types, and slightly higher rates of CO_2 efflux for maple than oak on day 3, both per g soil and per g TOC (**Table 1**). This suggests a slightly higher rate of C acquisition from maple litter by microbial biomass, although no difference in biomass between litter types was observed at any time (see below). Rinkes et al. (2013) found that the priming effect was slightly higher for maple than oak litter, which might explain why biomass: TOC ratios did not increase with stimulation of recalcitrant SOC turnover (below), as the assimilation efficiency is likely lower for the more recalcitrant material.

MICROBIAL BIOMASS

Microbial biomass per g soil ($\mu\text{g C}\cdot\text{g soil}^{-1}$) was initially greater for loam than sand. There were no differences between soil or litter types on day 3, but on day 14, biomass was again higher for loam than sand (**Table 1**). In contrast, microbial biomass per g TOC ($\text{mg C}\cdot\text{g C}^{-1}$) was greater for sand than loam on day 3, but there were no other differences between litter or soil types (**Table 1**). Apparently, differences that existed when biomass was estimated per g soil were due to the higher SOC and associated microorganisms present in loam than sand. When Rinkes et al. (2013) subtracted the amount of biomass in controls from treatments with added litter they found no differences between treatments at any time. However, they also estimated total biomass: TOC ratios and found higher values in sand than loam, and higher values on day 3 than day 14 for both soil types. These

Table 1 | Results of Two-Way ANOVA of independent and relational variables for soil and litter types were based on data collected by Rinkes et al. (2013).

Factor	Units	Day	Loam	Sand	Maple	Oak
Microbiota	$\mu\text{g C-g soil}^{-1}$	0	143.7 \pm 98.4**	22.7 \pm 27.9**	107.0 \pm 116.2	59.5 \pm 63.5
		3	356.1 \pm 175.4	315.1 \pm 248.9	334.2 \pm 224.6	337.0 \pm 207.7
		14	208.6 \pm 75.7**	114.8 \pm 107.6**	158.3 \pm 102.9	164.8 \pm 106.1
Microbiota	mg C-g C^{-1}	0	2.94 \pm 0.81	1.81 \pm 2.22	3.45 \pm 0.10	1.31 \pm 1.50
		3	7.33 \pm 2.34**	25.49 \pm 2.73**	16.51 \pm 10.57	16.31 \pm 9.96
		14	4.38 \pm 0.69	9.51 \pm 6.85	6.64 \pm 4.65	7.25 \pm 6.42
CO ₂ efflux	$\mu\text{g C-g soil}^{-1}\cdot\text{d}^{-1}$	1	100.70 \pm 68.95**	25.61 \pm 10.06**	70.10 \pm 67.42	56.21 \pm 56.16
		3	232.70 \pm 46.46**	167.16 \pm 33.17**	229.78 \pm 45.82**	172.70 \pm 41.62**
		14	67.73 \pm 6.67**	39.77 \pm 9.96**	56.95 \pm 16.68	51.82 \pm 16.06
CO ₂ efflux	$\text{mg C-g C}^{-1}\cdot\text{d}^{-1}$	1	2.07 \pm 1.41	2.03 \pm 0.77	2.26 \pm 1.14	1.84 \pm 1.09
		3	4.80 \pm 0.96**	13.54 \pm 2.75**	10.35 \pm 5.40**	7.86 \pm 4.01**
		14	1.41 \pm 0.15**	3.32 \pm 0.79**	2.34 \pm 1.00	2.30 \pm 1.24
CO ₂ efflux	$\text{g C-g Biomass C}^{-1}\cdot\text{d}^{-1}$	1	0.713 \pm 0.521	4.033 \pm 4.370	0.661 \pm 0.328*	4.085 \pm 4.340*
		3	0.713 \pm 0.291	0.534 \pm 0.102	0.725 \pm 0.263	0.522 \pm 0.142
		14	0.328 \pm 0.049	0.494 \pm 0.280	0.439 \pm 0.255	0.383 \pm 0.173
ΣCO_2 efflux	mg C-g soil^{-1}	1	5.14 \pm 3.51*	1.30 \pm 0.49*	3.57 \pm 3.45	2.88 \pm 2.86
		3	22.98 \pm 7.10**	17.21 \pm 5.60**	23.19 \pm 5.95**	17.25 \pm 6.74**
		14	73.03 \pm 11.00**	45.95 \pm 6.85**	66.81 \pm 15.17**	53.40 \pm 15.19**
Total C	mg C	0	2.490 ^a	0.640	2.490	0.640
		3	2.477 \pm 0.006**	0.630 \pm 0.005**	1.552 \pm 0.944*	1.555 \pm 0.943*
		14	2.441 \pm 0.010**	0.608 \pm 0.007**	1.603 \pm 0.934**	1.530 \pm 0.939**
Litter C	g C	0	0.440	0.440	0.440	0.440
		3	0.427 \pm 0.006§	0.430 \pm 0.005§	0.427 \pm 0.006*	0.430 \pm 0.005*
		14	0.391 \pm 0.010**	0.408 \pm 0.007**	0.394 \pm 0.010**	0.405 \pm 0.011**
AP activity	$\text{nmol-g soil}^{-1}\cdot\text{h}^{-1}$	0	613.6 \pm 81.9**	152.0 \pm 68.3**	349.6 \pm 223.3§	416.1 \pm 284.2§
		3	841.0 \pm 201.1**	158.1 \pm 99.5**	418.6 \pm 332.9**	580.5 \pm 411.2**
		14	737.9 \pm 262.2**	100.3 \pm 23.3**	384.6 \pm 304.9§	465.3 \pm 429.6§
NAG activity	$\text{nmol-g soil}^{-1}\cdot\text{h}^{-1}$	0	276.8 \pm 29.8**	40.6 \pm 10.3**	152.9 \pm 115.8	164.6 \pm 139.2
		3	337.0 \pm 56.1**	121.5 \pm 80.6**	254.2 \pm 112.3*	204.3 \pm 141.3*
		14	543.8 \pm 293.4**	320.3 \pm 128.0**	510.4 \pm 238.2*	361.6 \pm 248.4*
BG activity	$\text{nmol-g soil}^{-1}\cdot\text{h}^{-1}$	0	165.8 \pm 35.9**	80.9 \pm 31.4**	145.0 \pm 54.5**	101.7 \pm 48.6**
		3	86.8 \pm 23.6**	33.2 \pm 20.3**	72.7 \pm 34.1**	47.3 \pm 31.2**
		14	818.1 \pm 436.6**	496.4 \pm 142.1**	744.3 \pm 352.8	580.5 \pm 360.5
AP activity	$\mu\text{mol-g Biomass}^{-1}\cdot\text{h}^{-1}$	0	693.19 \pm 1947.32	682.63 \pm 1035.33	694.91 \pm 1946.65	680.91 \pm 1036.57
		3	2.94 \pm 1.44**	0.92 \pm 0.93**	1.68 \pm 1.60	2.19 \pm 1.54
		14	4.37 \pm 3.57	2.88 \pm 4.40	2.74 \pm 2.39	4.50 \pm 5.04
NAG activity	$\mu\text{mol-g Biomass}^{-1}\cdot\text{h}^{-1}$	0	339.52 \pm 954.51	143.43 \pm 199.26	339.42 \pm 954.55	143.54 \pm 199.16
		3	1.23 \pm 0.75	0.83 \pm 1.00	1.24 \pm 1.04§	0.82 \pm 0.69§
		14	3.13 \pm 2.18§	9.23 \pm 15.09§	6.04 \pm 8.09	6.19 \pm 13.37
BG activity	$\mu\text{mol-g Biomass}^{-1}\cdot\text{h}^{-1}$	0	258.26 \pm 727.29	238.78 \pm 349.57	260.21 \pm 726.52	236.83 \pm 351.05
		3	0.33 \pm 0.28	0.24 \pm 0.31	0.38 \pm 0.37*	0.19 \pm 0.15*
		14	4.85 \pm 3.46§	13.84 \pm 20.43§	9.92 \pm 16.61	8.61 \pm 13.69

(Continued)

Table 1 | Continued

Factor	Units	Day	Loam	Sand	Maple	Oak
PO_4^{3-}	$\mu\text{g P}\cdot\text{g soil}^{-1}$	0	$7.93 \pm 7.85^{**}$	$37.77 \pm 18.53^{**}$	$29.07 \pm 20.71^{\$}$	$16.62 \pm 19.90^{\$}$
		3	$0.35 \pm 0.45^{**}$	$5.23 \pm 4.93^{**}$	$4.53 \pm 4.53^{**}$	$1.05 \pm 3.17^{**}$
		14	$0.11 \pm 0.07^{**}$	$2.44 \pm 2.35^{**}$	$2.07 \pm 2.28^{**}$	$0.48 \pm 1.36^{**}$
NO_3^{-}	$\mu\text{g N}\cdot\text{g soil}^{-1}$	0	$163.55 \pm 15.13^{**}$	$13.76 \pm 0.96^{**}$	88.40 ± 81.16	88.91 ± 80.40
		3	$144.67 \pm 19.06^{**}$	$4.03 \pm 2.92^{**}$	73.56 ± 71.85	75.14 ± 74.35
		14	$94.92 \pm 28.36^{**}$	$0.97 \pm 1.01^{**}$	47.19 ± 50.14	48.70 ± 53.81
NH_4^{+}	$\mu\text{g N}\cdot\text{g soil}^{-1}$	0	$24.72 \pm 8.03^{**}$	$2.60 \pm 0.85^{**}$	$10.87 \pm 8.58^{**}$	$16.46 \pm 15.92^{**}$
		3	$21.83 \pm 10.28^{**}$	$0.10(\text{trace})^{**}$	$8.31 \pm 11.62^{**}$	$13.62 \pm 14.23^{**}$
		14	$1.81 \pm 0.29^{**}$	$0.26 \pm 0.15^{**}$	$0.96 \pm 0.84^*$	$1.11 \pm 0.80^*$
Vector length	Unitless	0	$0.665 \pm 0.155^{**}$	$2.058 \pm 0.515^{**}$	$1.596 \pm 0.896^{**}$	$1.126 \pm 0.685^{**}$
		3	$0.280 \pm 0.069^{**}$	$0.459 \pm 0.250^{**}$	$0.464 \pm 0.246^{**}$	$0.275 \pm 0.067^{**}$
		14	$2.886 \pm 4.931^{**}$	$5.604 \pm 2.004^{**}$	$4.358 \pm 2.548^{**}$	$4.080 \pm 5.067^{**}$
Vector angle	Degrees	0	$65.54 \pm 3.09^{**}$	$73.40 \pm 5.089^{**}$	67.93 ± 4.66	71.02 ± 6.60
		3	$67.64 \pm 4.11^{**}$	$52.67 \pm 21.63^{**}$	$50.50 \pm 18.85^{**}$	$69.82 \pm 7.19^{**}$
		14	$54.49 \pm 14.63^{**}$	$19.91 \pm 9.06^{**}$	$31.64 \pm 20.05^{**}$	$43.25 \pm 21.21^{**}$

Means \pm standard deviations for variables for each soil and litter type by day. Asterisks following means indicate significant differences between soil or litter types by day.

* $P \leq 0.05$.

** $P \leq 0.01$.

$\$P \leq 0.10$.

^aValues without standard deviations were for single observations.

relationships between biomass and potential substrate (SOC and litter C) provide the basic parameters for models (Figure 1).

Most studies report that microorganisms usually account for less than 2–3% of the total organic matter in soils (e.g., Anderson and Domsch, 1989; Wardle, 1998), and would represent an even smaller fraction of the total soil mass. For this reason, the higher SOC content (and presumably associated biomass) for loam could explain the differences between soils in biomass per g soil on day 14 (Table 1). The biomass: TOC value on day 14 averaged $6.9 \pm 5.6 \text{ mg C}\cdot\text{g C}^{-1}$ (ca. 0.7%) across all soil and litter types, suggesting a relatively consistent relationship regardless of soil or litter type. Nonetheless, we found that biomass: TOC ratios ($\text{mg C}\cdot\text{g C}^{-1}$) were higher for sand (2.5%) than loam (0.7%) on day 3, although there were no differences in biomass per g soil on day 3, nor were there differences in biomass: TOC on day 1. These results fell within observations by Rinkes et al. (2013) of 2.1–2.9% for sand and 0.7% for loam on day 3.

These results suggest an initial flush of microbial growth on fresh litter that was more apparent for sand than loam, because sand had a much lower SOC content. The much smaller amount of litter C (0.44 g C) supported a higher biomass: TOC ratio than the more recalcitrant SOC (0.20 g C for sand vs. 2.05 for loam) because C acquisition rate per g TOC (demonstrated by the difference in ratios between days 1 and 3), and thus per g biomass, was higher for sand. Wardle (1998) found that variation in soil biomass C across ecosystem types declined with increasing soil C, which is consistent with the differences between loam and sand we found in the present study. An unexpected result of

this study was that there were no differences in microbial biomass estimates between litter types although oak is usually considered more recalcitrant than maple, and CO_2 efflux was slightly higher for maple than oak (above).

ENZYME ACTIVITIES

Extracellular enzyme activities can be expressed per unit soil, per unit organic matter, and per unit microbial biomass. Rinkes et al. (2013) subtracted soil-only control values from enzyme activities reported for treatments, to focus on litter activities. We examined litter + soil enzyme activities per unit soil mass and microbial biomass to focus on the whole system dynamics. For this reason, our activity values were much higher than those reported by Rinkes et al. (2013). We did not estimate activities per g organic matter because although the ratio of biomass: TOC ($\text{mg C}\cdot\text{g C}^{-1}$) was greater in sand than loam on day 3 (Table 1), no other significant differences in this ratio existed between treatments.

In general, Rinkes et al. (2013) found that enzyme activities per g soil increased over time, but with few treatment effects. In comparison, we found that all enzymes (not subtracting soil-only control values) showed higher activity per g soil ($\text{nmol}\cdot\text{g soil}^{-1}\cdot\text{h}^{-1}$) in loam than sand on all days (Table 1). We also found that AP was higher for oak than maple litter on all days. In contrast, Rinkes et al. (2013) reported frequent interactions between litter particle size and soil type for AP, but consistently low activity for maple litter incubated in sand, and generally higher activity for loam than sand on day 14.

We found that β -glucosidase (BG) activity was higher for loam than sand on all days, whereas Rinkes et al. (2013) found that control-adjusted BG activities were higher for loam than sand only on day 14. We also found that BG was higher for maple than oak on days 0 and 3 (Table 1) whereas Rinkes et al. (2013) reported no difference between litter types.

Finally, we found that β -N-acetyl-glucosaminidase (NAG) activity was higher for loam than sand on all days, and for maple than oak on days 0 and 3 (Table 1). In contrast, Rinkes et al. (2013) found no differences in control-adjusted NAG activity between any treatments.

We calculated biomass-specific enzyme activities ($\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) by dividing enzyme activities ($\text{nmol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) by microbial biomass ($\mu\text{g C}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$). There were no differences between soils or litters for any enzyme on day 0, when average activities were high but extremely variable (Table 1). On day 3, biomass-specific activities for AP were higher for loam than sand, and both BG and NAG were higher for maple than oak. On day 14, both BG and NAG were higher for sand than loam. These results contrast with higher activities of all enzymes per g soil for loam than sand on all days (Table 1). Although enzyme activities per g soil or g TOC are useful tools to evaluate treatment effects, biomass-specific enzyme activities are necessary to develop and test enzyme-based models (Figure 1), and as our results indicate, are not necessarily consistent with activities per unit soil. For example, the differences between enzyme activities per g soil on day 0 were apparently due to the higher initial microbial biomass for loam rather than differences in enzyme expression by microorganisms (Figure 1).

As an aide to interpreting patterns of enzyme activities, we followed Sinsabaugh et al. (2008) in calculating the ratios of BG/NAG and BG/AP activities for each pair of observations, including each combination of litter + soil treatment on each day (Figure 2). For each locus in this enzyme activity “space” we calculated an enzyme activity vector as the distance and angle from the origin. Vector length increases with increasing enzyme production toward C acquisition relative to nutrients (N and P), and the steepness of the vector angle increases with increasing enzyme production toward P acquisition. Thus we interpret increasing vector length as a relative increase in C limitation, and increasing vector angle as a relative increase in P vs. N limitation. The rationale for interpreting relative C, N, and P limitations to microorganisms from the relative activities of C, N, and P acquiring enzyme activities is based on stoichiometric and metabolic theories of ecological systems (Sterner and Elser, 2002; Gillooly et al., 2005; Allison et al., 2010, 2011). In brief, microbial requirements are relatively constrained by their elemental composition and metabolic demands, and needed resources are typically obtained from environmental sources through the actions of extracellular enzymes (Sinsabaugh and Follstad Shah, 2012). The ratios of BG/NAG are often plotted against BG/AP to determine the relative C, N, or P limitations to microorganisms, given the patterns of these key enzyme activities with respect to each other (Figure 2). Translating these ratios into vector lengths and directions (angles) provides clear metrics of relative C limitation (length), and relative P vs. N

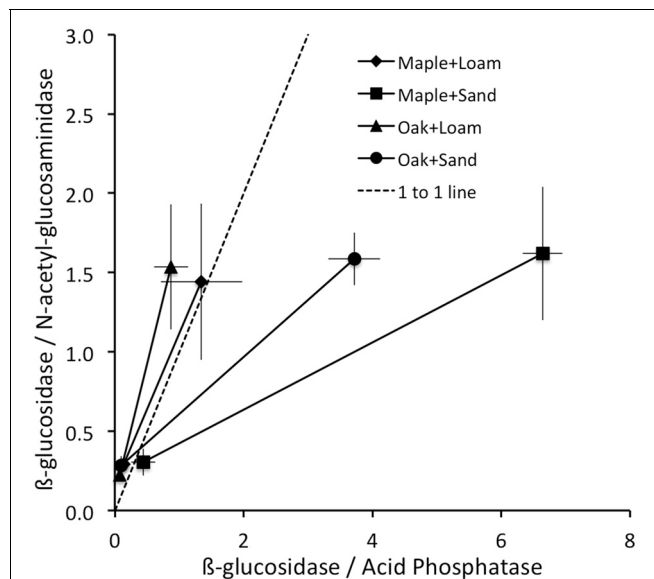


FIGURE 2 | Mean \pm stdev ratios of enzyme activities were lowest on day 3 (closest to origin) and greatest on day 14 for all litter + soil combinations; 1:1 line shown.

limitation (angle), but Rinkes et al. (2013) did not conduct these analyses.

Vector lengths were greater for maple than oak and greater for sand than loam on all days (Table 1). On day 0, vector angles were greater for sand than loam with no difference between litter types. In contrast, vector angles were greater for loam than sand and greater for oak than maple on both days 3 and 14 (Table 1). Vector lengths increased over time, and were significantly higher on day 14 than days 0 or 3 (Figure 2; all $P = 0.01$). Vector angles decreased over time, and were lower on day 3 than day 0 ($P = 0.10$), and lower on day 14 than days 0 and 3 (both $P = 0.01$). However, patterns differed by soil type. In sand, angles were significantly lower on day 3 than day 0, and lower on day 14 than day 3 (all $P = 0.01$). In loam, angles were only significantly lower on day 14 than day 3 ($P = 0.01$). In sand, lengths were higher on day 0 than day 3, and higher on day 14 than both days 0 and 3 (all $P \leq 0.05$). In loam, lengths were only higher on day 14 than day 3 ($P \leq 0.05$).

These patterns in enzyme activity vector length suggest that added litter provided a flush of soluble compounds driving biomass growth and concomitant immobilization of mineral nutrients, which could be obtained without enzyme activity. During this period of growth (day 0 to day 3), enzyme activity per unit biomass fell and relative C limitation declined (shorter vector lengths), although this pattern was stronger for sand than loam. By day 14, readily available soluble compounds from litter and mineral nutrients from soil may have been depleted and biomass-specific enzyme activities increased (along with vector lengths; Figure 2). Angles declined over time for both soil and litter types, but changed more for oak than maple litter (Figure 2, Table 1), suggesting that microorganisms became relatively more N limited for oak over time.

INTEGRATING ENZYME ACTIVITY, CO₂ EFFLUX, AND MICROBIAL GROWTH

We analyzed the incremental growth of microbial biomass ($\mu\text{g C}\cdot\text{g soil}^{-1}$) over time, which we calculated as the difference between sequential observations for each combination of litter, soil, and litter size treatments (Table 1). Cumulative enzyme activity ($\mu\text{mol}\cdot\text{g soil}^{-1}$) was calculated by multiplying the average activity of an enzyme between dates by the time span. Biomass growth estimates were compared to the lengths and angles of enzyme activity vectors at the time of observations (above), the cumulative activities of enzymes between observations, and the cumulative CO₂ efflux (mg C) during the same periods. Stepwise regression showed that the length of the enzyme activity vector and cumulative acid phosphatase (ΣAP) activity together explained most of the variation in microbial growth, and that growth was negatively related to both factors (Table 2). Thus, our results suggest that greater microbial growth was associated with lower microbial investment in C and P acquisition via enzyme production.

We also compared cumulative respiration (ΣCO_2) to cumulative enzyme activity (ΣNAG , ΣBG , and ΣAP). The underlying assumption for this comparison was that respiration is an index of microbial metabolism fueled by the actions of extracellular enzymes, and thus should be related to enzyme activity. The only direct measure of microbial activity in this study was CO₂ efflux, but other studies have shown cumulative enzyme activity to be positively correlated with various measures of decomposition (Sinsabaugh, 1994; Jackson et al., 1995; Amin et al., 2013). We found significant relationships between ΣCO_2 and both ΣNAG and ΣBG , with no significant effects of litter or soil type (Table 3). These consistent relationships between C and N acquiring enzyme activities across litter and soil types suggest that these relationships were very conservative within the constraints of this study.

Table 2 | Regression coefficients relating the length of the enzyme activity vector and cumulative acid phosphatase activity (ΣAP ; $\mu\text{mol}\cdot\text{g soil}^{-1}$) to change in biomass ($N = 24$, $R^2 = 0.722$).

Variable	Coefficient	Standard error	$P \leq$
Intercept	298.11	42.75	0.01
Length	-77.84	11.62	0.01
ΣAP	-1.35	0.32	0.01

Table 3 | Regressions of cumulative CO₂ efflux (mg C) against the cumulative activities of acid phosphatase (AP), β -N-acetyl-glucosaminidase (NAG) and β -glucosidase (BG) (μmol).

Parameter	BG	NAG	AP
N	28	28	28
Intercept	18.34	13.43	18.16
Slope	0.368	0.467	0.234
R^2	0.642	0.833	0.657
$P \leq$	0.01	0.01	0.01

In contrast, the relationship between ΣCO_2 and ΣAP was highly variable over time and between litter and soil types. Our analyses showed significant effects of both soil and litter types as well as interactions between all factors, suggesting complex, inconsistent relationships between microbial respiration and AP activity. Overall, these systems appeared to be more strongly P than N limited between days 0 and 3 (vector angles $>45^\circ\text{C}$; Table 4), especially for oak and loam (Figure 2, Table 1), but became more N limited by day 14 (vector angles $<45^\circ\text{C}$; Table 4), especially for sand (Figure 2, Table 1). In comparison, the change in microbial biomass between days 3 and 14 was negatively related to both the vector length and cumulative acid phosphatase (ΣAP) activity (Figure 3, Table 4). In fact, microbial growth was negatively related to the cumulative activity of each enzyme (not shown), which were highly correlated with one another. In essence, the negative relationship between growth and vector length also suggested an increasing C limitation over this time period (Figure 2, Table 4). The negative relationship between growth and cumulative enzyme activity suggests that less growth may occur when resources become more limiting and require an increase in relative enzyme production.

MINERAL NUTRIENT CONCENTRATIONS

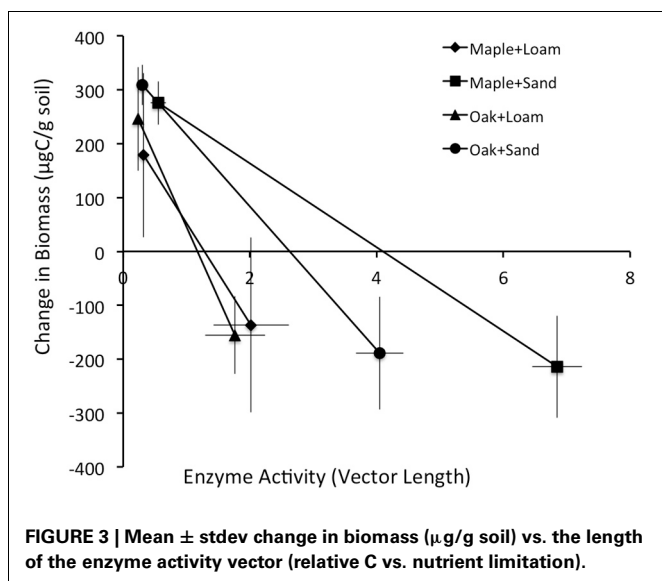
Rinkes et al. (2013) reported that prior to litter addition all soil properties differed between soil types except for pH and initial PO_4^{3-} concentrations. However, we found that immediately after litter addition (within 30 min), PO_4^{3-} , NO_3^- and NH_4^+ concentrations ($\mu\text{g}\cdot\text{g soil}^{-1}$) varied between both soil types and litter types, probably due to differences in nutrient contents of litter. PO_4^{3-} was significantly higher for sand than loam for all days (Table 1), as reported by Rinkes et al. (2013). However, we also found that PO_4^{3-} was higher for maple than oak litter on all days.

Our analyses showed that NO_3^- and NH_4^+ were higher for loam than sand on all days (Table 1), as reported by Rinkes et al. (2013). However, Rinkes et al. (2013) reported low overall concentrations of NH_4^+ for sand ($<5 \mu\text{g}\cdot\text{g soil}^{-1}$) throughout the experiment, but high initial concentrations for loam ($15\text{--}30 \mu\text{g}\cdot\text{g soil}^{-1}$) falling to $<5 \mu\text{g}\cdot\text{g soil}^{-1}$ by day 14, resulting in no differences between soils on day 14. We also found that NH_4^+ concentrations were higher for oak than maple litter on

Table 4 | Mean \pm stdev values for enzyme activity (BG, β glucosidase; NAG, β -N-acetyl-glucosaminidase; AP, acid phosphatase; $\text{mmol}\cdot\text{g C}^{-1}\cdot\text{h}^{-1}$), enzyme activity vector length ($\text{mol}\cdot\text{mol}^{-1}$) and angle (degrees), and microbial biomass ($\text{mg C}\cdot\text{g C}\cdot\text{h}^{-1}$).

Parameter	Day 0	Day 3	Day 14
BG	$5.98 \pm 8.78\text{ab}$	$0.19 \pm 0.13\text{a}$	$5.44 \pm 4.13\text{b}$
NAG	$4.15 \pm 4.92\text{b}$	$0.72 \pm 0.43\text{a}$	$3.41 \pm 2.26\text{b}$
AP	$16.05 \pm 28.83\text{b}$	$1.53 \pm 1.22\text{a}$	$2.51 \pm 1.86\text{a}$
Vector length	$1.35 \pm 0.86\text{a}$	$0.35 \pm 0.14\text{a}$	$3.67 \pm 2.16\text{b}$
Vector angle	$70.29 \pm 5.79\text{a}$	$60.44 \pm 17.26\text{a}$	$36.12 \pm 20.63\text{b}$
Biomass	$2.38 \pm 1.51\text{a}$	$16.41 \pm 9.79\text{b}$	$6.95 \pm 5.35\text{a}$

Different letters following means within rows indicate significant differences between days.



all days (Table 1), whereas Rinkes et al. (2013) reported higher control-adjusted concentrations for oak than maple only on day 0.

Another interesting result of this study was that neither the concentrations nor the dynamics of soil PO_4^{3-} , NO_3^- , and NH_4^+ pools provided much explanation for the dynamics of system C, microbial biomass, or enzyme dynamics. AP had a consistent, positive relationship to NH_4^+ (not shown), but it was the only measure of enzyme activity that showed any consistent relationship to any soil nutrient. Overall, the vector angle of enzyme activity was positively related to NO_3^- , and NH_4^+ , but this relationship varied by day.

INSIGHTS TO KEY RELATIONSHIPS

The primary goal of this paper is not to elucidate the effects of soil and litter types on decomposition processes, *per se*, but to illustrate how the interpretation of experimental data from a modeling perspective could differ from other approaches. We also tried to highlight the importance of collecting simultaneous, comparative measures of key model features, such as microbial biomass, enzyme activity, and respiratory output, by examining such data from the study by Rinkes et al. (2013). For example, many differences in microbial biomass, CO_2 efflux rates, and enzyme activities that existed between litter and soil types when observations were expressed per g soil were not apparent or were different when they were expressed per g TOC or per g microbial biomass (Table 1). Moreover, the relationships between enzymes, resources, and microorganisms were fairly tightly constrained, consistent with the theory of ecoenzymatic stoichiometry (Sinsabaugh and Follstad Shah, 2012).

Regardless of the reason why values of biomass: TOC were constrained (Wardle, 1998) any limit to biomass necessarily limits extracellular enzyme activities because they are produced by microorganisms and are likely to be related to other microbial activities (e.g., CO_2 efflux). Although many factors control the persistence and activity of enzymes in the environment

(Nannipieri and Gianfreda, 1998; Nannipieri et al., 2012; Burns et al., 2013), Sinsabaugh and Moorhead (1997) argued that a more rapid turnover of microbes than their enzymes would lead to an unstable system. Similarly, the model by Allison (2005) suggests that decoupling enzyme activity from the microorganisms that produced them would permit other microorganisms (i.e., “cheaters” that don’t produce enzymes) to potentially destabilize the system. Within this context, the observed biomass-specific respiration and enzyme activities represented the boundaries of these constraints for this study system. For example, microbial biomass: TOC was higher for sand (maximum 2.5%) than loam (maximum 0.7%) because the TOC of sand (0.64 g) was dominated by litter (0.44 g) whereas the TOC of loam (2.49 g) was dominated by more recalcitrant SOC (2.05 g). Thus the higher relative C availability in sand supported a higher relative biomass per unit substrate C, but only on day 3; by day 14 the ratio no longer differed between soils. This pulse of biomass growth for sand also provides an explanation for patterns of enzyme activity.

The patterns of biomass-specific enzyme activities indicated few differences in C, N, and P acquisition between soils or litter types. BG activity was higher for sand than loam on day 14, consistent with a greater enzyme activity vector length (Table 1), suggesting a higher relative C availability for loam. BG activity also was higher for maple than oak on day 3, again consistent with a greater vector length. AP activity was greater for loam than sand on day 3, suggesting a greater P demand for loam. Vector angle was also higher for loam than sand on day 3, also consistent with greater P vs. N demand. Finally, NAG was higher for oak than maple on day 3, and higher for sand than loam on day 14, suggesting greater N demands. Vector angles were lower for both of the latter cases, consistent with higher N vs. P demands. Thus patterns in biomass-specific enzyme activities were consistent with characteristics of enzyme vectors although vectors provided more detailed insights to microbial demands for C, N and P. For example, vector lengths indicated greater C than N or P limitation for sand on all days, suggesting that microorganisms were consistently more C limited in sand than loam, despite few differences in BG activities. In sand, the significant decrease in biomass: TOC between days 3 and 14 (not shown) suggested that increasing C limitation may have been responsible for the decline in biomass. At the same time, vector length increased 10-fold (Table 1). Vector angles indicated initially greater P vs. N limitation for sand than loam (day 0), but changed to a greater N vs. P limitation in sand for both days 3 and 14 (Figure 2, Table 1). Angles also indicated consistently greater P vs. N limitation in oak vs. maple litter. In contrast, biomass-specific AP and NAG activities showed few differences between litter or soil types. This pattern in vector angles was consistent with the higher PO_4^{3-} for sand and maple litter on days 3 and 14, concomitant with higher NO_3^- and NH_4^+ for loam than sand on all days (Table 1).

Understanding enzyme-biomass relationships needed for mechanistic models (Figure 1) requires understanding biomass-resource relationships that control the allocation of enzymes toward C, N, and P-acquisition (Sinsabaugh and Follstad Shah, 2012). Thus it is not simply the activities of enzymes in the environment or even the biomass-specific activities, but the balance between activities like those revealed herein by enzyme vectors.

Recently, Moorhead et al. (2012) were able to simulate differential allocation of C and N acquiring enzyme activities during decomposition in response to relative C and N availability by assuming that enzyme production was finite and that microorganisms optimized resource acquisition to maximize growth. The observations of Rinkes et al. (2013) verify these general assumptions, but require analysis of relational variables, such as C, N, and P acquiring enzyme activities to describe this balance.

This study also refutes a common assumption of enzyme-based models that enzyme production is roughly constitutive (Schimel and Weintraub, 2003; Moorhead et al., 2012), because a decline in enzyme activity occurred after the addition of fresh litter to microcosms despite an increase in biomass. Thus, biomass-specific activities of all enzymes were generally lowest on day 3 although biomass was greatest on day 3. If enzyme production were strictly constitutive, activity would increase with biomass. Instead, there appeared to be a shift in patterns of substrate use, with fresh litter driving a flush of CO₂ efflux and microbial growth. Enzyme activities then increased from day 3 to 14 despite a decline in biomass (Figure 2, Table 1), also suggesting a change in substrate use and changing emphasis on enzyme production. These results suggest that enzyme production was inducible, as modeled by Allison (2005), and responsive to differences in substrate characteristics (Berg, 2000; Berg and McClaugherty, 2008). In brief, microorganisms initially use simpler, easier to obtain resources from soluble litter fractions, shifting to increasingly more recalcitrant compounds as decomposition progresses (Van Hees et al., 2005; Glanville et al., 2012). This pattern also is consistent with the idea that different groups of microorganisms may be more active at different stages of litter decay (Allison and Martiny, 2008; Rinkes et al., 2011), including an initial burst of activity by some that may have little to no enzyme production (Allison, 2005).

Relationships between CO₂ efflux, biomass, and enzyme activities also addressed key model behaviors (Figure 1). For example, biomass-specific CO₂ efflux rates ranged from 32–85% of the standing biomass C per day (Table 1), with no differences between soil or litter types or any difference between day 3 and day 14. Thus the respiratory coefficient was consistent despite differences in B:C ratios between soils on day 3 and despite the very different total amounts of biomass in the two soil types (Table 1). A respiratory coefficient of this magnitude is much higher than basal metabolic rates of usually <1% used in models (Parton et al., 1987; Skjemstad et al., 2004; Moorhead and Sinsabaugh, 2006), suggesting high growth-associated respiration and turnover rates, and possibly overflow metabolism to maintain observed biomass: TOC ratios (e.g., Parnas, 1975; Schimel and Weintraub, 2003). However, Rinkes et al. (2013) did not apply an extraction efficiency coefficient (K_{ec}) to the amounts of microbial biomass C they extracted with chloroform fumigation. Thus their biomass estimates are probably low, which would inflate the respiratory coefficient.

We also found that ratios of $\sum \text{CO}_2\text{-efflux} : \sum \text{enzyme activity}$ declined through time, suggesting a decline in enzyme efficiency, although a progressive change in substrate composition and selective use by microorganisms could also contribute to this pattern

(see above). Moreover, this ratio is an ambiguous measure of enzyme efficiency (Sinsabaugh, 1994; Jackson et al., 1995), particularly when gross measures of decomposition are used in calculations (e.g., CO₂-efflux), because enzymes usually target a specific type of substrate. It would be a more accurate measure of enzyme efficiency to relate changes in specific substrates, like cellulose, to the activities of enzymes that degrade them, like β -glucosidase (e.g., Amin et al., 2013). As an aside, significant positive intercepts from regressions of cumulative decay (e.g., $\sum \text{CO}_2$ efflux) vs. cumulative enzyme activity are also consistent with the idea that initial decay is relatively high for low levels of enzyme activity (Table 3), again arguing for changing substrate use patterns. These empirical data suggest that enzyme-based models will need to include multiple substrates with inducible enzyme activities to accurately portray SEM dynamics (Figure 1).

In summary, much of the information used to develop enzyme-based models to date has been obtained from disparate studies, which separately focused on various aspects of decomposition, microbial metabolism, enzyme activities, etc. (e.g., Schimel and Weintraub, 2003). Although insights can be gained from cross-system analyses, uncertainty about key relationships is a drawback. For example, relatively few studies have examined biomass dynamics during litter decay with much resolution (but see Fioretto et al., 2001), which is critical to linking enzyme activities to decomposition processes. More comprehensive studies like the one performed by Rinkes et al. (2013) reduce uncertainty by simultaneously measuring key variables that in turn permits direct comparisons, as shown herein. Moreover, Rinkes et al. (2013) also measured respiration for control soils (no litter) and control litter (no soil) in parallel incubations (not shown), so that the system dynamics of litter, soil and litter + soil could be isolated, although a significant priming effect found for loam demonstrated a synergism between litter and soil systems (Kuzayakov, 2010).

CONCLUSIONS

Despite the information gained from this detailed study, other relationships needed to inform enzyme-based models remain unknown (Burns et al., 2013). Among the more important are turnover rates for both enzymes and biomass, which presumably enter the SOC pool and thus become available for decomposition. Also unknown is the relationship between enzyme activity, concentration, and mass. Although Wang et al. (2012) recently suggested ways of estimating kinetic coefficients for enzymes in the field from laboratory studies, the relationship between activity and concentration is uncertain and highly variable, due to the influences of many environmental factors (Nannipieri and Gianfreda, 1998; Nannipieri et al., 2012). Models that presume to calculate enzyme pool sizes (e.g., Sinsabaugh and Moorhead, 1997; Schimel and Weintraub, 2003; Allison, 2005; Moorhead et al., 2012, etc.), in fact balance allocation, production, turnover and resource acquisition without direct observations. Such observations are needed to more directly and precisely determine the cost-benefit relationships of microbial investments in extracellular enzymes.

Another important mechanism underlying decomposition not mentioned herein is the taxonomic composition of the

microbial community. Different microorganisms have different environmental responses, enzyme capabilities, and metabolic and stoichiometric characteristics (Berg and McClaugherty, 2008). Thus all three of the substrate, enzyme, and biomass pools in **Figure 1** must expand to capture the more complex realities of the SEM relationships driving decomposition, which greatly increases the demand for experimental data (see Moorhead and Sinsabaugh, 2006). As previously discussed, few studies have measured key features of the simple enzyme-based model represented in **Figure 1**. Even fewer have examined the composition of the microbial

community, and possibly no studies have obtained information on changes in key system characteristics (e.g., **Figure 1**) corresponding to community changes. This work is only beginning.

ACKNOWLEDGMENTS

This work was financially supported by the National Science Foundation Ecosystem Sciences program grants DEB-0918718 and DEB-0946257. We thank Marshall McDaniel and the two reviewers for their thoughtful comments on earlier versions of this manuscript.

REFERENCES

- Allison, S. D. (2005). Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. *Ecol. Lett.* 8, 626–626. doi: 10.1111/j.1461-0248.2005.00756.x
- Allison, S. D. (2006). Soil minerals and humic acids alter enzyme stability: implications for ecosystem processes. *Biogeochemistry* 81, 361–373. doi: 10.1007/s10533-006-9046-2
- Allison, S. D. (2012). A trait-based approach for modeling microbial litter decomposition. *Ecol. Lett.* 15, 1058–1070. doi: 10.1111/j.1461-0248.2012.01807.x
- Allison, S. D., and Martiny, J. B. H. (2008). Resistance, resilience, and redundancy in microbial communities. *Proc. Nat. Acad. Sci. U.S.A.* 115, 11512–11519. doi: 10.1073/pnas.0801925105
- Allison, S. D., Wallenstein, M. D., and Bradford, M. A. (2010). Soil carbon response to warming dependent on microbial physiology. *Nat. Geosci.* 3, 336–340. doi: 10.1038/ngeo846
- Allison, S. D., Weintraub, M. N., Gartner, T. B., and Waldrop, M. P. (2011). “Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function,” in *Soil Enzymology*, eds G. C. Shukla and A. Varma (New York, NY: Springer-Verlag), 229–243. doi: 10.1007/978-3-642-14225-3_12
- Amin, B. A. Z., Chabbert, B., Moorhead, D., and Bertrand, I. (2013). Impact of fine litter chemistry on lignocellulolytic enzyme efficiency during decomposition of maize leaf and root in soil. *Biogeochemistry*. doi: 10.1007/s10533-013-9856-y
- Anderson, T. H., and Domsch, K. H. (1989). Ratios of microbial biomass carbon to total organic-carbon in arable soils. *Soil Biol. Biochem.* 21, 471–479. doi: 10.1016/0038-0717(89)90117-X
- Berg, B. (2000). Litter decomposition and organic matter turnover in northern forest soils. *For. Ecol. Manag.* 133, 13–22.
- Berg, B., and McClaugherty, C. (2008). *Plant Litter: Decomposition, Humus Formation, Carbon Sequestration*. Berlin: Springer-Verlag.
- Blagodatskaya, E. V., and Kuzyakov, Y. (2008). Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biol. Fert. Soils* 45, 115–131. doi: 10.1007/s00374-008-0334-y
- Burns, R. G. (1983). “Extracellular enzyme-substrate interactions in soil,” in *Microbes in Their Natural Environments*, eds J. N. Slater, R. Whittenbury, and J. W. T. Wimpenny (Cambridge: Cambridge University Press), 249–298.
- Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stronberger, M. E., Wallenstein, M. D., et al. (2013). Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biol. Biochem.* 58, 216–234. doi: 10.1016/j.soilbio.2012.11.009
- Fioretto, A., Di Nardo, C., Papa, S., and Fuggi, A. (2005). Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem. *Soil Biol. Biochem.* 37, 1083–1091. doi: 10.1016/j.soilbio.2004.11.007
- Fioretto, A., Papa, S., Curcio, E., Sorrentina, G., and Fuggi, A. (2000). Enzyme dynamics on decomposing leaf litter of *Cistus incanus* and *Myrtus communis* in a Mediterranean ecosystem. *Soil Biol. Biochem.* 32, 1847–1855. doi: 10.1016/S0038-0717(00)00158-9
- Fioretto, A., Papa, S., Pellegrino, A., and Fuggi, A. (2003). Litter-fall and litter decomposition in a low Mediterranean shrubland. *Biol. Fert. Soils* 39, 37–44. doi: 10.1007/s00374-003-0675-5
- Fioretto, A., Papa, S., Pellegrino, A., and Fuggi, A. (2007). Decomposition dynamics of *Myrtus communis* and *Quercus ilex* leaf litter: mass loss, microbial activity and quality change. *Appl. Soil. Ecol.* 36, 32–40. doi: 10.1016/j.apsoil.2006.11.006
- Fioretto, A., Papa, S., Sorrentino, G., and Fuggi, A. (2001). Decomposition of *Cistus incanus* leaf litter in a Mediterranean maquis ecosystem: mass loss, microbial enzyme activities and nutrient changes. *Soil Biol. Biochem.* 33, 311–321. doi: 10.1016/S0038-0717(00)00142-5
- Folse, H. J., and Allison, S. D. (2012). Cooperation, competition, and coalitions in enzyme-producing microbes: social evolution and nutrient depolymerization rates. *Front. Microbiol.* 3:338. doi: 10.3389/fmicb.2012.00338
- Gessner, M. O. (2001). Mass loss, fungal colonisation and nutrient dynamics of *Phragmites australis* leaves during senescence and early aerial decay. *Aquat. Bot.* 69, 325–339. doi: 10.1016/S0304-3770(01)00146-2
- Gilloly, J. F., Allen, A. P., Brown, J. H., Elser, J. J., del Rio, C. M., Savage, V. M., et al. (2005). The metabolic basis of whole-organism RNA and phosphorus content. *Proc. Natl. Acad. Sci. U.S.A.* 102, 11923–11927. doi: 10.1073/pnas.0504756102
- Glanville, H., Rousk, J., Golyshin, P., and Jones, D. L. (2012). Mineralization of low molecular weight carbon substrates in soil solution under laboratory and field conditions. *Soil Biol. Biochem.* 48, 88–95. doi: 10.1016/j.soilbio.2012.01.015
- Jackson, C. R., Foreman, C. M., and Sinsabaugh, R. L. (1995). Microbial enzyme activities as indicators of organic matter processing rates in a Lake Erie coastal wetland. *Freshwat. Biol.* 34, 329–342. doi: 10.1111/j.1365-2427.1995.tb00892.x
- Kuehn, K. A., Lemke, M. J., Suberkropp, K., and Wetzel, R. G. (2000). Microbial biomass and production associated with decaying leaf litter of the emergent macrophytes *Juncus effusus*. *Limnol. Oceanogr.* 45, 862–870. doi: 10.4319/lo.2000.45.4.0862
- Kuzyakov, Y. (2010). Priming effects: interactions between living and dead organic matter. *Soil Biol. Biochem.* 42, 1363–1371. doi: 10.1016/j.soilbio.2010.04.003
- Lawrence, C. R., Neff, J. C., and Schimel, J. P. (2009). Does adding microbial mechanisms of decomposition improve soil organic matter models? a comparison of four models using data from a pulsed rewetting experiment. *Soil Biol. Biochem.* 41, 1923–1934. doi: 10.1016/j.soilbio.2009.06.016
- Manzoni, S., and Porporato, A. (2009). Soil carbon and nitrogen mineralization: theory and models across scales. *Soil Biol. Biochem.* 41, 1355–1379. doi: 10.1016/j.soilbio.2009.02.031
- Moorhead, D. L., Lashermes, G., and Sinsabaugh, R. L. (2012). A theoretical model of C- and N-acquiring exoenzyme activities, which balances microbial demands during decomposition. *Soil Biol. Biochem.* 53, 133–141. doi: 10.1016/j.soilbio.2012.05.011
- Moorhead, D. L., and Sinsabaugh, R. L. (2006). A theoretical model of litter decay and microbial interaction. *Ecol. Monogr.* 76, 151–174. doi: 10.1890/0012-9615(2006)076[0151:ATMOLD]2.0.CO;2
- Nannipieri, P., and Gianfreda, L. (1998). “Kinetics of enzyme reactions in soil environments,” in *Structure and Surface Reactions*, eds P. M. Huang, N. Senesi, and J. Buffle (New York, NY: John Wiley and Sons), 449–479.
- Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., Masciandaro, G., et al. (2012). Soil

- Enzymology: classical and molecular approaches. *Biol. Fertil. Soils* 48, 743–762. doi: 10.1007/s00374-012-0723-0
- Niu, S., Luo, Y., Fei, S., Yuan, W., Schimel, D., Amiro, B., et al. (2012). Thermal optimality of net ecosystem exchange of carbon dioxide and underlying mechanisms. *New. Phytol.* 194, 775–783. doi: 10.1111/j.1469-8137.2012.04095.x
- Parnas, H. (1975). Model for decomposition of organic material by microorganisms. *Soil Biol. Biochem.* 7, 161–169. doi: 10.1016/0038-0717(75)90014-0
- Parton, W. J., Schimel, D. S., Cole, C. V., and Ojima, D. S. (1987). Analysis of factors controlling soil organic matter levels in Great Plains Grasslands. *Soil Sci. Soc. Am. J.* 51, 1173–1179. doi: 10.2136/sssaj1987.03615995005100050015x
- Resat, H., Bailey, V., McCue, L. A., and Konopka, A. (2012). Modeling microbial dynamics in heterogeneous environments: Growth on soil carbon sources. *Microb. Ecol.* 63, 883–897. doi: 10.1007/s00248-011-9965-x
- Reynolds, J. F., and Leadley, P. W. (1992). “Modeling the response of arctic plants to changing climate,” in *Arctic Ecosystems in a Changing Climate: An Ecophysiological Perspective*, eds F. S. Chapin, III, R. L. Jefferies, F. J. Reynolds, G. R. Shaver, and J. Svoboda (New York, NY: Academic Press), 413–438.
- Rinkes, Z. L., Weintraub, M. N., DeForest, J. L., Grandy, A. S., and Moorhead, D. L. (2013). Interactions between leaf litter quality, particle size, and microbial community during the earliest stage of decay. *Biogeochemistry*. doi: 10.1007/s10533-013-9872-y
- Rinkes, Z. L., Weintraub, M. N., DeForest, J. L., and Moorhead, D. L. (2011). Microbial substrate preference and community dynamics during decomposition of *Acer saccharum*. *Fungal. Ecol.* 4, 396–407. doi: 10.1016/j.funeco.2011.01.004
- Schimel, J. P., and Weintraub, M. N. (2003). The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biol. Biochem.* 35, 549–563. doi: 10.1016/S0038-0717(03)00015-4
- Sinsabaugh, R. L. (1994). Enzymatic analysis of microbial pattern and process. *Biol. Fertil. Soils* 17, 69–74. doi: 10.1007/BF00418675
- Sinsabaugh, R. L., and Follstad Shah, J. (2012). Ecoenzymatic stoichiometry and ecological theory. *Annu. Rev. Ecol. Syst.* 43, 313–343. doi: 10.1146/annurev-ecolsys-071112-124414
- Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., Allison, S. D., Crenshaw, C., et al. (2008). Stoichiometry of soil enzyme activity at global scale. *Ecol. Lett.* 11, 1252–1264.
- Sinsabaugh, R. L., and Moorhead, D. L. (1997). “Synthesis of litter quality and enzyme approaches to decomposition modeling,” in *Driven by Nature: Plant Litter Quality and Decomposition*, eds G. Cadisch and K. Giller (London: CAB International), 363–375.
- Sinsabaugh, R., Manzoni, S., Moorhead, D. L., and Richter, A. (2013). Carbon use efficiency of microbial communities: Stoichiometry, methodology and modeling. *Ecol. Lett.* 16, 930–939. doi: 10.1111/ele.12113
- Skjemstad, J. O., Spouncer, L. R., Cowie, B., and Swift, R. S. (2004). Calibration of the Rothamsted organic carbon turn-over model (RothC ver. 26.3), using measurable soil organic carbon pools. *Aust. J. Soil Res.* 42, 79–88. doi: 10.1071/SR03013
- Sterner, R. W., and Elser, J. J. (2002). *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton: Princeton University
- Suberkropp, K. (2001). Fungal growth, production, and sporulation during leaf decomposition in two streams. *Appl. Environ. Microbiol.* 67, 5063–5068. doi: 10.1128/AEM.67.11.5063-5068.2001
- Van Hees, P. A. W., Jones, D. L., Finlay, R., Godbold, D. L., and Lundstrom, U. S. (2005). The carbon we do not see—the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. *Soil Biol. Biochem.* 37, 1–13. doi: 10.1016/j.soilbio.2004.06.010
- Vetter, Y. A., Deming, J. W., Jumars, P. A., and Krieger-Brockett, B. B. (1998). A predictive model of bacterial foraging by means of freely released extracellular enzymes. *Microb. Ecol.* 36, 75–92. doi: 10.1007/s002489900095
- Wang, G., and Post, W. M. (2012). A theoretical reassessment of microbial maintenance and implications for microbial ecology modeling. *REMS Microbiol. Ecol.* 81, 610–617. doi: 10.1111/j.1574-6941.2012.01389.x
- Wang, G., Post, W. M., Mayes, M. A. (2013). Development of microbial-enzyme-mediated decomposition model parameters through steady-state and dynamic analyses. *Ecol. Appl.* 23, 255–272. doi: 10.1890/12-0681.1
- Wang, G., Post, W. M., Mayes, M. A., Frerichs, J. T., and Sindhu, J. (2012). Parameter estimation for models of ligninolytic and cellulolytic enzyme kinetics. *Soil Biol. Biochem.* 48, 28–38. doi: 10.1016/j.soilbio.2012.01.011
- Wardle, D. A. (1998). Controls of temporal variability of the soil microbial biomass: a global-scale synthesis. *Soil Biol. Biochem.* 30, 1627–1637. doi: 10.1016/S0038-0717(97)00201-0

Conflict of Interest Statement: The authors declare that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 15 May 2013; accepted: 24 July 2013; published online: 12 August 2013.
Citation: Moorhead DL, Rinkes ZL, Sinsabaugh RL and Weintraub MN (2013) Dynamic relationships between microbial biomass, respiration, inorganic nutrients and enzyme activities: informing enzyme-based decomposition models. *Front. Microbiol.* 4:223. doi: 10.3389/fmicb.2013.00223

This article was submitted to *Frontiers in Terrestrial Microbiology*, a specialty of *Frontiers in Microbiology*.

Copyright © 2013 Moorhead, Rinkes, Sinsabaugh and Weintraub. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Environmental impacts on the diversity of methane-cycling microbes and their resultant function

Emma L. Aronson^{1,2*}, Steven D. Allison^{2,3} and Brent R. Helliker⁴

¹ Department of Plant Pathology and Microbiology, University of California, Riverside, CA, USA

² Department of Ecology and Evolutionary Biology, University of California, Irvine, CA, USA

³ Department of Earth System Science, University of California, Irvine, CA, USA

⁴ Department of Biology, University of Pennsylvania, Philadelphia, PA, USA

Edited by:

Per Bengtson, Lund University, Sweden

Reviewed by:

Hongchen Jiang, Miami University, USA

Brajesh Singh, University of Western Sydney, Australia

*Correspondence:

Emma L. Aronson, Department of Plant Pathology and Microbiology, University of California at Riverside, Boyce Hall 2491, Riverside, CA 92521, USA
e-mail: emma.aronson@gmail.com

Methane is an important anthropogenic greenhouse gas that is produced and consumed in soils by microorganisms responding to micro-environmental conditions. Current estimates show that soil consumption accounts for 5–15% of methane removed from the atmosphere on an annual basis. Recent variability in atmospheric methane concentrations has called into question the reliability of estimates of methane consumption and calls for novel approaches in order to predict future atmospheric methane trends. This review synthesizes the environmental and climatic factors influencing the consumption of methane from the atmosphere by non-wetland, terrestrial soil microorganisms. In particular, we focus on published efforts to connect community composition and diversity of methane-cycling microbial communities to observed rates of methane flux. We find abundant evidence for direct connections between shifts in the methane-cycling microbial community, due to climate and environmental changes, and observed methane flux levels. These responses vary by ecosystem and associated vegetation type. This information will be useful in process-based models of ecosystem methane flux responses to shifts in environmental and climatic parameters.

Keywords: methane, CH₄, methanotroph, biogeochemistry, soil, MOB, review

INTRODUCTION

Microorganisms have the potential to impact large-scale ecosystem functions that are relevant to the atmospheric composition of the Earth. In particular, microbial communities responsible for “narrow” processes, those that are phylogenetically and/or physiologically constrained, have been linked to corresponding process rates in nature (Schimel and Schaeffer, 2012). Schimel and Gullledge (1998) proposed studying methane-cycling microbial communities to demonstrate the connection between microbial community composition and ecosystem function. Environmental and climatic shifts can alter methane (CH₄) flux profiles of soils (Bender and Conrad, 1992; Willison et al., 1995; Aronson and Helliker, 2010), likely through shifts in microbial community structure and function. Since the publication of Schimel and Gullledge (1998), numerous technological advances have allowed for the direct analysis of the connection between environmental and climatic factors and microbial community composition. In addition, our understanding of how different members of the microbial community contribute to soil CH₄ flux has increased. In this review, we outline the responses of methane-cycling microbial community composition and abundance to environment and climate and how well these shifts correspond to changes in soil CH₄ flux profiles.

The goal of this review is to highlight the current state of, and recent advances in, our understanding of CH₄ consumption by microorganisms in terrestrial environments, as well as to point out areas where further study is needed. We hypothesized that net

CH₄ flux is correlated with the abundance and/or composition of methane-cycling microbes. We focus on non-wetland soils while touching on wetland and methanogen communities when relevant. To this end we discuss the main global changes that could impact methanotroph communities in particular. These changing environmental and climatic drivers include increased atmospheric CO₂ and CH₄ mixing ratios, increased temperature, changes in precipitation regimes, soil pH, and increased inorganic nitrogen (N) deposition to soil. In addition, we analyzed trends in CH₄ fluxes by ecosystem, climatic zone, and vegetation type. In order to organize the body of knowledge on this topic, a meta-dataset was created from the literature, which is published along with this review as supplemental data. We believe that this dataset can assist in identifying future experimental directions as well as modeling efforts of the relationships between environmental and climatic changes, methane-cycling microbial communities, and soil CH₄ fluxes.

BACKGROUND TO THE METHANE CYCLE

Methane is the 2nd most important anthropogenic greenhouse gas, responsible for 20–30% of total greenhouse gas radiative forcing since the industrial revolution (IPCC, 2007). Methane is currently about 200 times less concentrated in the atmosphere than is carbon dioxide, but each molecule of CH₄ is 25 times more potent in terms of heat-holding capacity (Lelieveld et al., 1998). Due to changes in human activity and land use, both carbon dioxide and CH₄ began to increase around 150 years ago,

as the industrial age began. Since that time, atmospheric CH₄ concentrations have increased ~150%; from a pre-industrial mixing ratio of about 0.7 ppm to ~1.8 ppm currently (Maxfield et al., 2006; Degelmann et al., 2010).

Variability in atmospheric methane concentrations

Atmospheric CH₄ concentrations became erratic and did not increase overall from 1997 until 2007, and then began increasing again around 2008 (Rigby et al., 2008) and continue to increase. The reason(s) for this shift is unknown, but several explanations have been proposed for the recent vagaries in atmospheric CH₄. Decreases in wetland sources have been proposed to explain the lack of growth in late 1990s and early 2000s (Bousquet et al., 2006). The patching of natural gas pipelines in Russia has also been proposed as an explanation for the change in atmospheric CH₄ concentrations, since these had become leaky after the collapse of the Soviet Union, losing an estimated 29–50 Tg CH₄ yr⁻¹ in the late 1980s–early 1990s (Reshetnikov et al., 2000), although these numbers have not been confirmed. A reduction in fossil fuel sources has also been implied as the cause by a study of ethane levels in Greenland and Antarctic firn (Aydin et al., 2011). Also proposed are variations in atmospheric concentration of OH⁻ radicals (Rigby et al., 2008), yet there did not appear to be any increase in atmospheric CH₄ destruction from these radicals recorded early in the duration of this decrease (Prinn, 2001) and there is an active debate over the reliability of past OH⁻ measurements (Lelieveld et al., 2004). Other explanations have focused on reduced rice agriculture and other microbial emissions, confirmed by isotopic measurements and models (Kai et al., 2011).

The wide range of potential explanations for past trends in atmospheric CH₄ indicates a lack of understanding of the interplay between biotic and abiotic controls on CH₄ cycling. The underlying biology of the microbial responses to environmental variables is still poorly understood (do Carmo et al., 2006). The non-wetland, terrestrial ecosystem CH₄ sink may be larger than suggested by top-down models suggest, possibly accounting for this missing sink, but this hypothesis can only be tested

with further study of soil methanotroph community composition and response to climatic and other variables. Indeed, the same isotopic fractionation evidence suggesting that reduced microbial sources may be responsible for the decline in atmospheric CH₄ growth (i.e., Kai et al., 2011) could also imply increased microbial consumption. Small advances in our understanding of any CH₄ source or sink will greatly improve our ability to budget this important greenhouse gas.

Atmospheric methane sources and sinks

Methane sources are variable but their number and magnitude appear to be on the rise, while CH₄ sinks are more uncertain. Total CH₄ emissions were calculated by Lelieveld et al. (1998) to be 600 Tg CH₄ yr⁻¹, and by Wang et al. (2004) to be 506 Tg CH₄ yr⁻¹, with most recent estimates falling between 503 and 610 Tg CH₄ yr⁻¹ (IPCC, 2007). **Figure 1** shows rough estimates of the relative contributions of CH₄ sources and sinks, based on Lelieveld et al. (1998), Wang et al. (2004), and Conrad (2009). The largest global CH₄ sources are natural and constructed wetlands, which contribute around 1/3 of annual emissions (IPCC, 2007). Anthropogenic sources, including rice paddies, domesticated animals, landfills, fossil fuel acquisition and burning, as well as biomass use for energy and agriculture, total at least 307 Tg CH₄ yr⁻¹, which could be over 60% of total emissions (Wang et al., 2004). There may be more sources than have been accounted for, as CH₄ has also been found to be produced aerobically in the ocean (Karl et al., 2008). Trees themselves have also been linked to CH₄ production (Keppler et al., 2006) through spontaneous UV-induced release and/or diffusion from dissolved soil CH₄ in leaf water (Nisbet et al., 2009), although the overall contribution of that source has been shown to be negligible (Dueck et al., 2007).

There are indications that CH₄ release from known sources was previously underestimated and has been on the rise with temperature increases in the last century. As high latitudes heat up in a generally warming climate, permafrost and accumulated ice thaw at accelerated rates (IPCC, 2007). This has caused the area of thermokarst lakes to increase, by at least double in the last 35

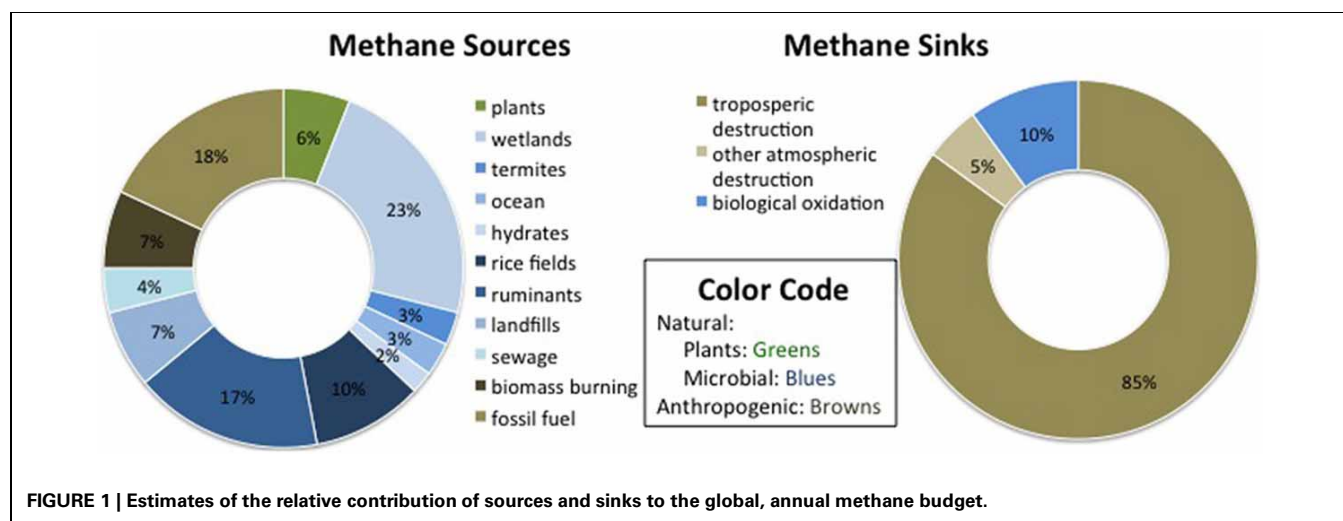


FIGURE 1 | Estimates of the relative contribution of sources and sinks to the global, annual methane budget.

years (Walter et al., 2006). Advances in measurements in high latitude lakes show that most CH₄ is released in rapid ebullition, a source type which was previously missed, and that the CH₄ being released is Pleistocene in age, indicating the release of old carbon stores. This source accounts for at least 3.7 Tg CH₄ yr⁻¹ previously omitted from global estimates (Walter et al., 2006). Also associated with the warming in these higher latitudes is geological CH₄ release from shallow hydrates, which may increase quickly as warming continues and could contribute up to 1.4×10^6 Tg CH₄ (Shakhova et al., 2010). Further, increased temperatures in wetlands around the globe will likely lead to large increases in CH₄ release, due to the sensitivity of methanogens to warming (Christensen et al., 2003).

The largest estimated CH₄ sinks include tropospheric destruction (approximately 80–90% annually) and oxidation in other parts of the atmosphere (5–10%), according to Lelieveld et al. (1998). The most common figure for gross oxidation by soil in terrestrial environments is $\sim 30 \pm 15$ Tg CH₄ (IPCC, 2007), which corresponds to 2.5–7.5% of the estimated 600 Tg CH₄ budget per year (Lelieveld et al., 1998). However, there has been some variation in this estimate, with a classic review of methanotrophy estimating soil consumption at 40–60 Tg yr⁻¹ (Hanson and Hanson, 1996). Of all the CH₄ sources and sinks, the biotic sink strength is the most responsive to variation in human activities (Dunfield et al., 2007).

The above figures for total consumption by the soil were not measured directly, but rather approximated by top-down, or inverse, global models (Wang et al., 2004). Inverse modeling solves for the sources and sinks based on observations of atmospheric chemical species over time and space while attempting to minimize uncertainty (Prinn, 2000). More recently, a meta-analysis by Dutaur and Verchot (2007) attempted to scale up from averages of local observations, resulting in an estimated consumption rate of ~ 34 Tg CH₄ yr⁻¹. Due to low consumption levels at atmospheric concentrations and high variability, the bottom-up approach of extrapolating from small-scale observations has had limited success in the past. However, the bottom-up approach should be applied more strenuously in the near future to take advantage of advances in technology and more widespread measurements. Future attempts to scale up from local observations should also account for the environmental factors and their impacts on microbial communities that govern CH₄ flux.

METHANE-CYCLING MICROORGANISMS

Soil exchange of CH₄ with the atmosphere is regulated by two groups of microorganisms, known as methanogens and methanotrophs. The disparate environmental requirements of these two groups, particularly oxygen concentration, temperature, water content, and nutrient availability, determine the net CH₄ flux of a given ecosystem. Methanogenic (CH₄ producing) archaea, active mainly in anaerobic conditions, produce CH₄ as a metabolic byproduct and are the main biological source of CH₄ in natural systems, landfills, and agriculture. Methanotrophic (CH₄ consuming) bacteria (sometimes referred to as CH₄ oxidizing bacteria or MOB) are active mainly in aerobic conditions and derive energy and carbon from the oxidation of CH₄ (Hanson and Hanson, 1996).

Methanogens

In natural systems, methanogens produce about 33% of emissions (Lelieveld et al., 1998). Most anthropogenic CH₄ emissions from waste management and agriculture are also due in large part to the action of methanogens. Most methanogens are anaerobic archaea, and there exists a large variety of methanogens that loosely fit into two main, non-phylogenetic categories: those that are hydrogenotrophic, i.e., produce CH₄ primarily using H₂ and CO₂; and those that are acetotrophic, i.e., use primarily acetate for metabolism that has been formed from previous decomposition activities (Le Mer and Roger, 2001). Most, if not all, known methanogens express an isozyme of methyl-coenzyme M reductase (MRT), of which the gene encoding the α subunit (*mcrA*) is present in most known methanogens (Shively et al., 2001).

Methanotrophs

The most common group of methane consumers is aerobic Methanotrophs (mostly methane oxidizing bacteria or MOB), which are generally found in oxic soils or microsites within anoxic soils. MOB are the only known biological sink for CH₄, as key organisms within a soil microbial consortium that derives energy from CH₄ conversion to carbon dioxide (Hanson and Hanson, 1996). Methanotrophs are a sub-group of the methylotrophs, which also contain methanol oxidizing bacteria (Kolb, 2009). There are 12 recognized genera of methanotrophs that are phylogenetically divided into type I (within the class *Gamma proteobacteria*) and type II (within the class *Alpha proteobacteria*; Mohanty et al., 2006). The key methanotrophic enzyme is CH₄ monooxygenase (MMO), which occurs as both particulate (pMMO) and soluble (sMMO) forms. The *pmoA* gene encodes the α subunit of pMMO, and is included in the genome of all most known methanotrophic species (Dedysh et al., 2000). Methanotrophs are divided into at least two functionally distinct groups, the high affinity group that uses CH₄ at very low concentrations, and the low affinity group that only uses CH₄ at high concentrations (Bender and Conrad, 1992). Most culturable methanotrophs are low affinity, which tend to be located near source environments (Reay et al., 2005). In addition to the more common CH₄ cyclers, a group of methanogen-like anaerobic CH₄ oxidizing archaea (MOA) has been described (Hallam et al., 2003). These MOA contain *mcrA* genes (Hallam et al., 2003) and many are involved in a consortium that couples denitrification with anaerobic CH₄ oxidation (Raghoebarsing et al., 2006).

MICROBIAL COMMUNITY COMPOSITION IMPACTS ON METHANE FLUX

The capacity to produce or consume CH₄ is distributed among relatively few microbial taxa that are phylogenetically distinct (Martiny et al., 2013). The narrow distributions of these traits imply that CH₄ production and consumption rates may be more closely tied to microbial community composition and abundance than other biogeochemical processes (Schimel, 1995). Genes involved in methane-cycling are found in deep-branching microbial clades, similar to other complex microbial traits such as oxygenic photosynthesis and sulfate reduction (Martiny et al., 2013). By contrast, genes involved in heterotrophic processing of

other carbon compounds are not highly conserved, and nearly all microbial taxa contribute to CO₂ production in upland soils.

For methanogenesis, studies have found variation in the strength of the link between community structure and function. In a peat soil microcosms, methogenesis correlated positively with *mcrA* gene expression, which was a better predictor than gene abundance (Freitag and Prosser, 2009). The pathway of methane production shows a clear dependence on microbial composition, with acetoclastic methanogenesis dependent on the *Methanosarcinaceae* and CO₂ reduction driven by groups such as the *Methanobacteriales* and *Methanosaetaceae*. These groups are sensitive to temperature, such that the CO₂/H₂ pathway becomes more dominant at higher temperatures (Fey and Conrad, 2000; Conrad et al., 2009). However, the temperature threshold for dominance varies from 15°C to 40°C across these studies, and both pathways are observed in peat soils with cooler average temperatures (Kotsyurbenko et al., 2004).

Other studies point to a more complex relationship between methane production and methanogen communities. Ramakrishnan et al. (2001) examined biogeographic patterns in methanogen communities across 11 rice field soils and found relatively similar microbial composition despite >10-fold differences in methane production rates. Similarly, Juottonen et al. (2008) observed relatively little change in methanogen abundance and composition across seasons in a boreal mire, but large variations in methane production that were likely due to increased substrate availability during winter. In a Siberian permafrost soil, Ganzert et al. (2007) found a shift from mesophilic to psychrophilic methanogens with depth, but no single group was clearly related to rates of methanogenesis, suggesting a degree of functional redundancy within methanogen communities.

As with methanogen communities, the link to functional rates is also variable for methanotroph communities. Some studies have found tight relationships between methane oxidation rates and community structure, often in the context of environmental change. In a temperate agricultural soil, long-term fertilization with ammonium nitrate reduced methanotroph abundance by >70%, resulting a similar decline in methane oxidation rates (Maxfield et al., 2008; Seghers et al., 2003a) observed a similar pattern that was associated with reductions in the abundance of low-affinity type I methanotrophs. Different groups of methanotrophs may show different responses to fertilization, as observed in rice field and forest soils where type II methanotrophs were more strongly inhibited by mineral N fertilization than type I methanotrophs (Mohanty et al., 2006). In contrast, organic fertilizer addition can increase methanotroph abundance and associated rates of methane oxidation (Seghers et al., 2005).

Gradient studies also suggest that variation in methanotroph abundance can correlate with functional rates. In a pine forest soil, methane oxidation rates across soil horizons were related to the abundance of a single PLFA marker identified with ¹³C stable isotope probing (Bengtson et al., 2009). Using a combination of molecular approaches and ¹³C tracers, Bodelier et al. (2013) found a tight link between methane consumption rates and the abundance of type I methanotrophs across a riparian floodplain. In contrast, studies in New Zealand have shown that

type II methanotrophs are linked to higher methane oxidation rates associated with afforestation and reforestation (Singh et al., 2007; Nazaries et al., 2011). A similar pattern was observed across a broader gradient of vegetation types in Scotland, with increased type II methanotroph abundance, lower overall methanotroph diversity, and increased rates of methane consumption associated with forest vegetation (Nazaries et al., 2013).

Not all studies show such tight relationships between methanotroph communities and methane oxidation. Bárcena et al. (2011) found *pmoA* genes associated with high-affinity methanotrophs in a glacial forefield in Greenland, but detected almost no methane oxidation. Jaatinen et al. (2004) measured increased methane oxidation following boreal forest fire but no associated change in communities of methane-oxidizing bacteria. Conversely, Seghers et al. (2003b) found differences in methanotroph community composition but no substantial difference in methane oxidation in response to chronic herbicide treatment.

Differences in community composition that are not associated with differences in methane-cycling could indicate a degree of functional redundancy among methane-cycling microbes. However, such conclusions could be misleading. In some studies, more direct links between composition and function might have been observed if methanogen or methanotroph abundance had been measured. Studies using group-specific primers can identify within-group shifts in composition but not overall changes in abundance that may be more important for functional rates (Seghers et al., 2003a). For example, Menyailo et al. (2008) found that reductions in methanotroph-derived PLFA markers largely explained a 3-fold reduction in soil methane consumption following reforestation of a Siberian grassland. Despite the overall reduction in biomass, there were no apparent shifts in methanotroph community composition.

In addition, microbes that appear functionally redundant in one environment may show distinct responses when the environment changes. For example, different methanotroph communities may oxidize CH₄ at similar rates in unfertilized soils (Seghers et al., 2003a), but communities dominated by type II methanotrophs could show much steeper declines in CH₄ oxidation in response to N deposition (Mohanty et al., 2006).

Overall, many studies we reviewed support the idea that CH₄ cycling depends on the composition and abundance of relatively narrow microbial groups. In addition, these studies demonstrate that environmental factors are important because they influence microbial communities. The abundances of methane-cycling microbes are often sensitive to environmental conditions such as temperature, precipitation, nutrient availability, CH₄ concentration, and plant species (Fey and Conrad, 2000; Henckel et al., 2000; Horz et al., 2005; Liebner and Wagner, 2007; Maxfield et al., 2008; Tsutsumi et al., 2009). In some cases, these factors impact CH₄ cycling through changes in microbial communities, but in other cases, environmental changes have important direct effects. For example, substrate availability and temperature both affect CH₄ cycling rates, independent of changes in community composition (Wagner et al., 2005; Juottonen et al., 2008). Thus, even if CH₄ cycling depends on narrow groups of methanogens and methanotrophs, the relationship between structure and function

will always be subject to modification by environmental factors (Nazaries et al., 2011). This complexity will require models of the CH₄ cycle that allow for feedbacks between microbial communities and environmental drivers.

ENVIRONMENTAL FACTORS AND THE METHANE CYCLE

There is no ecosystem for which all of the potential direct or indirect effects of environmental variables on CH₄ consumption of soil are understood, but many known interactions are summarized in **Table 1**. Conspicuously absent in **Table 1** are any trends in tropical grasslands or savannahs, as there were no studies available testing environmental effects in these ecosystems to review. In general, the effect of higher soil moisture and precipitation is a decrease in the sink strength of the soil, however as **Table 1** shows, even these impacts are not completely consistent. Other environmental variables that indirectly affect CH₄ flux due to their influence on soil moisture and oxygen content are aspect and catena position, position on slope, soil type, and water holding capacity. Due to varying microbial preferences in terms of optimal pH, there is also some variation in response of CH₄ flux to varying pH in the soil. Few general studies of distribution and activity of soil microbes as a whole have been done across catenas, slopes, or soil types, and many of those that have been done have not included methanotrophic or methanogenic organisms (Florinsky et al., 2004).

METHANE FLUX RESPONSES TO INCREASED METHANE CONCENTRATIONS

Although the average mixing ratio of CH₄ at the Earth's surface has risen from around 0.7 ppm during pre-industrial times to about 1.8 currently, there has been little direct study of the impacts of rising atmospheric CH₄ on the rate of consumption of CH₄ by upland soils. Bender and Conrad (1992) determined that there were two kinetic optima for methanotrophy. There was a

clear increase in the consumption of CH₄ by the soil with increasing CH₄ concentrations, indicating that the reaction is methane-limited at atmospheric oxygen levels (Bender and Conrad, 1992). However, they did not test consumption at CH₄ concentrations between 2 and 6 ppm, since this range is thought to fall between the two V_{max} values for methanotrophy. Yet, this range might be relevant for soil CH₄ consumption rates under global change. Most other investigations of methanotrophy responses to CH₄ concentration have used high concentrations, focused either on determining kinetic or potential rates of methanotrophy (Henckel et al., 2000; Tuomivirta et al., 2009; Tate et al., 2012).

Recently, one study showed that levels of CH₄ only slightly elevated above ambient can lead to markedly increased CH₄ consumption. Irvine et al. (2012) observed a strong direct relationship between ambient CH₄ concentrations at the start of CH₄ flux measurement and the rate of consumption in salt marsh soils. This result could indicate that increases in average ambient CH₄ concentrations will lead to a measurable increase in atmospheric CH₄ consumption across soils.

METHANE FLUX RESPONSES TO INCREASED CO₂ CONCENTRATIONS

Increases in CO₂ can lead to increased methanogeny, both indirectly through greater biomass production increasing acetotrophic metabolism, and directly from CO₂ stimulating hydrogenotrophic metabolism. In wetland areas the increased plant production due to elevated CO₂ leads to greater CH₄ release, likely due to acetotrophic metabolism (Dacey et al., 1994). Experiments in rice system soils have overwhelmingly agreed with these results (Ziska et al., 1998; Groot et al., 2003; Cheng et al., 2006). Whole soil and plant-facilitated emission of CH₄ increased up to 69% in a wetland glasshouse experiment with elevated CO₂ (Vann and Megonigal, 2003). However, plant facilitation may not add to this increase at all, as emissions facilitated by transport through wetland plants were not found to be changed

Table 1 | Summary of the impact of major environmental characteristics on methane uptake by soil.

Ecosystem/Biome	H ₂ O Content	Precipitation	Position on slope	pH
Boreal forest	low > high ¹ high > low ²	low > high ³	high > low ⁴ low > high ⁵	ND ⁶
Boreal Steppe/Tundra	NR	low > high ⁷	low > high ⁸	high > low ⁹
Temperate forest	low > high ¹⁰ ND ¹¹	low > high ¹² ND ¹³	high > low ¹⁴ low > high ¹⁵	high > low ¹⁶ low > high ¹⁷
Temperate grassland	low > high ¹⁸	low > high ¹⁹	high > low ²⁰ ND ²¹	NR
Tropical forest	low > high ²²	low > high ²³ high > low ²⁴	high/flat > low ²⁵ low > high ²⁶	high > low ²⁷
Shrubland/Desert	high > low ²⁸	low > high ²⁹ ND ³⁰	NR	high > low ³¹

High and low refer to the variables in the column headers.

does not include agricultural systems except tree plantations; NR indicates that there were no studies located reporting on the indicated effect in that ecosystem/biome; ND indicates those studies that found no difference in CH₄ flux with different values for that variable.

¹Adamsen et al., 1993; Borken and Beese, 2006, ²Ambus and Christensen, 1995; van Huissteden et al., 2008, ³Bowling et al., 2009; Koide et al., 2010, ⁴Borken et al., 2003, ⁵Sjogersten and Wookey, 2002; Borken et al., 2003, ⁶McNamara et al., 2008, ⁷West et al., 1999; Mariko et al., 2007, ⁸Sjogersten and Wookey, 2002, ⁹Menyailo et al., 2008, ¹⁰Castro et al., 1994, 1995; Klemmedtsson and Klemmedtsson, 1997; Prieme et al., 1997; Butterbach-Bahl and Papen, 2002; McLain et al., 2002; Borken et al., 2006; Rosenkranz et al., 2006; Aronson et al., 2012, ¹¹Prieme et al., 1997; Groffman et al., 2006, ¹²Castro et al., 1994; Bradford et al., 2000; Blankinship et al., 2010a; Xu and Luo, 2012, ¹³Borken et al., 2006, ¹⁴Castro et al., 1993; Hart, 2006, ¹⁵Yavitt et al., 1990, ¹⁶Born et al., 1990; Brumme and Borken, 1999, ¹⁷Sitaula et al., 1995; Prieme et al., 1997; Kolb et al., 2005, ¹⁸Neff et al., 1994; van den Pol-van Dasselaar et al., 1998, ¹⁹Blankinship et al., 2010b, ²⁰Mosier et al., 1991; Torn and Harte, 1996; Mosier et al., 1997a,b, ²¹Brady and Weil, 1999; Chen et al., 2011 ²²Keller et al., 1990; Jauhainen et al., 2005; Teh et al., 2005; Konda et al., 2010, ²³Werner et al., 2006, ²⁴Davidson et al., 2004, ²⁵Delmas et al., 1992; Singh et al., 1997; Verchot et al., 2000; Wolf et al., 2012, ²⁶Silver et al., 1999, ²⁷King and Nanba, 2008, ²⁸Angel and Conrad, 2009, ²⁹Anderson and Poth, 1998; Galbally et al., 2010; Hou et al., 2012, ³⁰Blankinship et al., 2010a, ³¹Angel and Conrad, 2009.

by increased CO₂ in a free-air CO₂ enrichment (FACE) experiment (Baggs and Blum, 2004).

Though not as widely studied in non-wetland ecosystems, a similar trend was observed in two FACE studies performed in temperate forests, where heightened CO₂ exposure resulted in an overall annual decrease in CH₄ uptake of up to 30% (Phillips et al., 2001) and 25% (McLain et al., 2002). Another FACE study in a temperate grassland also showed decreased consumption with elevated CO₂ (Ineson et al., 1998). It was hypothesized that these shifts were due to stimulation of methanogenesis by increased soil moisture in the lower soil layers (McLain et al., 2002; McLain and Ahmann, 2008; Dubbs and Whalen, 2010). However, elevated CO₂ caused decreased overall bacterial counts and *pmoA* abundances (by qPCR and FISH) in a meadow soil (Kolb et al., 2005), indicating direct negative impacts on methanotrophy. Some studies have contradicted this trend, such as an open top chamber experiment in a shortgrass steppe, which showed a slight increase in net CH₄ uptake that was not significant (Mosier et al., 2002). Similarly, elevated CO₂ increased CH₄ consumption in a grassland greenhouse study (Dijkstra et al., 2010). More analysis of the impact of elevated CO₂ on CH₄ flux in non-wetland terrestrial systems is needed before definitive conclusions can be drawn, specifically in the presence of other predicted global changes, such as warming.

SOIL MOISTURE

Studies of precipitation and soil moisture content show correlations between wetter sites and decreased CH₄ uptake or increased release (see Table 1), which is due in large part to the disparate environmental requirements of methanotrophs and methanogens. Throughfall exclusion in the Amazon basin caused CH₄ consumption to more than quadruple compared to plots receiving natural precipitation levels (Davidson et al., 2004). Many studies have found that increased soil moisture content negatively influences CH₄ consumption in ecosystems ranging from boreal, temperate, and tropical forests to shortgrass steppe, temperate farmland, and tundra (Adamsen and King, 1993; Castro et al., 1994; Klemmedtsson and Klemmedtsson, 1997; Epstein et al., 1998; Burke et al., 1999; West et al., 1999; McLain et al., 2002; Mosier et al., 2002).

However, there are intricacies that this generalization does not address. A dry tropical forest study showed that in the rainy season, CH₄ consumption was inversely related to water content and precipitation (Singh et al., 1997). In the dry season, the trend was reversed, likely because all microbial activities are decreased, and the input of rain to severely dry soil leads to an increase in microbial activity, including methanotrophy. Boreal forest sites without peat show no significant difference in CH₄ fluxes between inundated and dry soils. However, inundated peat soils released significantly more CH₄ than dry peat soils from the boreal forest (Oelbermann and Schiff, 2010), indicating a vital role of water holding capacity of soil and surrounding vegetation.

Position in landscape, aspect, and catena

Factors such as position in the landscape, aspect, and catena impact CH₄ flux indirectly, due to their impact on soil moisture

retention. A mixed shrub, herb, and tree community showed higher CH₄ consumption on North facing slopes (Burke et al., 1999). In a tundra study the results were mixed, with low snowmelt areas with high wind showing higher CH₄ consumption on the North facing slope and areas with more snowmelt and protection having lower consumption on North facing slopes (West et al., 1999). A study in the boreal forest, using many different measures of CH₄ flux and different tree communities showed that CH₄ consumption was consistently greater on South facing slopes (Whalen et al., 1992). South facing slopes may have higher rates of evaporation than North facing slopes in the Northern Hemisphere, where all of these studies were located. This difference should lead to higher CH₄ consumption on South facing slopes for more saturated soils, with the opposite effect for low water content soils, which does explain the mixed results seen in West et al. (1999). However, other factors may impact the effect of slope aspect, such as whether one slope receives higher precipitation due to orographic effects, as is known to occur in the Rocky Mountains of North America.

The impact of slope position is more variable, and more complete information is summarized in Table 1. For example, in the rainy season, dry tropical forest showed decreased CH₄ uptake with low position on slope, with no trend in the dry season (Singh et al., 1997), which was also seen in boreal forest stands (Gulledge and Schimel, 2000). This result is likely due to prolonged increases in soil water content corresponding to poor drainage conditions and lower exposure to evaporation at low slope positions relative to hilltops. In Puerto Rican rainforest, the higher cloud forests release copious amounts of CH₄, compared to the lower Tabanuco and Colorado forests which consume and release small amounts of CH₄, respectively (Silver et al., 1999).

Soil type

Soil type exerts strong controls on the water holding capacity of soil, as well as the diffusion of gases into soil, both of which lead to pronounced effects on CH₄ flux. Sandy soil (soil with larger particle size) has the lowest water holding capacity, followed by loam and then clay (Brady and Weil, 1999). The sand content of temperate grassland has been correlated with CH₄ consumption rates, with sandy soil consuming more CH₄ than loam, which in turn consumed more than clay (Born et al., 1990). Across terrestrial ecosystems, a recent meta-analysis performed by Dutaur and Verchot (2007) found that soil texture was one of the main factors correlated with CH₄ fluxes, with coarser and medium-textured (loam) soils consuming more CH₄ than fine (clay) soils (Dutaur and Verchot, 2007). Due to this recent meta-analysis, further discussion of the impact of soil type is limited in this review.

SOIL TEMPERATURE

The methane-cycling microorganism response to temperature varies more than the response to changes in soil moisture. Insofar as temperature can lead to greater evapotranspiration, it may lead to decreased soil moisture, which would increase CH₄ consumption. This trend was seen in multiple studies in temperate and boreal forests, which have found that higher observed soil

temperatures correlate with greater uptake rates of CH_4 (Castro et al., 1995; Klemetsson and Klemetsson, 1997; Bradford et al., 2001; Butterbach-Bahl and Papen, 2002; Rosenkranz et al., 2006). However, the enzymes involved in CH_4 oxidation have variable optimum temperatures, with the average optimum temperature at 25°C (Hanson and Hanson, 1996). The enzymes involved in the degradation of organic matter that eventually results in methanogenesis have optima between 30 and 40°C (Le Mer and Roger, 2001). Similarly, temperature and precipitation have been shown to change the standing and ephemeral microbial community structure (Pritchard and Rogers, 2000), with varied consequences. A soil warming study using infrared heating, a method that provides a good approximation of future global warming (Aronson and McNulty, 2009), found that with increases in growing season temperature of up to 4.1°C there was no change in the CH_4 flux of bog and fen mesocosms (Updegraff et al., 2001). However, higher temperatures (21°C vs. 14°C) caused significantly greater CH_4 release from inundated peat soils from the boreal forest (Oelbermann and Schiff, 2010). Results were similar in a soil warming study within a grassland system, with increased heating causing lower CH_4 uptake rates (Christensen et al., 1997).

NITROGEN AND FERTILIZER IN THE METHANE CYCLE

Global inorganic N input to non-wetland ecosystems from deposition, industry, and fertilizer use is projected to double from the 1990 levels by the year 2050 (Kroeze and Seitzinger, 1998). The effects of N on CH_4 uptake in the soil environment are more complex than other environmental variables. Compared to natural forest and grassland, cropland and pasture consume less CH_4 and show greater decreases in CH_4 consumption rates with increased nitrogen additions (Aronson and Helliker, 2010). In general, the conversion of native lands to row-crop agriculture has been found to lead to a seven-fold reduction in both methanotroph diversity and CH_4 consumption (Levine et al., 2011). The genetics and enzyme kinetics behind CH_4 oxidation show tight evolutionary and functional linkages between the enzymes that enable CH_4 and ammonia oxidation (Dunfield and Knowles, 1995). Methanotrophs and ammonia oxidizers are capable of switching substrates, which is a mechanism believed to be responsible for the inhibition of CH_4 uptake by soil exposed to high concentrations of ammonia (Hanson and Hanson, 1996). In a rice paddy soil, CH_4 oxidation and nitrification (i.e., ammonia oxidation) were inversely related in the presence of high N (Alam and Jia, 2012). In a wetland study by Baggs and Blum (2004), emissions facilitated by transport through plants were doubled with a four-fold increase in N deposition. However, laboratory experiments at elevated levels of ammonium showed that the inhibition of CH_4 oxidation did not correspond to a shift in methanotroph communities (Bykova et al., 2007).

Methanotrophs demonstrate N limitation of CH_4 uptake at low concentrations of available nitrogen relative to available CH_4 in both N-limited wetlands (Bodelier et al., 2000) and upland soils (Aronson et al., 2012). A potential mechanism for this observed stimulation of CH_4 oxidation with added inorganic N, in N-limited systems, was proposed by

Bodelier and Laanbroek (2004) to be the N-fixation pathway found in a subset of methanotrophs, specifically the nitrigenase pathway found in types II and X methanotrophs (Hanson and Hanson, 1996). Type X methanotrophs are closely related to type I, but share some metabolic similarities with type II (Macalady et al., 2002). Thus, it has been put forward that in N-limited conditions, methanotrophy is limited by the energy requirement of N fixation (Henckel et al., 2000). Evidence for stimulation of methanotrophy by addition of low levels of inorganic N has been found in some non-wetland terrestrial systems (Aronson and Helliker, 2010). In general, soil drainage condition may indicate whether N stimulates methanotrophy, inhibits it, or does not impact the CH_4 cycle at all (Aronson et al., 2013).

SOIL pH

Methanotrophs are more sensitive to acidic environments than are methanogens, although they are more tolerant of variations in pH through time (Le Mer and Roger, 2001). With the exception of variable responses to pH in the temperate forest, there was a general trend of increasing CH_4 consumption with higher pH (Table 1). There was also no clear trend in the boreal forest studied (McNamara et al., 2008).

ECOSYSTEM AND VEGETATION EFFECTS ON METHANE UPTAKE

We conducted a meta-analysis to determine ecosystem and vegetation impacts on CH_4 uptake in upland soils (methods in Appendix A, database in Appendix B). Across the ecosystems included in our meta-analysis, there exists a high variability in CH_4 flux by ecosystem type (Figure 2). The One-Way ANOVA performed across studies by ecosystem type found that there was a significant difference between ecosystem types ($p < 0.031$). Means comparisons using Student's *t* revealed that forests and grasslands consumed more CH_4 than tundra, with the other

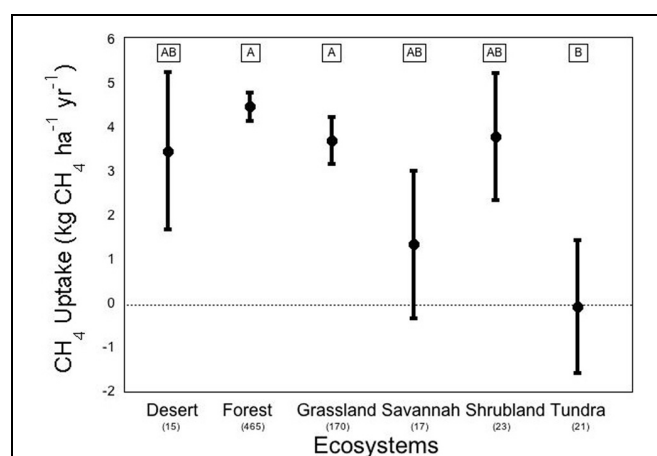


FIGURE 2 | Methane flux by ecosystem. Negative numbers indicate net release of methane by the soil. Averages are expressed bounded by standard errors of the means. The number of studies included in each average is listed in parentheses under each ecosystem type. Means with the same letter are not significantly different (Student's *t*-test).

ecosystems not different from each other. In addition, vegetation type (Figure 3), was significant by ANOVA ($p < 0.044$). Means comparisons showed that tundra, which released methane on average, differed significantly from all other vegetation types, which consumed methane.

On average, forest systems show the greatest CH_4 consumption capability of any ecosystem, at an average of about $-4.50 \pm 0.32 \text{ kg ha}^{-1} \text{ yr}^{-1}$. The variation between forest observations is great, even though the standard error is relatively low, due to the fact that the number of studies included in the database from forests is an order of magnitude greater than most other ecosystems. This rate can be much higher; a study of a New Zealand pine forest found an overall uptake of CH_4 at an annual rate of $-12.1 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Tate et al., 2006). At the extreme end, an early CH_4 uptake study in a British mixed-temperate forest on a single day found an uptake rate that would scale to $-165 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Willison et al., 1995). But not all forests consume CH_4 overall; a study of the CH_4 budget of a black spruce forest in Germany found an average CH_4 release of $54.5 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Fiedler et al., 2005). Tundra ecosystems (including “alpine” and “subarctic” tundra) on average were found to release CH_4 at a rate of $0.035 \text{ kg ha}^{-1} \text{ yr}^{-1}$. Tundra also displayed extremely high variation in uptake rates across various environmental conditions, which may be due to ebullition; the release of large amounts of CH_4 in bubbles from clathrate associations deep below the soil or water column (Shakhova et al., 2010). Vegetation height has also been found to be a good indicator of CH_4 release in varied wet tundra sites (von Fischer et al., 2010). Deserts displayed the greatest variation, with mean \pm standard error of desert flux found to be $3.49 \pm 1.79 \text{ kg ha}^{-1} \text{ yr}^{-1}$ across 9 studies, which may be due to more extreme responses to precipitation pulses. Alternately, this variation may be due fact that deserts over natural gas deposits have been shown to be CH_4 sources (Etiope and Klusman, 2010).

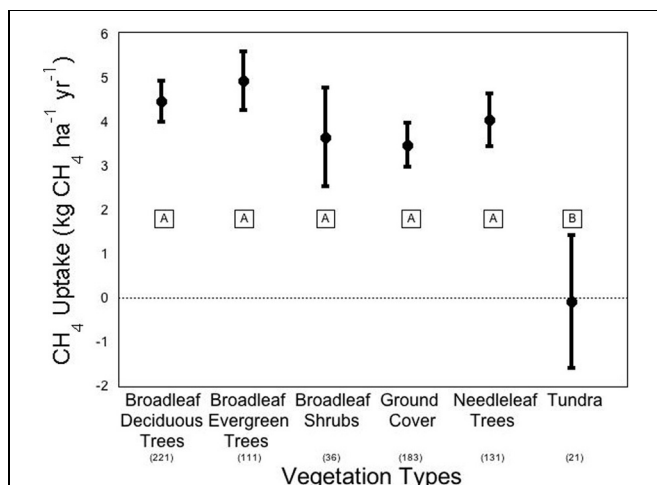


FIGURE 3 | Methane flux by vegetation types. Negative numbers indicate net release of methane by the soil. Averages are expressed bounded by standard errors of the means. The number of studies included in each average is listed in parentheses under each vegetation type. Means with the same letter are not significantly different (Student's t -test).

VEGETATION EFFECTS

Robust differences in CH_4 fluxes appear when separated by vegetation type (Figure 3; ANOVA $p = 0.009$). Individual plant species effects on CH_4 flux can be substantial, but most effects have been reported in wetland species. The most common species effects occur in some wetland plants that facilitate CH_4 entering and leaving the soil or sediment. For an example with the sedge plant type/functional type, there is a clear difference between *Carex scopulorum*, which allows the emission of CH_4 , and *Kobresia myosuroides*, which allowed the consumption of CH_4 (West et al., 1999). Confounding may frequently emerge in most experiments that report on the plant species and functional type causes of uptake because the effects of plant species are difficult to tease apart from the effects of environmental variables, which may in turn predict plant species colonization. For example, in West et al. (1999), the variation in amount of snowmelt received during the snow-free months in the alpine tundra predicted plant species dominance differences. The CH_4 uptake rate in these sites varied, but whether the variation was due to a species or environmental effect is ambiguous (West et al., 1999).

Generally when plant effects are observed, it is not specific species but plant functional type differences that are of interest, with the soil around trees associated with higher CH_4 consumption than shrubs, grasses, and sedges. Across studies, deciduous forests have higher CH_4 uptake rates than do coniferous forests (Degelmann et al., 2010), which is likely related to pH impacts. In the meta-analysis, we found broadleaf deciduous trees to consume $-4.51 \text{ kg CH}_4 \text{ ha}^{-1} \text{ yr}^{-1}$ compared to $-4.08 \text{ kg CH}_4 \text{ ha}^{-1} \text{ yr}^{-1}$ in needleleaf trees, however, this difference was not significant (Figure 3). There was also one study that directly tested the impact of tree proximity on CH_4 uptake rate and found that there is greater net uptake by soils that are closer to deciduous trees and further from coniferous trees (Butterbach-Bahl et al., 2002). There has also been an observed effect of grass functional diversity on CH_4 uptake in shortgrass steppe soils (Epstein et al., 1998). In clay soils, a mixture of C_3 and C_4 grasses appeared to consume more CH_4 than either grass type alone, though these results were not significant at the 5% level. In sandy clay soils, a different effect was observed with C_4 plants significantly increasing uptake of CH_4 compared to C_3 . Mixed grasses fell between the grass types and did not differ significantly from either C_3 or C_4 uptake (Epstein et al., 1998).

DISTURBANCE, BURNING, AND PLANT SUCCESSION

There has been limited study of the impacts of burning, grazing, plant removal, and other disturbances on CH_4 uptake by soils. There are no clear trends in a handful of studies on the effects of burning on CH_4 flux performed across multiple ecosystems. In tropical forests and temperate grasslands, burning increased consumption of CH_4 (Tate and Striegl, 1993; Poth et al., 1995). Burning results in vegetative cover removal that could increase the sunlight reaching the soil, therefore allowing for a lower water filled pore space and more consumption of CH_4 . However, in tropical savannas the impact of burning was decreased consumption (Prieme and Christensen, 1999). In boreal forests and Mediterranean shrublands, the response to fire was mixed or there

was no change at all (Gulledge et al., 1997; Anderson and Poth, 1998; Castaldi and Fierro, 2005).

The impact of non-fire vegetative removal has also been mixed across ecosystems. Grazing has been shown to increase CH₄ uptake in the boreal steppe (Geng et al., 2010). In temperate and tropical grasslands grazing generally decreased consumption (Zhou et al., 2008; Chen et al., 2010, 2011; Wang et al., 2012). Clipping was found to increase CH₄ consumption in tropical savannah (Sanhueza and Donoso, 2006). Thinning of the trees decreased CH₄ consumption in one temperate forest (Dannenmann et al., 2007), but not another (Wu et al., 2011). Clear-cutting reduced consumption in the boreal forest (Saari et al., 2004) and temperate forest (Wu et al., 2011).

Changes in CH₄ consumption are often observed during ecological succession following disturbance. Within forests, the climax (i.e., virgin or old-growth) vegetation is most often found to consume more CH₄ than early successional stages. This trend was found in two temperate forest studies of deciduous (Hudgens and Yavitt, 1997) and mixed deciduous and coniferous stands of various ages since disturbance (Brumme and Borken, 1999). Within tropical forests, old-growth forest was found to consume more CH₄ (Keller and Reiners, 1994; Verchot et al., 2000; Veldkamp et al., 2008; Zhang et al., 2008). MacDonald et al. (1999) had mixed results and MacDonald et al. (1998) and Goreau and Mello (1985) found that secondary forest consumed more CH₄ than old-growth forest. (Kruse and Iversen, 1995) found that in temperate grasslands, post-plow secondary growth soils consumed more CH₄ than both bare plowed soil and natural heathland. They also found that oaks invading the grassland consumed resulted in more CH₄ consumption than the nature heathland or secondary grasses, and that old-growth and established oak stands consumed even more CH₄ (Kruse and Iversen, 1995). In Mediterranean shrublands, old growth shrubs consumed more CH₄ than early and mid-succession (Price et al., 2010).

CONCLUSIONS

Methane-cycling microorganisms in soils have the potential to impact the atmospheric composition of the Earth. As a narrow process, we found the composition of the microbial communities responsible for CH₄ consumption and production have been linked to corresponding process rates in nature, as was proposed by Schimel and Gulledge (1998). We hypothesized that net CH₄ flux would be correlated with the abundance and/or composition of methane-cycling microbes. In fact we found prolific, although not entirely consistent, evidence that the impacts of environmental and climate drivers on net CH₄ flux are the result of changes in the methane-cycling microbial community.

REFERENCES

- Adamsen, A. P. S., and King, G. M. (1993). Methane consumption in temperate and sub-arctic forest soils – rates, vertical zonation, and responses to water and nitrogen. *Appl. Environ. Microbiol.* 59, 485–490.
- Alam, M. S., and Jia, Z. (2012). Inhibition of methane oxidation by nitrogenous fertilizers in a paddy soil. *Front. Microbiol.* 3:246. doi: 10.3389/fmicb.2012.00246
- Ambus, P., and Christensen, S. (1995). Spatial and seasonal nitrous oxide and methane fluxes in Danish forest-ecosystems, grassland-ecosystems, and agroecosystems. *J. Environ. Qual.* 24, 993–1001. doi: 10.2134/jeq1995.00472425002400050031x
- Anderson, I. C., and Poth, M. A. (1998). Controls on fluxes of trace gases from Brazilian cerrado soils. *J. Environ. Qual.* 27, 1117–1124. doi: 10.2134/jeq1998.00472425002700050017x
- Angel, R., and Conrad, R. (2009). *In situ* measurement of methane fluxes and analysis of transcribed particulate methane monooxygenase in desert soils. *Environ. Microbiol.* 11, 2598–2610. doi: 10.1111/j.1462-2920.2009.01984.x
- Aronson, E. L., Dubinsky, E. A., and Helliher, B. R. (2013). Effects of nitrogen addition on soil microbial diversity and methane cycling capacity depend on drainage conditions in a pine forest soil. *Soil Biol. Biochem.* 62, 119–128. doi: 10.1016/j.soilbio.2013.03.005

However, we found fewer studies that linked these changes to overall abundance of methanotrophs and/or methanogens, or specific phylogenetic lineages within these groups. This is an area of study ripe for investigation, and we believe that coupled with the knowledge of the impact of shifts in community composition, this data on abundance could complete the picture of the role of microorganisms in the global CH₄ cycle.

Combined with information on microbial community impacts on CH₄ flux, the dataset created for this review can assist in future modeling efforts. In particular, it demonstrates relationships between environmental and climatic changes, methane-cycling microbial communities, and soil CH₄ fluxes. Process-based and ecosystem-specific models of CH₄ flux are necessary to predict ecosystem CH₄ fluxes in response to environmental and climatic changes. In order to create these models, certain ecosystems deserve further study, either because they consume large amounts of CH₄ or because they are understudied. In particular, attention should be focused tropical grasslands and savannahs. Secondly, some attention should be paid to the impact of pH in boreal forest and soil moisture content in boreal steppe/tundra, as well as the impacts of temperature across the boreal landscape, as research on these topics is lacking and most warming is expected to occur in high latitudes where these ecosystems are prevalent (IPCC, 2007).

Finally, it is important to decrease the uncertainty regarding CH₄ sources and sinks in order to improve predictions of future global warming. We now have the tools necessary to answer questions about recent fluctuations in the CH₄ growth rate in the atmosphere and predict the CH₄ budget. The increasing use of eddy covariance techniques for regional scale estimates of CH₄ fluxes can assist these global inventories, but should be paired with chamber-based flux measurements to account for the effects of environmental variation. Small-scale process-based models, global inventories, and global inverse models have all approached this issue with limited success. The next generation of models must use process-based and microbial community knowledge to account for seasonal and inter-annual variation in global CH₄ budgets.

ACKNOWLEDGMENTS

The authors would like to thank the Air and Waste Management Association's Air Pollution Education and Research Grant, the NASA Graduate Student Researchers Program, as well as the NOAA Climate and Global Change Postdoctoral Fellowship for supporting this research.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/Terrestrial_Microbiology/10.3389/fmicb.2013.00225/abstract

- Aronson, E. L., and Helliker, B. R. (2010). Methane flux in non-wetland soils in response to nitrogen addition: a meta-analysis. *Ecology* 91, 3242–3251. doi: 10.1890/09-2185.1
- Aronson, E. L., and McNulty, S. G. (2009). Appropriate experimental ecosystem warming methods by ecosystem, objective, and practicality. *Agricult. Forest Meteorol.* 149, 1791–1799. doi: 10.1016/j.agrformet.2009.06.007
- Aronson, E. L., Vann, D. R., and Helliker, B. R. (2012). Methane flux response to nitrogen amendment in an upland pine forest soil and riparian zone. *J. Geophys. Res.* 117, G03012. doi: 10.1029/2012JG001962
- Aydin, M., Verhulst, K. R., Saltzman, E. S., Battle, M. O., Montzka, S. A., Blake, D. R., et al. (2011). Recent decreases in fossil-fuel emissions of ethane and methane derived from firn air. *Nature* 476, 198–201. doi: 10.1038/nature10352
- Baggs, E. M., and Blum, H. (2004). CH₄ oxidation and emissions of CH₄ and N₂O from Lolium perenne swards under elevated atmospheric CO₂. *Soil Biol. Biochem.* 36, 713–723. doi: 10.1016/j.soilbio.2004.01.008
- Bárcena, T. G., Finster, K. W., and Yde, J. C. (2011). Spatial patterns of soil development, methane oxidation, and methanotrophic diversity along a receding glacier forefield, south-east greenland. *Arct. Antarct. Alp. Res.* 43, 178–188. doi: 10.1657/1938-4246.43.2.178
- Bender, M., and Conrad, R. (1992). Kinetics of CH₄ oxidation in oxic soils exposed to ambient air or high CH₄ mixing ratios. *FEMS Microbiol. Ecol.* 101, 261–270. doi: 10.1016/0168-6496(92)90043-S
- Bengtson, P., Basiliko, N., Dumont, M. G., Hills, M., Murrell, J. C., Roy, R., et al. (2009). Links between methanotroph community composition and CH₄ oxidation in a pine forest soil. *FEMS Microbiol. Ecol.* 70, 356–366. doi: 10.1111/j.1574-6941.2009.00751.x
- Blankinship, J. C., Brown, J. R., Dijkstra, P., Allwright, M. C., and Hungate, B. A. (2010a). Response of terrestrial CH₄ uptake to interactive changes in precipitation and temperature along a climatic gradient. *Ecosystems* 13, 1157–1170. doi: 10.1007/s10021-010-9391-9
- Blankinship, J. C., Brown, J. R., Dijkstra, P., and Hungate, B. A. (2010b). Effects of interactive global changes on methane uptake in an annual grassland. *J. Geophys. Res.* 115, G02008. doi:10.1029/2009JG001097
- Bodelier, P. L. E., and Laanbroek, H. J. (2004). Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiol. Ecol.* 47, 265–277. doi: 10.1016/S0168-6496(03)00304-0
- Bodelier, P. L. E., Meima-Franke, M., Hordijk, C. A., Steenbergh, A. K., Hefting, M. M., Bodrossy, L., et al. (2013). Microbial minorities modulate methane consumption through niche partitioning. *ISME J.* 2013, 1–15.
- Bodelier, P. L. E., Roslev, P., Henckel, T., and Frenzel, P. (2000). Stimulation by ammonium-based fertilizers of methane oxidation in soil around rice roots. *Nature* 403, 421–424. doi: 10.1038/35000193
- Borken, W., and Beese, F. (2006). Methane and nitrous oxide fluxes of soils in pure and mixed stands of European beech and Norway spruce. *Eur. J. Soil Sci.* 57, 617–625. doi: 10.1111/j.1365-2389.2005.00752.x
- Borken, W., Davidson, E. A., Savage, K., Sundquist, E. T., and Steudler, P. (2006). Effect of summer through-fall exclusion, summer drought, and winter snow cover on methane fluxes in a temperate forest soil. *Soil Biol. Biochem.* 38, 1388–1395. doi: 10.1016/j.soilbio.2005.10.011
- Borken, W., Xu, Y. J., and Beese, F. (2003). Conversion of hardwood forests to spruce and pine plantations strongly reduced soil methane sink in Germany. *Glob. Change Biol.* 9, 956–966. doi: 10.1046/j.1365-2486.2003.00631.x
- Born, M., Dorr, H., and Levin, I. (1990). Methane consumption in aerated soils of the temperate zone. *Tellus* 42, 2–8. doi: 10.1034/j.1600-0889.1990.00002.x
- Bousquet, P., Ciais, P., Miller, J. B., Dlugokencky, E. J., Hauglustaine, D. A., Prigent, C., et al. (2006). Contribution of anthropogenic and natural sources to atmospheric methane variability. *Nature* 443, 439–443. doi: 10.1038/nature05132
- Bowling, D. R., Miller, J. B., Rhodes, M. E., Burns, S. P., Monson, R. K., and Baer, D. (2009). Soil, plant, and transport influences on methane in a subalpine forest under high ultraviolet irradiance. *Biogeosciences* 6, 1311–1324. doi: 10.5194/bg-6-1311-2009
- Bradford, M. A., Ineson, P., Wookey, P. A., and Lappin-Scott, H. M. (2000). Soil CH₄ oxidation: response to forest clearcutting and thinning. *Soil Biol. Biochem.* 32, 1035–1038. doi: 10.1016/S0038-0717(00)00007-9
- Bradford, M. A., Wookey, P. A., Ineson, P., and Lappin-Scott, H. M. (2001). Controlling factors and effects of chronic nitrogen and sulphur deposition on methane oxidation in a temperate forest soil. *Soil Biol. Biochem.* 33, 93–102. doi: 10.1016/S0038-0717(00)00118-8
- Brady, N. C., and Weil, R. R. (1999). *The Nature and Properties of Soils, 12th Edn.* Toronto, ON: Macmillan.
- Brumme, R., and Borken, W. (1999). Site variation in methane oxidation as affected by atmospheric deposition and type of temperate forest ecosystem. *Global Biogeochem. Cycles* 13, 493–501. doi: 10.1029/1998GB000017
- Brummell, M. E., Farrell, R. E., and Siciliano, S. D. (2012). Greenhouse gas soil production and surface fluxes at a high arctic polar oasis. *Soil Biol. Biochem.* 52, 1–12.
- Burke, R. A., Meyer, J. L., Cruse, J. M., Birkhead, K. M., and Paul, M. J. (1999). Soil-atmosphere exchange of methane in adjacent cultivated and floodplain forest soils. *J. Geophys. Res. Atmos.* 104, 8161–8171. doi: 10.1029/1999JD900015
- Butterbach-Bahl, K., and Papen, H. (2002). Four years continuous record of CH₄-exchange between the atmosphere and untreated and limed soil of a N-saturated spruce and beech forest ecosystem in Germany. *Plant Soil* 240, 77–90. doi: 10.1023/A:1015856617553
- Butterbach-Bahl, K., Rothe, A., and Papen, H. (2002). Effect of tree distance on N₂O and CH₄-fluxes from soils in temperate forest ecosystems. *Plant Soil* 240, 91–103. doi: 10.1023/A:1015828701885
- Bykova, S., Boeckx, P., Kravchenko, I., Galchenko, V., and Cleemput, O. V. (2007). Response of CH₄ oxidation and methanotrophic diversity to NH₄ and CH₄ mixing ratios. *Biol. Fertil. Soils* 43, 341–348. doi: 10.1007/s00374-006-0114-5
- Castaldi, S., and Fierro, A. (2005). Soil-atmosphere methane exchange in undisturbed and burned Mediterranean shrubland of southern Italy. *Ecosystems* 8, 182–190. doi: 10.1007/s10021-004-0093-z
- Castro, M. S., Melillo, J. M., Steudler, P. A., and Chapman, J. W. (1994). Soil-moisture as a predictor of methane uptake by temperate forest soils. *Can. J. For. Res.* 24, 1805–1810. doi: 10.1139/x94-233
- Castro, M. S., Steudler, P. A., Melillo, J. M., Aber, J. D., and Bowden, R. D. (1995). Factors controlling atmospheric methane consumption by temperate forest soils. *Glob. Biogeochem. Cycles* 9, 1–10. doi: 10.1029/94GB02651
- Castro, M. S., Steudler, P. A., Melillo, J. M., Aber, J. D., and Millham, S. (1993). Exchange of N₂O and CH₄ between the atmosphere and soils in Spruce-Fir forests in the Northeastern United-States. *Biogeochemistry* 18, 119–135. doi: 10.1007/BF00003273
- Chen, W., Wolf, B., Zheng, X., Yao, Z., Butterbach-Bahl, K., Brüggemann, N., et al. (2011). Annual methane uptake by temperate semiarid steppes as regulated by stocking rates, aboveground plant biomass and topsoil air permeability. *Glob. Change Biol.* 17, 2803–2816. doi: 10.1111/j.1365-2486.2011.02444.x
- Chen, W. W., Wolf, B., Yao, Z. S., Brüggemann, N., Butterbach-Bahl, K., Liu, C. Y., et al. (2010). Annual methane uptake by typical semiarid steppe in Inner Mongolia. *J. Geophys. Res. Atmos.* 115, D15108.
- Cheng, W. G., Yagi, K., Sakai, H., and Kobayashi, K. (2006). Effects of elevated atmospheric CO₂ concentrations on CH₄ and N₂O emission from rice soil: An experiment in controlled-environment chambers. *Biogeochemistry* 77, 351–373. doi: 10.1007/s10533-005-1534-2
- Christensen, T. R., Ekberg, A., Strom, L., Mastepanov, M., Panikov, N., Oquist, M., et al. (2003). Factors controlling large scale variations in methane emissions from wetlands. *Geophys. Res. Lett.* 30. doi: 10.1029/2002GL016848
- Christensen, T. R., Michelsen, A., Jonasson, S., and Schmidt, I. K. (1997). Carbon dioxide and methane exchange of a subarctic heath in response to climate change related environmental manipulations. *Oikos* 79, 34–44. doi: 10.2307/3546087
- Conrad, R. (2009). The global methane cycle: recent advances in understanding the microbial processes involved. *Environ. Microbiol. Rep.* 1, 285–292. doi: 10.1111/j.1758-2229.2009.00038.x
- Conrad, R., Klose, M., and Noll, M. (2009). Functional and structural response of the methanogenic microbial community in rice field soil to temperature change. *Environ. Microbiol.* 11, 1844–1853. doi: 10.1111/j.1462-2920.2009.01909.x
- Dacey, J. W. H., Drake, B. G., and Klug, M. J. (1994). Stimulation of methane emission by carbon-dioxide enrichment of marsh vegetation. *Nature* 370, 47–49. doi: 10.1038/370047a0

- Dannenmann, M., Gasche, R., Ledebuhr, A., Holst, T., Mayer, H., and Papen, H. (2007). The effect of forest management on trace gas exchange at the pedosphere-atmosphere interface in beech (*Fagus sylvatica* L.) forests stocking on calcareous soils. *Eur. J. For. Res.* 126, 331–346. doi: 10.1007/s10342-006-0153-3
- Davidson, E. A., Ishida, F. Y., and Nepstad, D. C. (2004). Effects of an experimental drought on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest. *Glob. Change Biol.* 10, 718–730. doi: 10.1111/j.1365-2486.2004.00762.x
- Dedysh, S. N., Liesack, W., Khmelenina, V. N., Suzina, N. E., Trotsenko, Y. A., Semrau, J. D., et al. (2000). *Methylocella palustris* gen. nov., sp. nov., a new methane-oxidizing acidophilic bacterium from peat bogs, representing a novel subtype of serine-pathway methanotrophs. *Int. J. Syst. Evol. Microbiol.* 50, 955–969. doi: 10.1099/00207713-50-3-955
- Degelmann, D. M., Borken, W., Drake, H. L., and Kolb, S. (2010). Different Atmospheric Methane-Oxidizing Communities in European Beech and Norway Spruce Soils. *Appl. Environ. Microbiol.* 76, 3228–3235. doi: 10.1128/AEM.02730-09
- Delmas, R. A., Servant, J., Tathy, J. P., Cros, B., and Labat, M. (1992). Sources and sinks of methane and carbon-dioxide exchanges in mountain forest in Equatorial Africa. *J. Geophys. Res. Atmos.* 97, 6169–6179. doi: 10.1029/90JD02575
- Dijkstra, F. A., Morgan, J. A., LeCain, D. R., and Follett, R. F. (2010). Microbially mediated CH₄ consumption and N₂O emission is affected by elevated CO₂, soil water content, and composition of semi-arid grassland species. *Plant Soil* 329, 269–281. doi: 10.1007/s11104-009-0152-5
- do Carmo, J. B., Keller, M., Dias, J. D., de Camargo, P. B., and Crill, P. (2006). A source of methane from upland forests in the Brazilian Amazon. *Geophys. Res. Lett.* 33, L04809. doi: 10.1029/2005GL025436
- Dubbs, L. L., and Whalen, S. C. (2010). Reduced net atmospheric CH₄ consumption is a sustained response to elevated CO₂ in a temperate forest. *Biol. Fert. Soils* 46, 597–606. doi: 10.1007/s00374-010-0467-7
- Dueck, T. A., de Visser, R., Poorter, H., Persijn, S., Gorissen, A., de Visser, W., et al. (2007). No evidence for substantial aerobic methane emission by terrestrial plants: a C-13-labelling approach. *New Phytol.* 175, 29–35. doi: 10.1111/j.1469-8137.2007.02103.x
- Dunfield, P., and Knowles, R. (1995). Kinetics of inhibition of methane oxidation by nitrate, nitrite, and ammonium in a humisol. *Appl. Environ. Microbiol.* 61, 3129–3135.
- Dunfield, P. E., Yuryev, A., Senin, P., Smirnova, A. V., Stott, M. B., Hou, S. B., et al. (2007). Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. *Nature* 450, U879–U818. doi: 10.1038/nature06411
- Dutaur, L., and Verchot, L. V. (2007). A global inventory of the soil CH₄ sink. *Global Biogeochem. Cycles* 21, GB4013. doi: 10.1029/2006GB002734
- Epstein, H. E., Burke, I. C., Mosier, A. R., and Hutchinson, G. L. (1998). Plant functional type effects on trace gas fluxes in the shortgrass steppe. *Biogeochemistry* 42, 145–168. doi: 10.1023/A:1005959001235
- Etiopie, G., and Klusman, R. W. (2010). Methane microseepage in drylands: soil is not always a CH₄ sink. *J. Integr. Environ. Sci.* 7, 31–38. doi: 10.1080/19438151003621359
- Fey, A., and Conrad, R. (2000). Effect of Temperature on Carbon and Electron Flow and on the Archaeal Community in Methanogenic Rice Field Soil. *Appl. Environ. Microbiol.* 66, 4790–4797. doi: 10.1128/AEM.66.11.4790-4797.2000
- Fiedler, S., Holl, B. S., and Jungkunst, H. F. (2005). Methane budget of a Black Forest spruce ecosystem considering soil pattern. *Biogeochemistry* 76, 1–20. doi: 10.1007/s10533-005-5551-y
- Florinsky, I. V., McMahon, S., and Burton, D. L. (2004). Topographic control of soil microbial activity: a case study of denitrifiers. *Geoderma* 119, 33–53. doi: 10.1016/S0016-7061(03)00224-6
- Freitag, T. E., and Prosser, J. I. (2009). Correlation of Methane Production and Functional Gene Transcriptional Activity in a Peat Soil. *Appl. Environ. Microbiol.* 75, 6679–6687. doi: 10.1128/AEM.01021-09
- Ganzert, L., Jurgens, G., Mänster, U., and Wagner, D. (2007). Methanogenic communities in permafrost-affected soils of the Laptev Sea coast, Siberian Arctic, characterized by 16S rRNA gene fingerprints. *FEMS Microbiol. Ecol.* 59, 476–488. doi: 10.1111/j.1574-6941.2006.00205.x
- Galbally, I., Meyer, C. P., Wang, Y. P., and Kirstine, W. (2010). Soil-atmosphere exchange of CH₄, CO₂, N₂O and NO_x and the effects of land-use change in the semi-arid Mallee system in Southeastern Australia. *Glob. Change Biol.* 16, 2407–2419.
- Geng, Y., Luo, G., and Yuan, G. (2010). CH₄ uptake flux of *Leymus chinensis* steppe during rapid growth season in Inner Mongolia, China. *Sci. China Earth Sci.* 53, 977–983. doi: 10.1007/s11430-010-3082-4
- Goreau, T. J., and Mello, W. Z. (1985). “Effects of deforestation on sources and sinks of atmospheric CO₂, N₂O, and CH₄ from central Amazonian soils and biota during the dry season,” in *Workshop on Biogeochemistry of Tropical Rain Forests*. Piracicaba.
- Groffman, P. M., Hardy, J. P., Driscoll, C. T., and Fahey, T. J. (2006). Snow depth, soil freezing, and fluxes of carbon dioxide, nitrous oxide and methane in a northern hardwood forest. *Glob. Change Biol.* 12, 1748–1760. doi: 10.1111/j.1365-2486.2006.01194.x
- Groot, T. T., van Bodegom, P. M., Harren, F. J. M., and Meijer, H. A. J. (2003). Quantification of methane oxidation in the rice rhizosphere using C-13-labelled methane. *Biogeochemistry* 64, 355–372. doi: 10.1023/A:1024921714852
- Gulledge, J., Doyle, A. P., and Schimel, J. P. (1997). Different NH₄⁺-inhibition patterns of soil CH₄ consumption: A result of distinct CH₄-oxidizer populations across sites? *Soil Biol. Biochem.* 29, 13–21. doi: 10.1016/S0038-0717(96)00265-9
- Gulledge, J., and Schimel, J. P. (2000). Controls on soil carbon dioxide and methane fluxes in a variety of taiga forest stands in interior Alaska. *Ecosystems* 3, 269–282. doi: 10.1007/s100210000025
- Hallam, S. J., Girguis, P. R., Preston, C. M., Richardson, P. M., and DeLong, E. F. (2003). Identification of methyl coenzyme M reductase A (mcrA) genes associated with methane-oxidizing archaea. *Appl. Environ. Microbiol.* 69, 5483–5491. doi: 10.1128/AEM.69.9.5483-5491.2003
- Hanson, R. S., and Hanson, T. E. (1996). Methanotrophic bacteria. *Microbiol. Rev.* 60, 439.
- Hart, S. C. (2006). Potential impacts of climate change on nitrogen transformations and greenhouse gas fluxes in forests: a soil transfer study. *Glob. Change Biol.* 12, 1032–1046. doi: 10.1111/j.1365-2486.2006.01159.x
- Henckel, T., Jackel, U., Schnell, S., and Conrad, R. (2000). Molecular analyses of novel methanotrophic communities in forest soil that oxidize atmospheric methane. *Appl. Environ. Microbiol.* 66, 1801–1808. doi: 10.1128/AEM.66.5.1801-1808.2000
- Horz, H. P., Rich, V., Avrahami, S., and Bohannan, B. J. M. (2005). Methane-oxidizing bacteria in a California upland grassland soil: diversity and response to simulated global change. *Appl. Environ. Microbiol.* 71, 2642–2652. doi: 10.1128/AEM.71.5.2642-2652.2005
- Hou, L.-Y., Wang, Z.-P., Wang, J.-M., Wang, B., Zhou, S.-B., and Li, L.-H. (2012). Growing season *in situ* uptake of atmospheric methane by desert soils in a semi-arid region of northern China. *Geoderma* 189–190, 415–422. doi: 10.1016/j.geoderma.2012.05.012
- Hudgens, D. E., and Yavitt, J. B. (1997). Land-use effects on soil methane and carbon dioxide fluxes in forests near Ithaca, New York. *Ecoscience* 4, 214–222.
- Hutchinson, G. L., and Mosier, A. R. (2002). Improved Soil Cover Method for Field Measurement of Nitrous Oxide Fluxes. *Soil Sci. Soc. Am. J.* 45, 311–316.
- Ineson, P., Benham, D. G., Poskitt, J., Harrison, A. F., Taylor, K., and Woods, C. (1998). Effects of climate change on nitrogen dynamics in upland soils. 2. A soil warming study. *Glob. Change Biol.* 4, 153–161. doi: 10.1046/j.1365-2486.1998.00119.x
- IPCC, (2007). *The Physical Science Basis, Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, eds S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, H. L. Miller, (Cambridge and New York: Cambridge University Press).
- Irvine, I. C., Vivanco, L., Bentley, P. N., and Martiny, J. B. H. (2012). The effect of nitrogen enrichment on C1-cycling microorganisms and methane flux in salt marsh sediments. *Front. Microbiol.* 3, 1–10. doi: 10.3389/fmicb.2012.00090
- Jatunen, K., Knief, C., Dunfield, P. F., Yrjölä, K., and Fritze, H. (2004). Methanotrophic bacteria in boreal forest soil after fire. *FEMS Microbiol. Ecol.* 50, 195–202. doi: 10.1016/j.femsec.2004.06.013
- Jauhainen, J., Takahashi, H., Heikkinen, J. E. P., Martikainen, P. J., and Vasander, H. (2005). Carbon fluxes from a tropical

- peat swamp forest floor. *Glob. Change Biol.* 11, 1788–1797. doi: 10.1111/j.1365-2486.2005.001031.x
- Juottonen, H., Tuittila, E.-S., Juutinen, S., Fritze, H., and Yrjölä, K. (2008). Seasonality of rDNA- and rRNA-derived archaeal communities and methanogenic potential in a boreal mire. *ISME J.* 2, 1157–1168. doi: 10.1038/ismej.2008.66
- Kai, F. M., Tyler, S. C., Randerson, J. T., and Blake, D. R. (2011). Reduced methane growth rate explained by decreased Northern Hemisphere microbial sources. *Nature* 476, 194–197. doi: 10.1038/nature10259
- Karl, D. M., Beversdorf, L., Bjorkman, K. M., Church, M. J., Martinez, A., and DeLong, E. F. (2008). Aerobic production of methane in the sea. *Nat. Geosci.* 1, 473–478. doi: 10.1038/ngeo234
- Keller, M., Mitre, M. E., and Stallard, R. F. (1990). Consumption of atmospheric methane in soils of central Panama: effects of agricultural development. *Glob. Biogeochem. Cycles* 4, 21–27. doi: 10.1029/GB004i001p00021
- Keller, M., and Reiners, W. A. (1994). Soil atmosphere exchange of nitrous-oxide, nitric-oxide, and methane under secondary succession of pasture to forest in the Atlantic lowlands of Costa Rica. *Global Biogeochem. Cycles* 8, 399–409. doi: 10.1029/94GB01660
- Keppeler, F., Hamilton, J. T. G., Brass, M., and Rockmann, T. (2006). Methane emissions from terrestrial plants under aerobic conditions. *Nature* 439, 187–191. doi: 10.1038/nature04420
- King, G. M., and Nanba, K. (2008). Distribution of atmospheric methane oxidation and methanotrophic communities on Hawaiian volcanic deposits and soils. *Microb. Environ.* 23, 326–330. doi: 10.1264/jsme2.ME08529
- Klemedtsson, A. K., and Klemedtsson, L. (1997). Methane uptake in Swedish forest soil in relation to liming and extra N-deposition. *Biol. Fert. Soils* 25, 296–301. doi: 10.1007/s003740050318
- Koide, T., Saito, H., Shirota, T., Iwahana, G., Lopez, M. L., Maximov, T. C., et al. (2010). Effects of changes in the soil environment associated with heavy precipitation on soil greenhouse gas fluxes in a Siberian larch forest near Yakutsk. *Soil Sci. Plant Nutr.* 56, 645–662. doi: 10.1111/j.1747-0765.2010.00484.x
- Kolb, S. (2009). Aerobic methanol-oxidizing Bacteria in soil. *FEMS Microbiol. Ecol.* 300, 1–10. doi: 10.1111/j.1574-6968.2009.01681.x
- Kolb, S., Carbrera, A., Kammann, C., Kampfer, P., Conrad, R., and Jackel, U. (2005). Quantitative impact of CO₂ enriched atmosphere on abundances of methanotrophic bacteria in a meadow soil. *Biol. Fert. Soils* 41, 337–342. doi: 10.1007/s00374-005-0842-y
- Konda, R., Ohta, S., Ishizuka, S., Heriyanto, J., and Wicaksono, A. (2010). Seasonal changes in the spatial structures of N₂O, CO₂, and CH₄ fluxes from *Acacia mangium* plantation soils in Indonesia. *Soil Biol. Biochem.* 42, 1512–1522. doi: 10.1016/j.soilbio.2010.05.022
- Kotsyurbenko, O. R., Chin, K.-J., Glagolev, M. V., Stubner, S., Simankova, M. V., Nozhevnikova, A. N., et al. (2004). Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an acidic West-Siberian peat bog. *Environ. Microbiol.* 6, 1159–1173. doi: 10.1111/j.1462-2920.2004.00634.x
- Kroeze, C., and Seitzinger, S. P. (1998). Nitrogen inputs to rivers, estuaries and continental shelves and related nitrous oxide emissions in 1990 and 2050: a global model. *Nutr. Cycl. Agroecosyst.* 52, 195–212. doi: 10.1023/A:1009780608708
- Kruse, C. W., and Iversen, N. (1995). Effect of plant succession, plowing, and fertilization on the microbial oxidation of atmospheric methane in a heathland soil. *FEMS Microbiol. Ecol.* 18, 121–128. doi: 10.1111/j.1574-6941.1995.tb00169.x
- Lelieveld, J., Crutzen, P. J., and Dentener, F. J. (1998). Changing concentration, lifetime and climate forcing of atmospheric methane. *Tellus B Chem. Phys. Meteorol.* 50, 128–150. doi: 10.1034/j.1600-0889.1998.t01-1-00002.x
- Lelieveld, J., Dentener, F. J., Peters, W., and Krol, M. C. (2004). On the role of hydroxyl radicals in the self-cleansing capacity of the troposphere. 1–8. *Atmos. Chem. Phys.* 4, 2337–2344. doi: 10.5194/acp-4-2337-2004
- Le Mer, J., and Roger, P. (2001). Production, oxidation, emission and consumption of methane by soils: A review. *Eur. J. Soil Biol.* 37, 25–50. doi: 10.1016/S1164-5563(01)01067-6
- Levine, U. Y., Teal, T. K., Robertson, G. P., and Schmidt, T. M. (2011). Agriculture impact on microbial diversity and associated fluxes of carbon dioxide and methane. *ISME J.* 5, 1683–1691. doi: 10.1038/ismej.2011.40
- Liebner, S., and Wagner, D. (2007). Abundance, distribution and potential activity of methane oxidizing bacteria in permafrost soils from the Lena Delta, Siberia. *Environ. Microbiol.* 9, 107–117. doi: 10.1111/j.1462-2920.2006.01120.x
- Macalady, J. L., McMillan, A. M. S., Dickens, A. F., Tyler, S. C., and Scow, K. M. (2002). Population dynamics of Type I and II methanotrophic bacteria in rice soils. *Environ. Microbiol.* 4, 148–157. doi: 10.1046/j.1462-2920.2002.00278.x
- MacDonald, J. A., Eggleton, P., Bignell, D. E., Forzi, F., and Fowler, D. (1998). Methane emission by termites and oxidation by soils, across a forest disturbance gradient in the Mbalmayo Forest Reserve, Cameroon. *Glob. Change Biol.* 4, 409–418. doi: 10.1046/j.1365-2486.1998.00163.x
- MacDonald, J. A., Jeeva, D., Eggleton, P., Davies, R., Bignell, D. E., Fowler, D., et al. (1999). The effect of termite biomass and anthropogenic disturbance on the CH₄ budgets of tropical forests in Cameroon and Borneo. *Glob. Change Biol.* 5, 869–879. doi: 10.1046/j.1365-2486.1999.00279.x
- Mariko, S., Urano, T., and Asanuma, J. (2007). Effects of irrigation on CO₂ and CH₄ fluxes from Mongolian steppe soil. *J. Hydrol.* 333, 118–123. doi: 10.1016/j.jhydrol.2006.07.027
- Martiny, A. C., Treseder, K., and Pusch, G. (2013). Phylogenetic conservatism of functional traits in microorganisms. *ISME J.* 7, 830–838. doi: 10.1038/ismej.2012.160
- Maxfield, P. J., Hornibrook, E. R. C., and Evershed, R. P. (2006). Estimating high-affinity methanotrophic bacterial biomass, growth, and turnover in soil by phospholipid fatty acid C-13 labeling. *Appl. Environ. Microbiol.* 72, 3901–3907. doi: 10.1128/AEM.02779-05
- Maxfield, P. J., Hornibrook, E. R. C., and Evershed, R. P. (2008). Acute impact of agriculture on high-affinity methanotrophic bacterial populations. *Environ. Microbiol.* 10, 1917–1924. doi: 10.1111/j.1462-2920.2008.01587.x
- McLain, J. E. T., and Ahmann, D. M. (2008). Increased moisture and methanogenesis contribute to reduced methane oxidation in elevated CO₂ soils. *Biol. Fert. Soils* 44, 623–631. doi: 10.1007/s00374-007-0246-2
- McLain, J. E. T., Kepler, T. B., and Ahmann, D. M. (2002). Belowground factors mediating changes in methane consumption in a forest soil under elevated CO₂. *Global Biogeochem. Cycles* 16, 1050. doi: 10.1029/2001GB001439
- McNamara, N. P., Black, H. I. J., Pearce, T. G., Reay, D. S., and Ineson, P. (2008). The influence of afforestation and tree species on soil methane fluxes from shallow organic soils at the UK Gisburn Forest Experiment. *Soil Use Manage.* 24, 1–7. doi: 10.1111/j.1475-2743.2008.00147.x
- Menyailo, O. V., Hungate, B. A., Abraham, W.-R., and Conrad, R. (2008). Changing land use reduces soil CH₄ uptake by altering biomass and activity but not composition of high-affinity methanotrophs. *Glob. Change Biol.* 14, 2405–2419. doi: 10.1111/j.1365-2486.2008.01648.x
- Mohanty, S. R., Bodelier, P. L. E., Floris, V., and Conrad, R. (2006). Differential effects of nitrogenous fertilizers on methane-consuming microbes in rice field and forest soils. *Appl. Environ. Microbiol.* 72, 1346–1354. doi: 10.1128/AEM.72.2.1346-1354.2006
- Mosier, A. R., Delgado, J. A., Cochran, V. L., Valentine, D. W., and Parton, W. J. (1997a). Impact of agriculture on soil consumption of atmospheric CH₄ and a comparison of CH₄ and N₂O flux in subarctic, temperate and tropical grasslands. *Nutr. Cycl. Agroecosyst.* 49, 71–83. doi: 10.1023/A:1009754207548
- Mosier, A. R., Morgan, J. A., King, J. Y., LeCain, D., and Milchunas, D. G. (2002). Soil-atmosphere exchange of CH₄, CO₂, NO_x, and N₂O in the Colorado shortgrass steppe under elevated CO₂. *Plant Soil* 240, 201–211. doi: 10.1023/A:1015783801324
- Mosier, A. R., Parton, W. J., Valentine, D. W., Ojima, D. S., Schimel, D. S., and Heinemeyer, O. (1997b). CH₄ and N₂O fluxes in the Colorado shortgrass steppe. 2. Long-term impact of land use change. *Glob. Biogeochem. Cycles* 11, 29–42. doi: 10.1029/96GB03612
- Mosier, A. R., Schimel, D., Valentine, D., Bronson, K., and Parton, W. (1991). Methane and nitrous-oxide fluxes in native, fertilized and cultivated grassland. *Nature* 350, 330–332. doi: 10.1038/350330a0
- Nazarides, L., Pan, Y., Bodrossy, L., Baggs, E. M., Millard, P., Murrell, J. C., et al. (2013). Evidence of Microbial Regulation of Biogeochemical Cycles from a Study on Methane Flux and

- Land Use Change. *Appl. Environ. Microbiol.* 79, 4013–4040. doi: 10.1128/AEM.00095-13
- Nazarides, L., Tate, K. R., Ross, J. D., and Singh, J. et al. (2011). Response of methanotrophic communities to afforestation and reforestation in New Zealand. *ISME J.* 5, 1832–1836. doi: 10.1038/ismej.2011.62
- Neff, J. C., Bowman, W. D., Holland, E. A., Fisk, M. C., and Schmidt, S. K. (1994). Fluxes of nitrous-oxide and methane from nitrogen-amended soils in a Colorado alpine ecosystem. *Biogeochemistry* 27, 23–33. doi: 10.1007/BF00002569
- Nisbet, R. E. R., Fisher, R., Nimmo, R. H., Bendall, D. S., Crill, P. M., Gallego-Sala, A. V., et al. (2009). Emission of methane from plants. *Proc. R. Soc. B Biol. Sci.* 276, 1347–1354. doi: 10.1098/rspb.2008.1731
- Oelbermann, M., and Schiff, S. L. (2010). Inundating contrasting boreal forest soils: CO₂ and CH₄ production rates. *Ecoscience* 17, 216–224. doi: 10.2980/17-2-3245
- Phillips, R. L., Whalen, S. C., and Schlesinger, W. H. (2001). Influence of atmospheric CO₂ enrichment on methane consumption in a temperate forest soil. *Glob. Change Biol.* 7, 557–563. doi: 10.1046/j.1354-1013.2001.00432.x
- Poth, M., Anderson, I. C., Miranda, H. S., Miranda, A. C., and Riggan, P. J. (1995). The magnitude and persistence of soil NO, N₂O, CH₄, and CO₂ fluxes from burned tropical savanna in Brazil. *Global Biogeochem. Cycles* 9, 503–513. doi: 10.1029/95GB02086
- Price, S., Whitehead, D., Sherlock, R., McSeveny, T., and Rogers, G. (2010). Net exchange of greenhouse gases from soils in an unimproved pasture and regenerating indigenous *Kunzea ericoides* shrubland in New Zealand. *Aust. J. Soil Res.* 48, 385–394. doi: 10.1071/SR09156
- Prieme, A., and Christensen, S. (1999). Methane uptake by a selection of soils in Ghana with different land use. *J. Geophys. Res. Atmos.* 104, 23617–23622. doi: 10.1029/1999JD900427
- Prieme, A., Christensen, S., Dobbie, K. E., and Smith, K. A. (1997). Slow increase in rate of methane oxidation in soils with time following land use change from arable agriculture to woodland. *Soil Biol. Biochem.* 29, 1269–1273. doi: 10.1016/S0038-0717(97)00017-5
- Prinn, R. G. (2000). “Measurement equation for trace chemicals in fluids and solution of its inverse,” in *Inverse Methods*, eds
- P. Kasibhatla, M. Heimann, P. Rayner, N. Mahowald, R. G. Prinn, and D. E. Hartley (Washington, DC: American Geophysical Union), 3–18.
- Prinn, R. G. (2001). Evidence for substantial variations of atmospheric hydroxyl radicals in the past two decades. *Science* 293, 1048.
- Pritchard, S. G., and Rogers, S. G. (2000). Spatial and temporal deployment of crop roots in CO₂-enriched environments. *New Phytol.* 147, 55–71. doi: 10.1046/j.1469-8137.2000.00678.x
- Purbopuspito, J., Veldkamp, E., Brumme, R., and Murdiyarso, D. (2006). Trace gas fluxes and nitrogen cycling along an elevation sequence of tropical montane forests in Central Sulawesi, Indonesia. *Glob. Biogeochem. Cycles* 20, GB3010. doi:10.1029/2005GB002516
- Raghoebarsing, A. A., Pol, A., van de Pas-Schoonen, K. T., Smolders, A. J. P., Ettwig, K. F., Rijpstra, W. I. C., et al. (2006). A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature* 440, 918–921. doi: 10.1038/nature04617
- Ramakrishnan, B., Lueders, T., Dunfield, P. F., Conrad, R., and Friedrich, M. W. (2001). Archaeal community structures in rice field soils from different geographical regions before and after initiation of methane production. *FEMS Microbiol. Ecol.* 37, 175–186. doi: 10.1111/j.1574-6941.2001.tb00865.x
- Reay, D. S., Nedwell, D. B., McNamara, N., and Ineson, P. (2005). Effect of tree species on methane and ammonium oxidation capacity in forest soils. *Soil Biol. Biochem.* 37, 719–730. doi: 10.1016/j.soilbio.2004.10.004
- Reshetnikov, A. I., Paramonova, N. N., and Shashkov, A. A. (2000). An evaluation of historical methane emissions from the Soviet gas industry. *J. Geophys. Res. Atmos.* 105, 3517–3529. doi: 10.1029/1999JD900761
- Rigby, M., Prinn, R. G., Fraser, P. J., Simmonds, P. G., Langenfelds, R. L., Huang, J., et al. (2008). Renewed growth of atmospheric methane. *Geophys. Res. Lett.* 35, L22805. doi: 10.1029/2008GL036037
- Rosenkranz, P., Bruggemann, N., Papen, H., Xu, Z., Horvath, L., and Butterbach-Bahl, K. (2006). Soil N and C trace gas fluxes and microbial soil N turnover in a sessile oak [*Quercus petraea* (Matt.) Liebl.] forest in Hungary. *Plant Soil* 286, 301–322. doi: 10.1007/s11104-006-9045-z
- Saari, A., Smolander, A., and Martikainen, P. J. (2004). Methane consumption in a frequently nitrogen-fertilized and limed spruce forest soil after clear-cutting. *Soil Use Manage.* 20, 65–73. doi: 10.1079/SUM2004224
- Sanhueza, E., and Donoso, L. (2006). Methane emission from tropical savanna *Trachypogon* sp. grasses. *Atmos. Chem. Phys.* 6, 5315–5319. doi: 10.5194/acp-6-5315-2006
- Schimel, J. (1995). “Ecosystem consequences of microbial diversity and community structure,” in *Arctic and Alpine Biodiversity: Patterns, Causes, and Ecosystem Consequences*, eds F. S. Chapin III and C. Körner (New York, NY: Springer-Verlag), 239–254. doi: 10.1007/978-3-642-78966-3_17
- Schimel, J. P., and Gullledge, J. (1998). Microbial community structure and global trace gases. *Glob. Change Biol.* 4, 745–758. doi: 10.1046/j.1365-2486.1998.00195.x
- Schimel, J. P., and Schaeffer, S. M. (2012). Microbial control over carbon cycling in soil. *Front. Microbiol.* 3:348. doi: 10.3389/fmicb.2012.00348
- Seghers, D., Siciliano, S. D., Top, E. M., and Verstraete, W. (2005). Combined effect of fertilizer and herbicide applications on the abundance, community structure and performance of the soil methanotrophic community. *Soil Biol. Biochem.* 37, 187–193. doi: 10.1016/j.soilbio.2004.05.025
- Seghers, D., Top, E. M., Reheul, D., Bulcke, R., Boeckx, P., Verstraete, W., et al. (2003a). Long-term effects of mineral versus organic fertilizers on activity and structure of the methanotrophic community in agricultural soils. *Environ. Microbiol.* 5, 867–877. doi: 10.1046/j.1462-2920.2003.00477.x
- Seghers, D., Verthä, K., Reheul, D., Bulcke, R., Siciliano, S. D., Verstraete, W., et al. (2003b). Effect of long-term herbicide applications on the bacterial community structure and function in an agricultural soil. *FEMS Microbiol. Ecol.* 46, 139–146. doi: 10.1016/S0168-6496(03)00205-8
- Shakhova, N., Semiletov, I., Leifer, I., Salyuk, A., Rekan, P., and Kosmach, D. (2010). Geochemical and geophysical evidence of methane release over the East Siberian Arctic Shelf. *J. Geophys. Res.-Oceans* 115, C08007. doi: 10.1029/2009JC005602
- Shively, J. M., English, R. S., Baker, S. H., and Cannon, G. C. (2001). Carbon cycling: the prokaryotic contribution. *Curr. Opin. Microbiol.* 4, 301–306. doi: 10.1016/S1369-5274(00)00207-1
- Silver, W. L., Lugo, A. E., and Keller, M. (1999). Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils. *Biogeochemistry* 44, 301–328. doi: 10.1007/BF00996995
- Singh, B. K., Tate, K. R., Kolipaka, G., Hedley, C. B., Macdonald, C. A., and Millard, P. (2007). Effect of afforestation and reforestation of pastures on the activity and population dynamics of methanotrophic bacteria. *Appl. Environ. Microbiol.* 73, 5153–5161. doi: 10.1128/AEM.00620-07
- Singh, J. S., Singh, S., Raghubanshi, A. S., Singh, S., Kashyap, A. K., and Reddy, V. S. (1997). Effect of soil nitrogen, carbon and moisture on methane uptake by dry tropical forest soils. *Plant Soil* 196, 115–121. doi: 10.1023/A:1004233208325
- Sitaula, B. K., Bakken, L. R., and Abrahamsen, G. (1995). CH₄ uptake by temperate forest soil – effect of N input and soil acidification. *Soil Biol. Biochem.* 27, 871–880. doi: 10.1016/0038-0717(95)00017-9
- Sjogersten, S., and Wookey, P. A. (2002). Spatio-temporal variability and environmental controls of methane fluxes at the forest-tundra ecotone in the Fennoscandian mountains. *Glob. Change Biol.* 8, 885–894. doi: 10.1046/j.1365-2486.2002.00522.x
- Tate, C. M., and Striegl, R. G. (1993). Methane consumption and carbon-dioxide emission in tallgrass prairie – effects of biomass burning and conversion to agriculture. *Global Biogeochem. Cycles* 7, 735–748. doi: 10.1029/93GB02560
- Tate, K. R., Ross, D. J., Scott, N. A., Rodda, N. J., Townsend, J. A., and Arnold, G. C. (2006). Post-harvest patterns of carbon dioxide production, methane uptake and nitrous oxide production in a *Pinus radiata* D. Don plantation. *For. Ecol. Manage.* 228, 40–50. doi: 10.1016/j.foreco.2006.02.023
- Tate, K. R., Walcroft, A. S., and Pratt, C. (2012). Varying atmospheric methane concentrations affect soil methane oxidation rates and methanotroph populations in pasture, an adjacent pine forest, and a landfill. *Soil Biol. Biochem.*

- 52, 75–81. doi: 10.1016/j.soilbio.2012.04.011
- Teh, Y. A., Silver, W. L., and Conrad, M. E. (2005). Oxygen effects on methane production and oxidation in humid tropical forest soils. *Glob. Change Biol.* 11, 1283–1297. doi: 10.1111/j.1365-2486.2005.00983.x
- Torn, M. S., and Harte, J. (1996). Methane consumption by montane soils: implications for positive and negative feedback with climatic change. *Biogeochemistry* 32, 53–67. doi: 10.1007/BF00001532
- Tsutsumi, M., Kojima, H., Uemura, S., Ono, K., Sumida, A., Hara, T., et al. (2009). Structure and activity of soil-inhabiting methanotrophic communities in northern forest of Japan. *Soil Biol. Biochem.* 41, 403–408. doi: 10.1016/j.soilbio.2008.12.005
- Tuomivirta, T. T., Yrjala, K., and Fritze, H. (2009). Quantitative PCR of pmoA using a novel reverse primer correlates with potential methane oxidation in Finnish fen. *Res. Microbiol.* 160, 751–756. doi: 10.1016/j.resmic.2009.09.008
- Udegraff, K., Bridgman, S. D., Pastor, J., Weishampel, P., and Harth, C. (2001). Response of CO₂ and CH₄ emissions from peatlands to warming and water table manipulation. *Ecol. Appl.* 11, 311–326.
- van den Pol-van Dasselaar, A., van Beusichem, M. L., and Oenema, O. (1998). Effects of soil moisture content and temperature on methane uptake by grasslands on sandy soils. *Plant Soil* 204, 213–222. doi: 10.1023/A:1004371309361
- van Huissteden, J., Maximov, T. C., Kononov, A. V., and Dolman, A. J. (2008). Summer soil CH₄ emission and uptake in taiga forest near Yakutsk, Eastern Siberia. *Agric. For. Meteorol.* 148, 2006–2012. doi: 10.1016/j.agrformet.2008.08.008
- Vann, C. D., and Megonigal, J. P. (2003). Elevated CO₂ and water depth regulation of methane emissions: Comparison of woody and non-woody wetland plant species. *Biogeochemistry* 63, 117–134. doi: 10.1023/A:1023397032331
- Veldkamp, E., Purbopuspito, J., Corre, M. D., Brumme, R., and Murdiyarso, D. (2008). Land use change effects on trace gas fluxes in the forest margins of Central Sulawesi, Indonesia. *J. Geophys. Res. Biogeosci.* 113, G02003. doi: 10.1029/2007JG000522
- Verchot, L. V., Davidson, E. A., Cattaneo, J. H., and Ackerman, I. L. (2000). Land-use change and biogeochemical controls of methane fluxes in soils of eastern Amazonia. *Ecosystems* 3, 41–56. doi: 10.1007/s100210000009
- von Fischer, J. C., Rhew, R. C., Ames, G. M., Fosdick, B. K., and von Fischer, P. E. (2010). Vegetation height and other controls of spatial variability in methane emissions from the Arctic coastal tundra at Barrow, Alaska. *J. Geophys. Res.-Biogeosci.* 115, G00103. doi: 10.1029/2009JG001283
- Wagner, D., Lipski, A., Embacher, A., and Gatteringer, A. (2005). Methane fluxes in permafrost habitats of the Lena Delta: effects of microbial community structure and organic matter quality. *Environ. Microbiol.* 7, 1582–1592. doi: 10.1111/j.1462-2920.2005.00849.x
- Walter, K. M., Zimov, S. A., Chanton, J. P., Verbyla, D., and Chapin, F. S. (2006). Methane bubbling from Siberian thaw lakes as a positive feedback to climate warming. *Nature* 443, 71–75. doi: 10.1038/nature05040
- Wang, C.-J., Tang, S.-M., Wilkes, A., Jiang, Y.-Y., Han, G.-D., and Huang, D. (2012). Effect of Stocking Rate on Soil-Atmosphere CH₄ Flux during Spring Freeze-Thaw Cycles in a Northern Desert Steppe, China. *PLoS ONE* 7:e36794. doi: 10.1371/journal.pone.0036794
- Wang, J. S., Logan, J. A., McElroy, M. B., Duncan, B. N., Megretskaia, I. A., and Yantosca, R. M. (2004). A 3-D model analysis of the slowdown and interannual variability in the methane growth rate from 1988 to 1997. *Global Biogeochem. Cycles* 18, GB3011. doi: 10.1029/2003GB002180
- Werner, C., Zheng, X. H., Tang, J. W., Xie, B. H., Liu, C. Y., Kiese, R., et al. (2006). N₂O, CH₄ and CO₂ emissions from seasonal tropical rainforests and a rubber plantation in Southwest China. *Plant Soil* 289, 335–353. doi: 10.1007/s11104-006-9143-y
- West, A. E., Brooks, P. D., Fisk, M. C., Smith, L. K., Holland, E. A., Jaeger, C. H., et al. (1999). Landscape patterns of CH₄ fluxes in an alpine tundra ecosystem. *Biogeochemistry* 45, 243–264. doi: 10.1007/BF00993002
- Whalen, S. C., Reeburgh, W. S., and Barber, V. A. (1992). Oxidation of methane in boreal forest soils – a comparison of 7 measures. *Biogeochemistry* 16, 181–211. doi: 10.1007/BF00002818
- Willison, T. W., Goulding, K. W. T., and Powlson, D. S. (1995). Effect of land-use change and methane mixing-ratio on methane uptake from United-Kingdom soil. *Glob. Change Biol.* 1, 209–212. doi: 10.1111/j.1365-2486.1995.tb00022.x
- Wolf, K., Flessa, H., and Veldkamp, E. (2012). Atmospheric methane uptake by tropical montane forest soils and the contribution of organic layers. *Biogeochemistry* 111, 469–483. doi: 10.1007/s10533-011-9681-0
- Wu, X., Brüggemann, N., Gasche, R., Papen, H., Willibald, G., and Butterbach-Bahl, K. (2011). Long-term effects of clear-cutting and selective cutting on soil methane fluxes in a temperate spruce forest in southern Germany. *Environ. Pollut.* 159, 2467–2475. doi: 10.1016/j.envpol.2011.06.025
- Xu, X., and Luo, X. (2012). Effect of wetting intensity on soil GHG fluxes and microbial biomass under a temperate forest floor during dry season. *Geoderma* 170, 118–126. doi: 10.1016/j.geoderma.2011.11.016
- Yavitt, J. B., Lang, G. E., and Sextone, A. J. (1990). Methane fluxes in wetland and forest soils, beaver ponds, and low-order streams of a temperate forest ecosystem. *J. Geophys. Res. Atmos.* 95, 22463–22474. doi: 10.1029/JD095iD13p22463
- Zhang, W., Mo, J. M., Zhou, G. Y., Gundersen, P., Fang, Y. T., Lu, X. K., et al. (2008). Methane uptake responses to nitrogen deposition in three tropical forests in southern China. *J. Geophys. Res. Atmos.* 113, D11116. doi: 10.1029/2007JD009195
- Zhou, X.-Q., Wang, Y.-F., Huang, X.-Z., Tian, J.-Q., and Hao, Y.-B. (2008). Effect of grazing intensities on the activity and community structure of methane-oxidizing bacteria of grassland soil in Inner Mongolia. *Nutr. Cycl. Agroecosyst.* 80, 145–152. doi: 10.1007/s10705-007-9127-1
- Ziska, L. H., Moya, T. B., Wassmann, R., Namuco, O. S., Lantin, R. S., Aduna, J. B., et al. (1998). Long-term growth at elevated carbon dioxide stimulates methane emission in tropical paddy rice. *Glob. Change Biol.* 4, 657–665. doi: 10.1046/j.1365-2486.1998.00186.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 15 June 2013; accepted: 25 July 2013; published online: 14 August 2013.
 Citation: Aronson EL, Allison SD and Helliker BR (2013) Environmental impacts on the diversity of methane-cycling microbes and their resultant function. *Front. Microbiol.* 4:225. doi: 10.3389/fmicb.2013.00225
 This article was submitted to *Frontiers in Terrestrial Microbiology*, a specialty of *Frontiers in Microbiology*.
 Copyright © 2013 Aronson, Allison and Helliker. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

APPENDIX A

META-ANALYSIS: METHODS OF DATABASE CREATION

Methane flux data were extracted from published studies in non-wetland terrestrial ecosystems and farming systems, and are listed in Appendix B, using the same methods as in Aronson and Helliker (2010). Analysis was limited to the measurement of net flux due to combined methanogeny and aerobic oxidation of CH₄ under ambient CH₄ concentrations; uptake by anaerobic oxidation or under elevated [CH₄] was not considered. All included studies used intact soil, mostly *in situ* with the exception of (Kruse and Iversen, 1995) and Willison et al. (1995), which used intact soil cores exposed to atmospheric CH₄ concentrations soon after removal from the field. All studies used static (mostly vented) chambers (Hutchinson and Mosier, 2002) or flow-through auto-chambers (e.g., Brummell et al., 2012). All the original data were extracted from figures, tables, and text in the published papers. The studies were located using review papers (Le Mer and Roger, 2001; Dutaur and Verchot, 2007) and ISI Web of Knowledge using search terms: “methane” and “uptake,” “oxidation,” “flux” or “consumption.” In particular, all applicable studies from Dutaur and Verchot (2007) were included in the database if the original article could be located. Unpublished data from the dataset published with Dutaur and Verchot (2007) were not used.

The resultant database (Appendix B) from 194 papers, consisted of 716 entries, each containing a methane flux measurement matched with ancillary information. There were multiple entries from many studies due to differing environmental information associated with each methane flux measurement. The annual CH₄ uptake averages presented in primary or secondary literature were used when applicable, while averages were calculated based on figures if no yearly average was provided. All flux measurements were standardized to a flux density of CH₄ in kg ha⁻¹ yr⁻¹. Ancillary information from each data source, included: latitude, longitude, and location information;

average annual temperature and precipitation; elevation; soil type or description; duration of study; start year; climatic zone; ecosystem type (as described in the reference); vegetation type; season(s) studied; environmental and fertilization information; replication information; plant type and species; and collection method and intervals.

The ecosystem types in **Figure 2** were gathered from the references. The climatic zones were also taken from the references, when this information was provided. When the reference did not state climatic zone, it was based on latitude (up to 25 degrees was considered tropical, 25°–50° was considered temperate, and 50°–70° was boreal, greater than 70° was tundra). The vegetation types from **Figure 3** are groupings of the dominant plants associated with each methane flux measurement. The types broadleaf deciduous, broadleaf evergreen, and needleleaf trees were taken directly from plant types the text, or inferred based on the ecosystem type listed. However, broadleaf shrubs were considered to include desert vegetation, chaparral and some grassland-type sites where shrubs were listed as dominant, in addition to shrubland. Tundra vegetation was variable, and the vegetation type classification was always given to that ecosystem/climatic zone. Ground cover included grasslands, heathland, steppe, and savannah. Agricultural systems were excluded from the comparisons shown in **Figures 2, 3**, since their flux profiles may not follow with natural gradients. In some cases the numbers associated with each ecosystem and vegetation type (in **Figures 2, 3**) differ from the number of those methane flux measurements due to the removal of a 5 outlier points (an order of magnitude greater consumption or release than the others) for statistical purposes.

DATA ANALYSIS

All statistical analysis was performed using JMP Pro 10 (SAS, Inc.). The statistical tests performed included One-Way ANOVA, as well as *post-hoc* Student's *t*-test comparisons. The significance cut-off was $p < 0.05$.



Controls on bacterial and archaeal community structure and greenhouse gas production in natural, mined, and restored Canadian peatlands

Nathan Basiliko^{1,2*}, Kevin Henry³, Varun Gupta¹, Tim R. Moore⁴, Brian T. Driscoll⁵ and Peter F. Dunfield^{2,6}

¹ Department of Geography, University of Toronto Mississauga, Mississauga, ON, Canada

² Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany

³ Department of Geography, University of Utah, Salt Lake City, UT, USA

⁴ Department of Geography, McGill University, Montreal, QC, Canada

⁵ Department of Natural Resource Sciences, McGill University, Ste. Anne de Bellevue, QC, Canada

⁶ Department of Biological Sciences, University of Calgary, Calgary, AB, Canada

Edited by:

Per Bengtson, Lund University, Sweden

Reviewed by:

Steffen Kolb, University of Bayreuth, Germany

Lisa Y. Stein, University of Alberta, Canada

*Correspondence:

Nathan Basiliko, Department of Geography, University of Toronto Mississauga, 3359 Mississauga Road North, Mississauga, ON L5L 1C6, Canada
e-mail: nathan.basiliko@utoronto.ca

Northern peatlands are important global C reservoirs, largely because of their slow rates of microbial C mineralization. Particularly in sites that are heavily influenced by anthropogenic disturbances, there is scant information about microbial ecology and whether or not microbial community structure influences greenhouse gas production. This work characterized communities of bacteria and archaea using terminal restriction fragment length polymorphism (T-RFLP) and sequence analysis of 16S rRNA and functional genes across eight natural, mined, or restored peatlands in two locations in eastern Canada. Correlations were explored among chemical properties of peat, bacterial and archaeal community structure, and carbon dioxide (CO₂) and methane (CH₄) production rates under oxic and anoxic conditions. Bacteria and archaea similar to those found in other peat soil environments were detected. In contrast to other reports, methanogen diversity was low in our study, with only 2 groups of known or suspected methanogens. Although mining and restoration affected substrate availability and microbial activity, these land-uses did not consistently affect bacterial or archaeal community composition. In fact, larger differences were observed between the two locations and between oxic and anoxic peat samples than between natural, mined, and restored sites, with anoxic samples characterized by less detectable bacterial diversity and stronger dominance by members of the phylum *Acidobacteria*. There were also no apparent strong linkages between prokaryote community structure and CH₄ or CO₂ production, suggesting that different organisms exhibit functional redundancy and/or that the same taxa function at very different rates when exposed to different peat substrates. In contrast to other earlier work focusing on fungal communities across similar mined and restored peatlands, bacterial and archaeal communities appeared to be more resistant or resilient to peat substrate changes brought about by these land uses.

Keywords: archaea, bacteria, carbon dioxide, decomposition, methane, methanogen

INTRODUCTION

Northern peatlands are important long-term sinks of atmospheric carbon dioxide (CO₂) due to net imbalances between primary production and heterotrophic mineralization of soil organic matter (peat) and plant litters (Roulet et al., 2007). They are also persistent sources of methane (CH₄) due to waterlogging of soil profiles that helps sustain methanogenesis. Soil microorganisms, including bacteria and archaea, are largely responsible for the production of both of these greenhouse gases through decomposition processes. However, we do not fully understand the factors that control microbial community structure at these sites, nor can we yet make meaningful linkages between diversity and activities that ultimately result in greenhouse gas emissions (Andersen et al., 2013).

Microbial community controls may be particularly important to greenhouse gas flux dynamics in sites that are commercially mined (or “cutover”) for horticultural substrates and soil amendments (Andersen et al., 2013). In these sites the microbial environment is dramatically altered through removal of newly-formed peat and exposure of biorecalcitrant peat that is thousands of years old and the resulting hydrological and plant community alterations (Andersen et al., 2006; Basiliko et al., 2007). Depending on the method of peat extraction, natural restoration of mined peatlands can lead to the relatively rapid formation of new peat (Robert et al., 1999). However, active restoration as a tool to return peatlands mined using contemporary methods to sinks for CO₂ and small sources of CH₄ has produced variable results (Tuittila et al., 1999; McNeil and Waddington,

2003; Marinier et al., 2004; Waddington et al., 2010). Restoration efforts likely can set peatlands on a trajectory toward renewed carbon sequestration, however because this is a process that would subsequently takes thousands of years, there is a clear trade-off between loss of contemporary carbon stocks and provision of peat as a natural resource. Croft et al. (2001) first demonstrated that numbers of cultivatable bacteria were reduced after mining and Glatzel et al. (2004) later suggested that understanding the microbial role in both the impacts of mining and the effectiveness of restoration is essential.

In restored or revegetated mined peatlands, functional microbial fingerprints using physiological profiling techniques have linked substrate utilization abilities to the newly established vegetation on peatland surfaces (Artz et al., 2008; Yan et al., 2008). Previous work in mined and/or restored peatlands has also explored the role of decomposer community structure. Litter chemistry and plant communities have been linked to changes in fungal communities and carbon loss in mined peat surfaces recently colonized by different plant functional groups (Artz et al., 2007; Trinder et al., 2008, 2009). However, recent work has suggested that bacterial activities and numbers predominate over those of fungi across natural North American peatlands, including non-saturated surface peat of acidic bogs and poor fens (Winsborough and Basiliko, 2010; Lin et al., 2012; Myers et al., 2012), although mining and restoration impacts on bacterial communities have not been extensively studied. Across other non-impacted peat environments it has been shown that bacterial and archaeal decomposer communities can be quite similar despite different vegetation and litter chemistry and even when exhibiting different rates of activity (Kim et al., 2008; Preston et al., 2012). Given the importance of wetlands in global CH₄ emissions, methanogenic archaea in peatlands have been the focus of study for some time (e.g., Williams and Crawford, 1984; Hales et al., 1996; Basiliko et al., 2003; Bräuer et al., 2006), including sites that have been drained for forestry and reflooded (Galand et al., 2005b; Juottonen et al., 2012). Restoration of peatlands used for forestry in Finland re-established methanogen communities similar to those in undisturbed sites, though methanogen abundance and CH₄ emissions rates remained lower, despite generally similar water table positions (Juottonen et al., 2012). However, the impacts of horticultural mining and subsequent restoration on methanogens remain unknown.

The objectives of this work were to characterize communities of bacteria and archaea across eight natural, mined, or restored peatlands in two eastern Canada locations using terminal restriction fragment length polymorphism (T-RFLP) fingerprinting combined with sequence analyses of small sub-unit ribosomal RNA and methyl-coenzyme M reductase (*mcrA*) gene fragments. Correlations were explored among chemical properties of peat that are altered through land-use changes, bacterial and archaeal community structure, and CO₂ and CH₄ production rates. We predicted that that organic substrate quality and nutrient availability, which are reduced by mining and potentially enhanced through restoration, would link to differences in bacterial and archaeal community structure. In particular we predicted that mined sites, relative to natural and restored sites, would have low

detectable bacterial and archaeal richness and evenness, concomitant with low rates of greenhouse gas production. Because peat substrate might be a stronger control on community structure than dispersal constraints for prokaryotes, we predicted that land-use effects on community structure within a location would be greater than differences in similar sites across locations.

METHODS

STUDY SITES, SAMPLING, AND PEAT PHYSICOCHEMICAL ANALYSES

Canadian peatlands near Rivière du Loup, QC and Shippagan, NB used in the present study were previously described in detail by Basiliko et al. (2007). In each location, we sampled natural sites, actively mined sites, mined sites that had been abandoned for ~30 years and did not have post-harvest peat accumulation, and reflooded block-cut mined sites that had accumulated ~35 cm of *Sphagnum*-dominated peat over ~30 years. Natural and restored sites were dominated by *Sphagnum* moss and shrub vegetation characteristic of bogs or poor fens, and peat in all sites appeared to be dominated by *Sphagnum* remains (Basiliko et al., 2007). The 20–30 and 30–40 cm depth segments were chosen for oxic and anoxic incubations and community analysis. The 30-cm depth was the approximate water-table position at sampling time in the natural, abandoned, and restored sites. Samples were taken in early September and frozen at –20°C and thawed for 3 days at 4°C prior to subsequent analyses. Peat properties characterized by Basiliko et al. (2007) with fresh samples were used in correlation analyses described below. Briefly, microbial biomass and extractable organic C, N, and P, and inorganic N and P were determined using a CHCl₃-fumigation, K₂SO₄ extraction procedure. Peat organic chemistry was characterized using FTIR-spectral analysis to determine the relative concentrations of organic acids or polysaccharides to aromatic molecules, and through differential solvent (diethyl ether and CHCl₃) extraction of lipids. The humic acid fraction of water-extractable dissolved organic C (DOC) was measured through acid precipitation methods, and the physical degree of humification was measured using the Von Post humification index. Peat moisture content was measured and pH determined in a 4:1 water:peat mixture. Water-extractable inorganic ions (Na⁺, K⁺, Mg²⁺, Ca²⁺, and SO₄^{2–}) were measured using ion chromatography.

MICROBIAL ACTIVITY, COMMUNITY STRUCTURE, AND PHYLOGENETIC CHARACTERIZATION

After thawing, peat was incubated under oxic and anoxic conditions at 20°C to restore microbial activity and standardize temperature and O₂ availability, and CO₂ and CH₄ exchange was measured following methods from Glatzel et al. (2004) and Basiliko et al. (2005). The rate of aerobic CO₂ production following the final aeration (incubation day 9–10) and the rate of anaerobic CH₄ and CO₂ production from day 25 to 30 were chosen to represent aerobic and anaerobic production, respectively and are expressed per g dry peat per day. Immediately following the final gas measurements, DNA was extracted from each of the 48 samples using the FastDNA SPIN Kit for Soil (Qbiogene, Carlsbad, CA, USA) according to the manufacturer's instructions, except that DNA was washed four times with 0.5 ml of guanidine thiocyanate (5 M) to remove humic substances (Bengtson

et al., 2009). Fragments of genes encoding for bacterial 16S rRNA were amplified from the oxic and anoxic peat DNA using PCR protocols described by Lukow et al. (2000), except that 27f and 1492r PCR primers were used (Preston et al., 2012). Fragments of genes encoding for the alpha-subunit of methanogen-specific methyl-coenzyme M reductase (*mcrA*) and archaeal 16S rRNA genes were amplified from the anoxic peat DNA using protocols from Lueders et al. (2001), and Ramakrishnan et al. (2001). Archaeal 16S rRNA genes could not be amplified from any samples from the mined site at Shippagan and one sample from the mined site at Rivière du Loup. T-RFLP analysis of *mcrA*, bacterial 16S rRNA, and archaeal 16S rRNA gene products followed Ramakrishnan et al. (2001) and Lukow et al. (2000). Relative abundances of individual operational taxonomic units (OTUs) were calculated as the intensities of single peaks larger than 50 bp as a fraction of the sum of all peaks. Peaks with relative intensities less than 1% of the total were removed.

Individual bacterial and archaeal 16S rDNA sequences were isolated from PCR products from anoxic samples with the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA). Cells from transformed colonies were used in direct-colony PCRs using the included vector-specific M13 primer pair for bacterial 16S rRNA genes or the original 109af-912ar primer pair for archaeal 16S rRNA genes. Ninety-eight bacterial and 138 archaeal PCR products with the correct-size amplification products were purified with the GenElute PCR CleanUp-Kit and sequenced with a 48 lane ABI 377 sequencing instrument (P-E Applied Biosystems, Foster City, CA). Sequence similarity was determined using the contig formation function in SeqMan (Lasergene, DNASTAR, Madison, WI, USA) and in cases where sequences were >98% similar over at least 600 bp length, one sequence was chosen for further phylogenetic analysis. For bacteria, the longest sequence with few base-assignment uncertainties was chosen within each >98%-similar contig for further phylogenetic analysis. For archaeal sequences, reverse sequences were also obtained and assembled with the forward sequence. Sequences were deposited in GenBank under the accession numbers JQ934752–JQ934792. Similar sequences from environmental samples and cultured organisms were identified using BLASTN 2.2.21+ searches (Zhang et al., 2000) and included in further analysis. Alignment and phylogenetic tree construction used the MEGA v5 software package with neighbor-joining and maximum composite likelihood methods (Tamura et al., 2011). GeneBank sequences had at least 598 bp of coverage with those from this study. *In silico* T-RFLP analysis was performed on cloned sequences using restriction digest tools in BioEdit Sequence Alignments Editor v 7.0.9.0 (Hall, 1999), and real T-RFs were putatively phylogenetically identified when possible.

STATISTICAL ANALYSES

T-RFLP data alone (i.e., no clone library data) were used for all community structure analyses. OTU richness, evenness, and Simpson's diversity (Simpson, 1949) indices were calculated. Evenness refers to the pattern of distribution of the individuals between the OTUs and compares the observed Shannon diversity index against an equal distribution of OTUs that would maximize diversity (Krebs, 1999). Analyses of variance with Tukey *post-hoc*

tests were performed on SYSTAT 10 (SPSS Inc. Chicago, IL, USA) to compare inter-site gas fluxes and diversity indices within and between Rivière du Loup and Shippagan. Links between peat properties or CO₂ and CH₄ fluxes and diversity indices were explored through Pearson correlation with Bonferroni probabilities. Agglomerative hierarchical cluster analysis (CA) was performed using Ward's method among samples to characterize similarities based on bacterial and archaeal OTU presence and abundance using PC ORD version 4.0 software (MJN Software Design, Gleneden Beach OR, USA). To further examine differences or similarities between sites and samples and to determine if any peat properties, including CO₂ and CH₄ fluxes in incubations, potentially described (predicted) any of the variation in the OTU data, Canonical correspondence analysis (CCA) was performed on CANOCO version 4.0 (Microcomputer Power, Ithaca, NY, USA).

RESULTS

PHYLOGENETIC CHARACTERIZATION OF BACTERIA AND ARCHAEA

Bacteria from 5 phyla were detected and many of the sequences were similar to sequences or isolates from other peat environments and mineral soils distributed globally (Dedysh, 2011; Figure 1). Six identical or nearly identical bacterial sequences were found in both Shippagan and Rivière du Loup (Table 1). On average 57 and 75% of T-RFs could be linked to particular clone sequences for oxic and anoxic samples, respectively. These T-RFs represented 7 of 8 encountered classes or phyla (no T-RFs corresponding to *Verrucomicrobia* were greater than 1% of total community peak height); however, there were no apparent land-use, inter-site, or inter-location differences based on *in silico* community composition analyses (Figure 2, Table 1). The T-RFs corresponding to the phylum Acidobacteria represented the dominant OTU in 3 out of 8 sites in the oxic incubations and in all sites in the anoxic incubations (Figure 2). All of the main bacterial groups were represented in both oxic and anoxic samples except the class *Clostridia*, which was not present in oxic samples (Figure 2). In general, the relative abundance of *Actinobacteria* and *Betaproteobacteria* decreased in anoxic compared to oxic samples (Figures 2A,B).

Euryarchaeota detected in the anoxic samples were closely related to members of the genus *Methanobacterium* and similar sequences retrieved from North American, Finnish, and German peat soils and rice field soils (Figure 3). Others were related to members of the methanogenic group Rice Cluster II that have previously been detected in North American and UK peat bogs (Hales et al., 1996; Basiliko et al., 2003; Cadillo-Quiroz et al., 2008; Figure 3). Sequences were also retrieved that have no close relatives among described archaea, but are similar to sequences detected in rich field soil, a Finnish fen and a USA mine biofilm (Lu and Conrad, 2005; Baker et al., 2006; Conrad et al., 2008; Juottonen et al., 2008; Figure 3). Crenarchaeotal 16S rRNA gene sequences were closely related to some other sequences detected in soils including moorlands (Jurgens and Saano, 1999; Kemnitz et al., 2007; Nicol et al., 2007; Lesaulnier et al., 2008; Figure 3). No single archaeal T-RF was dominant in most samples; however, unknown *Euryarchaeota* were generally relatively more abundant



FIGURE 1 | Bacterial 16S rRNA gene-based phylogenetic tree (neighbor-joining method) of representative sequences retrieved from sites at Rivière du Loup and Shippagan in bold and similar sequences from GenBank. Distances were computed using the maximum composite likelihood method in the MEGA v5 package. Vertical bars and labels refer to phyla. In reference to Figure 2, sequences with open circles, black squares,

and black upward pointing triangles represent classes in the phylum Proteobacteria, black circles represent the phylum Acidobacteria, black diamonds represent the phylum Actinobacteria, open and black upward pointing triangles represent classes in the phylum Firmicutes, and open squares represent the phylum Verrucomicrobia. Scale bar units are the number of base substitutions per site.

Table 1 | Terminal restriction fragment lengths, taxonomic affiliations, and source sites of sequences.

Bacteria				Archaea			
Seq no.	T-RF (bp)	Taxonomic affiliation	Found in:	Seq no.	T-RF (bp)	Taxonomic affiliation	Found in:
1	265	<i>Acidobacteria</i>	RDL Abd, SHP Nat	2	490	Rice Cluster II	SHP Nat
2	262	<i>Acidobacteria</i>	RDL Nat, RDL Har, SHP Nat, SHP Rst	4	184	unknown <i>Crenarchaeota</i>	RDL Nat, RDL Rst
3	262	<i>Acidobacteria</i>	RDL Abd, SHP Abd, SHP Rst	6	391	Rice Cluster II	RDL Abd
4	264	<i>Acidobacteria</i>	RDL Nat, RDL Rst	8	184	unknown <i>Crenarchaeota</i>	RDL Nat
5	261	<i>Acidobacteria</i>	RDL Har	9	184	unknown <i>Crenarchaeota</i>	RDL Nat, RDL Rst
6	267	<i>Acidobacteria</i>	RDL Har, SHP Rst	10	89	<i>Methanobacteria</i>	SHP Rst
7	264	<i>Acidobacteria</i>	SHP Rst	11	184	unknown <i>Crenarchaeota</i>	RDL Nat
8	264	<i>Acidobacteria</i>	RDL Abd	12	184	unknown <i>Crenarchaeota</i>	RDL Rst
9	149	<i>Acidobacteria</i>	SHP Rst	13	184	unknown <i>Crenarchaeota</i>	RDL Nat
10	265	<i>Acidobacteria</i>	SHP Rst	14	184	unknown <i>Crenarchaeota</i>	RDL Rst
11	264	<i>Acidobacteria</i>	RDL Nat	17	89	<i>Methanobacteria</i>	SHP Rst
12	94	<i>Acidobacteria</i>	RDL Adb, RDL Rst	21	184	unknown <i>Crenarchaeota</i>	RDL Rst
16	156	<i>Alphaproteobacteria</i>	RDL Nat, SHP abd	22	184	unknown <i>Crenarchaeota</i>	RDL Nat
18	148	<i>Alphaproteobacteria</i>	RDL Rst	31	805	unknown <i>Euryarchaeota</i>	RDL Min
19	69	<i>Actinobacteria</i>	RDL Har	32	184	unknown <i>Euryarchaeota</i>	SHP Rst
21	145	<i>Actinobacteria</i>	RDL Abd, SHP Abd				
22	135	<i>Betaproteobacteria</i>	RDL Abd, RDL Rst				
24	134	<i>Betaproteobacteria</i>	RDL Nat				
25	437	<i>Alphaproteobacteria</i>	SPH Abd				
27	140	<i>Betaproteobacteria</i>	RDL Har				
28	490	<i>Gammaproteobacteria</i>	SHP Nat				
29	134	<i>Actinobacteria</i>	SHP Nat				
31	181	<i>Acidobacteria</i>	RDL Rst				
39	276	<i>Clostridia</i>	RDL Nat, RDL Rst				
43	119	<i>Bacilli</i>	RDL Nat				
47	597	<i>Verrucomicrobia</i>	RDL Rst				

in the Rivière du Loup sites and the *Methanobacteria* were generally more abundant at the Shippagan sites (Figure 2C). Gene fragments of *mcrA* could only be amplified from the natural and restored samples from Shippagan. Samples from the Shippagan

natural site were dominated with a T-RF that corresponded to a group of methanogenic *Euryarchaeota* known as Rice Cluster I with recent isolates from the newly described order *Methanocellales* and the family *Methanobacteriaceae* (Lueders et al.,

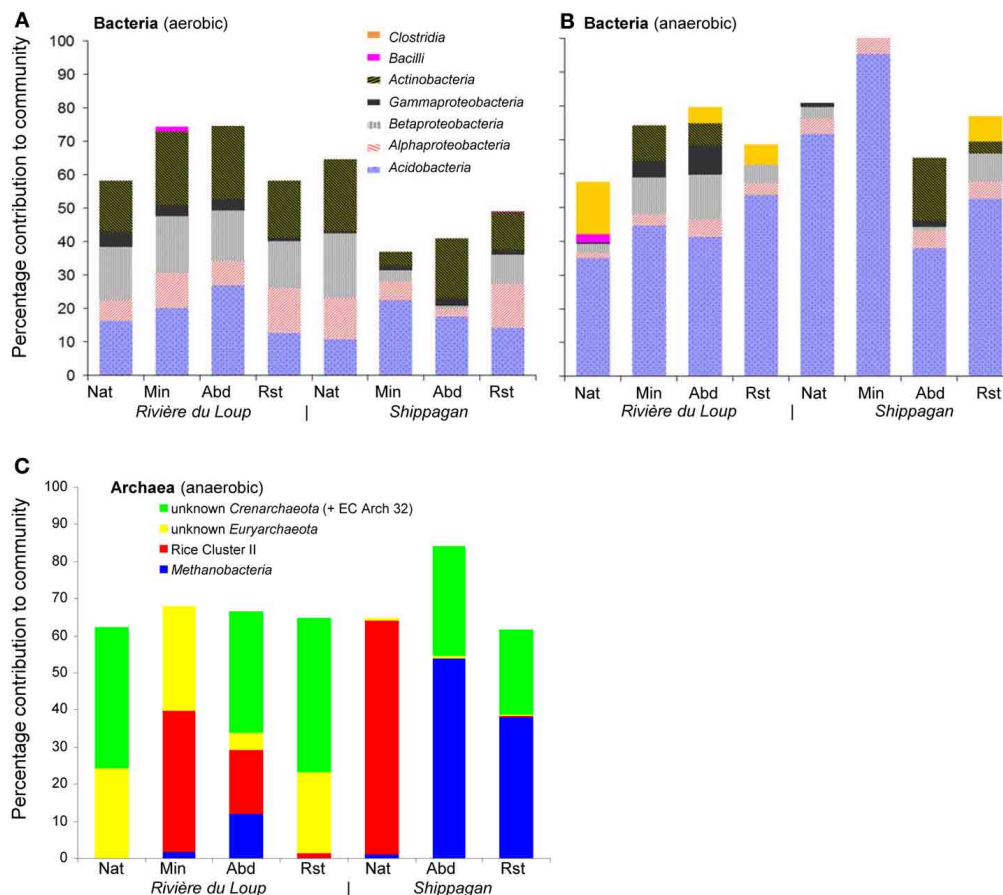


FIGURE 2 | Bacterial (A and B) and archaeal (C) community compositions (averaged across replicates) based on *in silico* mapping of terminal restriction sites of cloned sequences across natural (Nat), actively mined (Min), mined and abandoned (Abd), and mined and

restored (Rst) sites at Rivière du Loup and Shippagan. Unlabeled portions of each community (i.e., where bars did not add up to 100%) were a result of not being able to assign T-RFs to specific clone library sequences isolated from the anoxic samples.

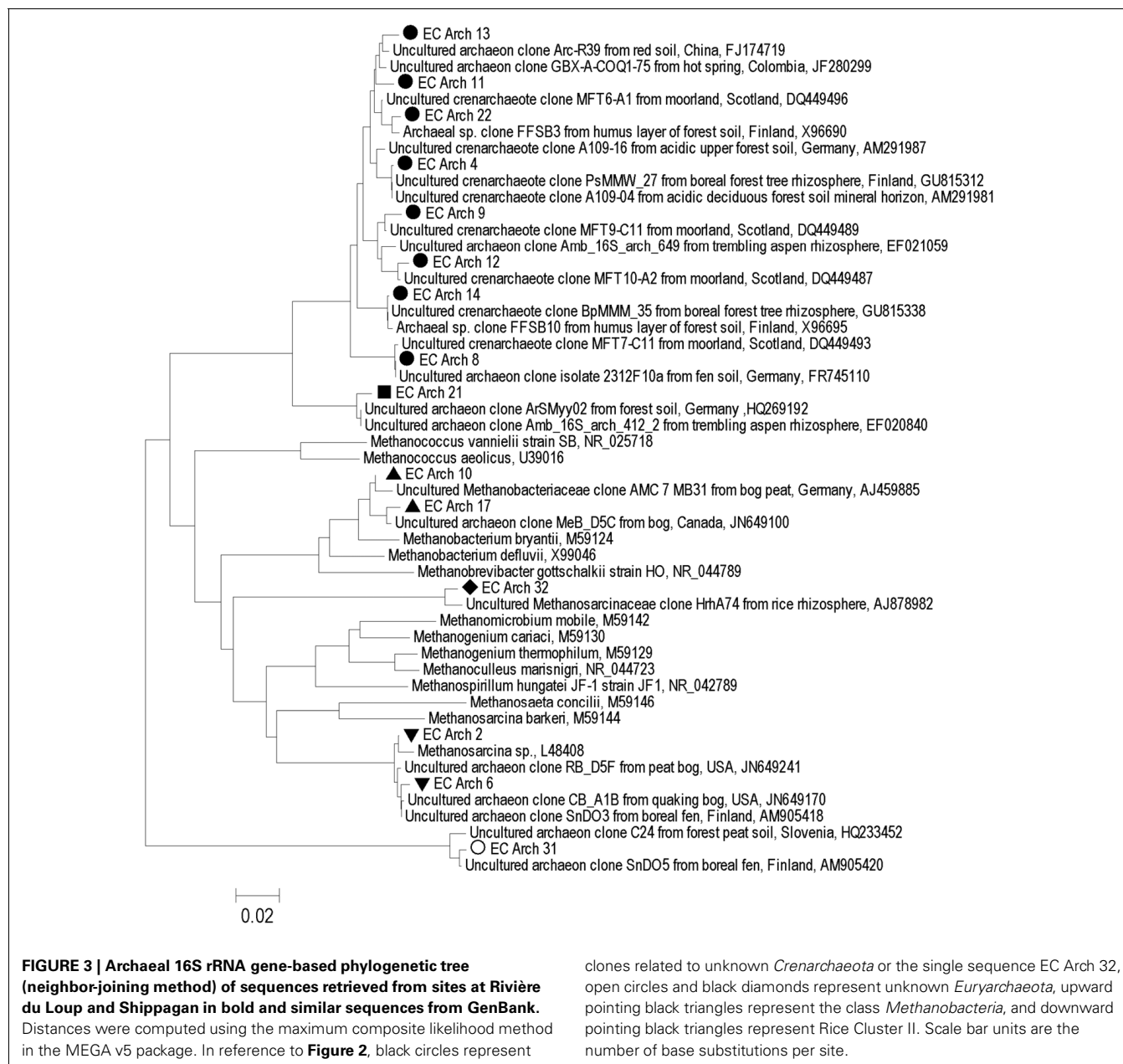
2001), despite our analyses of 16S rRNA gene clone sequences and T-RFLP analysis not detecting the former group. The 237-bp T-RF might also indicate members of Rice Cluster II or other poorly described methanogenic *Euryarchaeota*.

LAND USE EFFECTS ON MICROBIAL COMMUNITY AND LINKAGES AMONG MEASURED VARIABLES

CA and CCA indicated that community structure varied with land-use and between locations (Figures 4, 5, Table 2). In particular, CA of aerobic bacterial taxa grouped most replicate samples together within sites and Rivière du Loup and Shippagan did not have similar community structures (Figure 4). CCA of aerobic bacterial taxa clearly separated mined and abandoned sites from natural and restored sites at Shippagan, indicating that mining and abandonment led to changes in community structure, while restoration returned communities to a state similar more to natural communities (Figure 5). Differences in sites at Rivière du Loup could not be resolved well on the same CCA plot, indicating that community changes through mining and restoration were more pronounced at Shippagan, consistent with CA results. For anaerobic bacterial communities, CA indicated more similarity

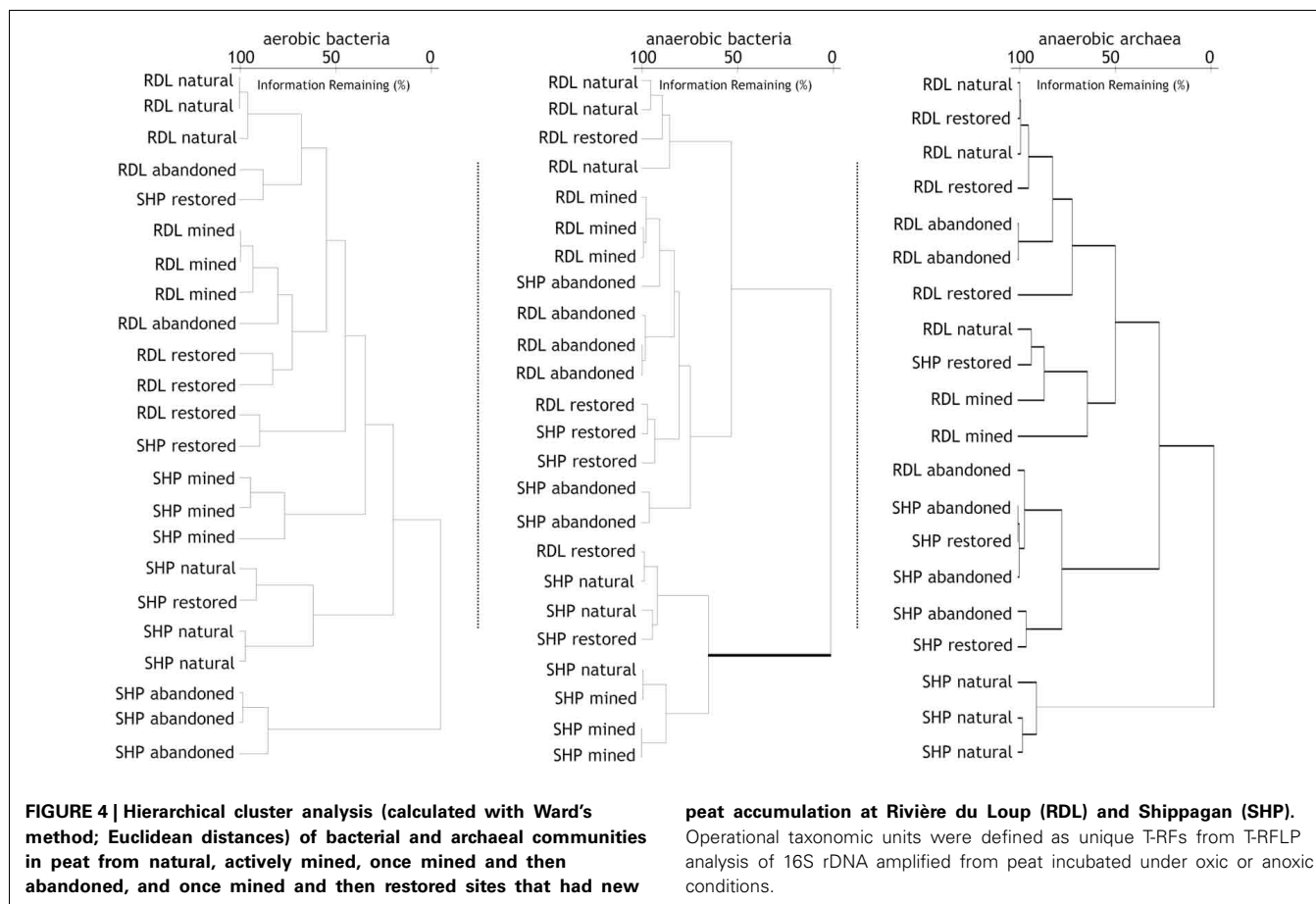
between sites, likely resulting from substantially increased dominance of *Acidobacteria* in all samples. CCA illustrated clearer differences between sites and locations, indicating both land-use and geographical differences, with the exception of the restored sites that had similar community structure and were clustered together. Although this contrasts with CA results, it is important to note that in defining the axes, CCA down-weighted the importance of the universally-dominant OTU, resulting in less apparent similarity between sites and locations; CCA axes were most heavily defined by members of the class *Gammaproteobacteria* and the phylum *Actinobacteria* in the anoxic incubations. Archaeal communities were largely different between samples from Rivière du Loup and Shippagan based on CA and CCA clustering patterns, however with the exceptions of the replicates at the natural sites clustering together in the CA, restoration did not lead to clear changes in archaeal community structure resolved in CA or CCA.

Diversity indices generally did not illustrate effects of mining, abandonment, and restoration within Rivière du Loup or Shippagan for aerobic and anaerobic bacterial communities (Table 3). Geographic differences were also not strong. The abandoned site at Rivière du Loup had the greatest archaeal OTU



richness, evenness, and Simpson's diversity and had significantly greater values for richness than all other sites. The natural and abandoned sites at Shippagan had significantly greater values for evenness and Simpson's diversity (**Table 3**). The relationships between peat properties and diversity indices (richness, evenness, Simpson's) were markedly different between oxic and anoxic samples (**Table 4**). Aerobic bacterial OTU diversity and evenness correlated positively with peat properties characteristic of greater bioavailability, and negatively with properties characteristic of biorecalcitrance. These and similar properties correlated with primary CCA axes. In contrast, diversity and evenness of anaerobic bacteria and archaea were in some cases positively correlated with peat properties characterized as biorecalcitrant by Basiliko et al. (2007) such as the total lipid content or the humic acid fraction of

extractable dissolved organic matter. Correlation of properties on CCA axes defined by anaerobic bacterial communities, which separates sites, indicated that diversity in natural and restored sites correlated with peat properties indicative of bioavailability such as the proportion of polysaccharide to aromatic molecules and total extractable K^+ , while diversity in mined and abandoned sites correlated to peat properties indicative of biorecalcitrance (**Figure 5**, **Table 2**). Factors that might structure communities therefore appeared to be dependent on land-use, regardless of the location (Rivière du Loup or Shippagan) in which land-use occurred. Anoxic conditions led to less diverse communities dominated by *Acidobacteria*-like organisms, while the bacterial community under oxic conditions was characterized by a more even distribution of taxa. While diversity indices correlated positively to peat



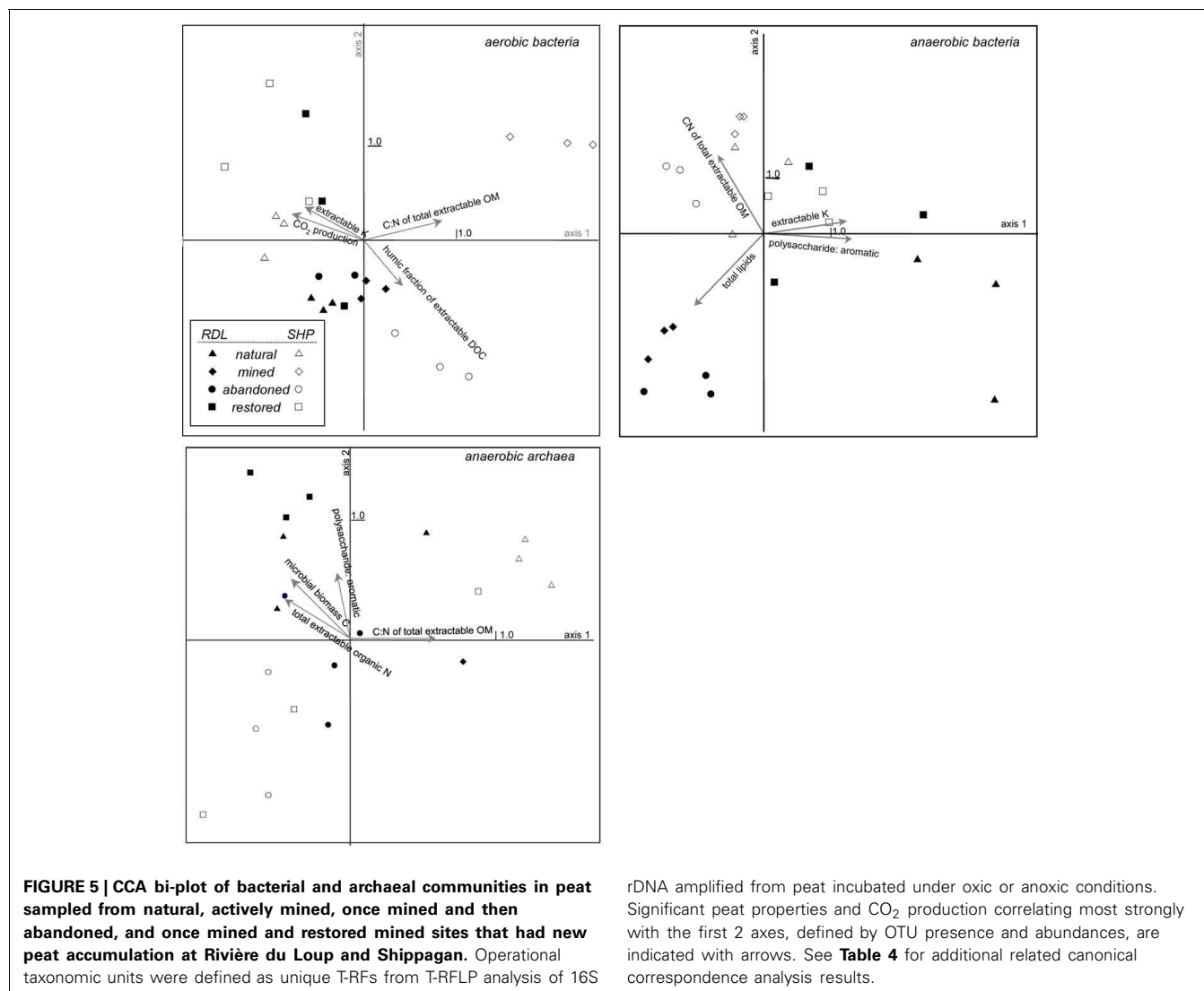
properties related to substrate bioavailability and microbial CO₂ and CH₄ production, direct correlations between diversity and activity were not significant. Aerobic CO₂ production correlated significantly with the first two CCA axes defined by aerobic bacterial OTUs, and varied primarily with bacterial communities of samples from natural and restored sites (Figure 5, Table 2).

DISCUSSION

LAND USE EFFECTS ON MICROBIAL COMMUNITY AND LINKAGES AMONG MEASURED VARIABLES

Horticultural peat mining and restoration strongly impacts substrate availability (Basiliko et al., 2007) and rates of microbial C mineralization across the eight sites in this study. However, these land-use practices did not consistently affect bacterial or archaeal diversity indices or community composition based on relative proportions of broad phylogenetic groups (phylum and/or class level) of bacteria or archaea. Multivariate analyses based on T-RF-defined OTU presence and abundance could resolve differences between some sites but not clearly across land-uses. Community structure differences across the sites were small compared to overall community structure differences between the 2 locations and between oxic and anoxic incubations. The latter point is somewhat surprising given the relatively short incubation time and known insensitivity of DNA-based T-RFLP approaches (e.g., over rRNA) to detect changes in bacterial communities following

flooding (Noll et al., 2005). Although not consistent with our initial predictions, observing only weak effects of land-use change is consistent with studies in other non-mined/restored North American and European bogs and fens, where bacterial community structure was similar across sites that differed in peat physicochemical characteristics and vegetation (Kim et al., 2008; Preston et al., 2012). Across peatlands in the James Bay lowlands region of Canada a similar pattern was also described for archaeal community structure (Preston et al., 2012). Considering that plant communities were similar across the four natural and restored sites in this study, patterns observed for bacterial and archaeal communities contrast with previously reported controls on fungal communities where vegetation was a strong predictor of community structure. In particular, litter chemistry has been shown to play a larger role than water table position in structuring fungal communities (Trinder et al., 2008). Artz et al. (2007) studied a set of mined peatlands, including some that had revegetated a few years previously to others that revegetated >50 years previously, and linked fungal community structure primarily to the successional stage of the plant communities and related chemical differences. It is becoming clear that fungal community structure and function are strongly related to vegetation on previously mined and restoring peatlands (Artz et al., 2007; Trinder et al., 2008, 2009). However, based on the present work, the same patterns are not strong for prokaryotic decomposers.



Although land-use changes across the two locations did not lead to consistent changes in community structure, there were relationships between peat substrate characteristics and bacterial or archaeal community structure across all samples analyzed. That the same factors did not predict high OTU richness or evenness in the oxic vs. anoxic incubations perhaps highlights the importance of water table position and oxygen availability or other short-term conditions over broader land-use related peat substrate changes as a control on microbial community structure. Across other soils types, links between substrate availability and richness or evenness have also been inconsistent (e.g., Nüsslein and Tiedje, 1998; Zhou et al., 2002). In this study we used 4 metrics of characterizing and comparing prokaryote communities based on T-RFLP data (CA, correspondence analysis, single-value diversity metrics, and comparison of proportions of coarse-scale taxonomic groups), and there were some subtle differences in patterns across land-use between the different data analysis techniques. However, differences across location and between aerobic vs. anaerobic communities were more consistent

regardless of the metric. This highlights that land-use effects on prokaryotic community structure were indeed small.

LINKING MICROBIAL COMMUNITY STRUCTURE AND GREENHOUSE GAS PRODUCTION

We had predicted clearer patterns between bacterial and archaeal OTU richness and community structure and rates of CO₂ and CH₄ production, as has been reported for methanogens and methanogenesis rates across peatland trophic gradients using T-RFLP (e.g., Godin et al., 2012) and other community fingerprinting techniques (e.g., Basiliko et al., 2003). However, only aerobic CO₂ production correlated significantly with changes in bacterial community structure elucidated with CCA, but not with any other metric. One potential explanation for the lack of observed relationships might be that we did not target the entire decomposer community, which would have included microfauna and fungi. A second explanation might be that the same taxa can potentially function at a large range of rates across different environments. A third explanation might

Table 2 | Canonical correspondence analysis results related to Figure 5: (A) inter-set correlation coefficients of variables (CO₂ production potential and peat properties) correlating significantly and most strongly with the first and second axes and (B) axis summary statistics for aerobic bacteria (1), anaerobic bacteria (2), and anaerobic archaea (3).

	Axis 1	Axis 2	Axis 3	Axis 4	Total inertia
(A)					
Aerobic bacteria					
Humic fraction of DOC	0.4333	−0.5639	0.1017	0.2183	
Water-extractable K ⁺	−0.5885	0.4775	0.0733	−0.4254	
C:N of total extractable DOM	0.8605	0.2133	0.1655	0.1512	
CO ₂ production potential	−0.6076	0.4418	−0.0571	−0.2436	
Anaerobic bacteria					
Total lipids	−0.6394	−0.7246	0.0091	−0.2104	
Polysaccharide: aromatic	0.8849	−0.0364	0.3875	0.0508	
Water-extractable K ⁺	0.8947	0.1093	−0.2826	−0.0232	
C:N of total extractable DOM	−0.4202	0.7629	0.4151	−0.224	
Archaea					
Polysaccharide: aromatic	−0.1363	0.4924	0.1029	0.2064	
Microbial biomass C	−0.4522	0.4385	0.1435	0.3374	
Total extractable organic N	−0.5246	0.3478	0.1323	0.2877	
C:N of total extractable DON	0.6648	−0.0204	−0.3333	0.4097	
(B)					
Aerobic bacteria					
Eigenvalues	0.226	0.163	0.102	0.082	1.195
OTUs-peat property correlations	0.966	0.953	0.956	0.949	
Cumulative percentage variance of:					
OTU data	18.9	32.6	41.1	47.9	
OTU-environment relation	31.1	53.5	67.5	78.7	
Sum of all unconstrained eigenvalues					1.195
Sum of all canonical eigenvalues					0.727
Anaerobic bacteria					
Eigenvalues	0.303	0.208	0.107	0.061	1.81
OTUs-peat property correlations	0.926	0.955	0.827	0.691	
Cumulative percentage variance of:					
OTU data	16.7	28.2	34.2	37.6	
OTU-environment relation	38.1	64.3	77.8	85.5	
Sum of all unconstrained eigenvalues					1.81
Sum of all canonical eigenvalues					0.795
Archaea					
Eigenvalues	0.546	0.484	0.445	0.403	5.094
OTUs-peat property correlations	0.923	0.865	0.944	0.857	
Cumulative percentage variance of:					
OTU data	10.7	20.2	29	36.9	
OTU-environment relation	21.2	40	57.3	072.9	
Sum of all unconstrained eigenvalues					5.094

involve limitations of our community fingerprinting methodology. Although T-RFLP analysis is a widely used rapid microbial community profiling approach that can illustrate differences in community structure of dominant taxa, T-RF-defined OTUs using SSU rDNA might not clearly separate functionally different groups of bacteria or archaea based on rates of CO₂ or CH₄ production. Other studies of community structure-activity relationships across peat and mineral soil environmental gradients

relying on coarse-scale resolution of defined taxa have reported functional redundancy (Rousk et al., 2009; Myers et al., 2012). Use of high-throughput sequencing approaches might have overcome some of the limitations of T-RFLP analyses, with both lower detection limits and the ability to define OTUs based on partial DNA sequences, rather than restriction fragments that do not necessarily correspond to functional differences among defined taxa.

Table 3 | Carbon dioxide and CH₄ flux potentials and OTU richness, evenness, and Simpson's diversity of microorganisms in peat from Rivière du Loup and Shippagan.

	Rivière du Loup				Shippagan			
	NAT	MIN	ABD	RST	NAT	MIN	ABD	RST
Aerobic CO ₂ production	0.26 (0.00) ^a	0.05 (0.01) ^b	0.05 (0.00) ^b	0.23 (0.03) ^a	0.21 (0.02) ^a	0.09 (0.01) ^b	0.03 (0.01) ^b	0.46 (0.10) ^c
Bacterial richness	23 (1) ^a	21 (1) ^a	22 (6) ^a	22 (4) ^a	19 (2) ^a	19 (1) ^a	18 (5) ^a	19 (1) ^a
Bacterial evenness	0.77 (0.01) ^a	0.70 (0.02) ^{a,b}	0.70 (0.10) ^{a,b}	0.71 (0.04) ^{a,b}	0.67 (0.00) ^{a,b}	0.69 (0.02) ^{a,b}	0.63 (0.06) ^b	0.67 (0.00) ^{a,b}
Simpson's index	17.5 (0.6) ^a	12.2 (2.1) ^{a,b}	11.8 (5.2) ^{a,b}	13.7 (2.3) ^{a,b}	11.0 (0.8) ^b	11.5 (1.3) ^b	8.8 (1.7) ^b	10.4 (0.3) ^b
Anaerobic CO ₂ production	0.09 (0.01) ^{a,b}	0.03 (0.00) ^{a,c}	0.03 (0.01) ^{a,c}	0.10 (0.03) ^{a,b}	0.07 (0.01) ^{a,c}	0.07 (0.00) ^{a,c}	0.03 (0.00) ^c	0.14 (0.06) ^b
CH ₄ production	0.01 (0.006) ^{a,b}	0.003 (0.003) ^{a,b}	0.001 (0.001) ^{a,b}	0.480 (0.510) ^a	0.890 (0.754) ^a	0.000 (0.005) ^b	0.001 (0.002) ^{a,b}	523 (220) ^c
Bacterial richness	13 (2) ^{a,b}	15 (2) ^a	15 (3) ^a	9 (1) ^{a,b}	7 (3) ^b	1 (1) ^c	11 (4) ^{a,b}	10 (4) ^{a,b}
Bacterial evenness	0.54 (0.03) ^a	0.54 (0.04) ^a	0.58 (0.02) ^a	0.42 (0.07) ^{a,b}	0.28 (0.12) ^b	0.04 (0.06) ^c	0.50 (0.06) ^a	0.44 (0.13) ^{a,b}
Simpson's index	5.9 (0.9) ^a	4.8 (0.8) ^a	6.2 (0.3) ^a	3.5 (1.1) ^a	2.0 (0.7) ^{b,c}	1.1 (0.2) ^{b,c}	5.0 (1.1) ^a	3.7 (1.5) ^{a,c}
Archaeal richness	7 (1) ^a	7 (1) ^a	12 (3) ^b	7 (2) ^a	6 (2) ^a	n.d. n.d.	6 (1) ^a	6 (1) ^a
Archaeal evenness	0.36 (0.05) ^{a,b}	0.35 (0.03) ^{a,b}	0.48 (0.02) ^b	0.33 (0.03) ^{a,b}	0.28 (0.11) ^a	n.d. n.d.	0.27 (0.08) ^a	0.33 (0.06) ^{a,b}
Simpson's index	3.5 (1.0) ^{a,b}	3.0 (0.4) ^{a,b}	4.7 (0.1) ^b	2.9 (0.4) ^{a,b}	2.5 (1.3) ^a	n.d. n.d.	2.4 (0.8) ^a	3.0 (0.8) ^{a,b}

Sites were natural (NAT), actively mined (MIN), mined and then abandoned (ABD), and restored trenches at block-cut mined sites that had new peat accumulation (RST). Fluxes are mg CO₂ g⁻¹ peat day⁻¹ and μg CH₄ g⁻¹ peat day⁻¹. Operational taxonomic units were defined as unique T-RFs from T-RFLP analysis of bacterial and archaeal 16S rDNA amplified from peat incubated under oxic (bacterial) and anoxic (bacterial and archaeal) conditions. Standard deviations of 3 replicates are in parentheses, and different superscripted letters denote significant differences between sites at $P < 0.05$.

PHYLOGENETIC DIVERSITY OF BACTERIA AND ARCHAEA AND SIMILARITIES TO OTHER SOIL ENVIRONMENTS

Although our relatively small clone library could not identify all taxa identified in the T-RFLP patterns, bacteria identified were generally similar to those isolated from northern peatlands, supporting the hypothesis that constraint on microbial distribution, or the “mass effect”, does not restrict potential community structure, but rather detectable members of a community arise due to local substrate and/or environmental conditions, also known as “species sorting” (Mouquet and Loreau, 2002; Van der Gucht et al., 2007; Andersen et al., 2013). That six bacterial 16S rDNA sequences were the same across the two locations that were >400 km apart also might support species sorting over the mass effect as a driver of bacterial community structure. Across sites and the two locations, members of the phylum *Acidobacteria* often predominated in bacterial communities. *Acidobacteria* are widely distributed soil bacteria capable of growth under acidic and low nutrient concentrations (Ward et al., 2009). Particularly members of the

order *Acidobacteriales* have been shown to be dominant community members in Russian and Slovenian peatlands (Dedysh et al., 2006; Kraigher et al., 2006; Ausec et al., 2009; Dedysh, 2011). Some *Acidobacteria* isolated from peat are strictly aerobic (Kulichevskaya et al., 2010) and dominant *in situ* community members under drained conditions (Ausec et al., 2009) in contrast to our findings of increased relative importance under anoxic conditions. *Clostridia*, which were not detected in oxic samples, are known to be obligate anaerobes. That *Actinobacteria* and *Betaproteobacteria* abundance decreased in anoxic (compared to oxic) samples is also consistent with a general, broad understanding of members of these classes in soils (Killham and Prosser, 2007).

Methanogen diversity as detected with T-RFLP analysis and an archaeal 16S rDNA clone library was low across locations and sites compared to other surveys of peatlands and did not include members from the families *Methanosarcinaceae* and *Methanosaetaceae* capable of growth on acetate found in peatlands elsewhere (Basiliko et al., 2003; Galand et al., 2005a;

Table 4 | Pearson correlation coefficients between peat properties and OTU richness, evenness, and Simpson's diversity among all sites at Rivière du Loup and Shippagan.

	Richness	Evenness	Simpson's diversity
AEROBIC BACTERIA			
Carboxyl: aromatic	0.459	0.549	0.667
Polysaccharide: aromatic	0.373 ($P = 0.08$)	0.532	0.686
von Post humification index		-0.375 ($P = 0.07$)	-0.484
Microbial C			0.419
Microbial N			0.428
N:P of total extractable DOM			-0.514
ANAEROBIC BACTERIA			
Extractable lipids	0.534	0.434	0.486
Humic fraction of DOC	0.576	0.542	0.619
von Post humification index	0.368 ($P = 0.08$)		
Microbial C:N	-0.475	-0.540	-0.398 ($P = 0.06$)
C:N of total extractable DOM	-0.621	-0.618	
Microbial N:P	-0.478	-0.518	
ARCHAEA			
Extractable lipids	0.618	0.598	0.524
Humic fraction of DOC	0.508	0.431 ($P = 0.06$)	
von Post humification index	0.509	0.483	0.481
C:N of total extractable DOM		-0.585	-0.512
Microbial N:P		-0.582	-0.468
Na ⁺	-0.570	-0.516	-0.396 ($P = 0.08$)
Moisture	-0.560	-0.541	-0.475

Operational taxonomic units were defined as unique T-RFs from T-RFLP analysis of bacterial and archaeal 16S rRNA genes amplified from peat incubated under aerobic (bacterial) and anaerobic (bacterial and archaeal) conditions. Only values with $P < 0.10$ are included in the table, and unless specified, all relationships are significant ($P < 0.05$).

Cadillo-Quiroz et al., 2008; Godin et al., 2012). This finding is consistent with little to no sedge presence even in the natural or restored sites, as sedges have been previously reported as a key control on acetoclastic methanogenesis (Rooney-Varga et al., 2007; Hines et al., 2008). Obligate or purportedly obligate CO₂ reducers such as members of the family *Methanomicrobiaceae* or Rice Cluster I (including new isolates of the order *Methanocellales*) common in other peatlands were also not detected. That both methanogen and crenarchaeal sequences were similar to those found in similar soil environments elsewhere gives support to species sorting (i.e., local environmental and substrate characteristics) over the mass effect (i.e., distributional constraints) in structuring the archaeal communities in the peatlands. Our community fingerprinting approach combined the detection of both methanogenic members of the phylum *Euryarchaeota* and non-methanogenic members of the *Crenarchaeota*, which might explain the lack of correlation between archaeal community structure and methanogenesis, particularly as the functional roles of the crenarchaea in peat soils are not yet known. Another factor that might have obscured relationships between the archaeal community and methanogenesis is the occurrence of anaerobic CH₄ oxidation that would have lowered observed rates (Smemo and Yavitt, 2011). Also, methanogens rely on specific substrates supplied by other microbes. Recent innovative reports by Wüst et al. (2009) and Drake et al. (2009) have demonstrated the importance of trophic interactions between fermenters and methanogens in

controlling methanogenesis, factors that were not investigated in this study.

SUMMARY

Across eight natural, mined, and restored eastern Canadian peatlands, the detected bacteria and archaea were similar to those found in other peatlands and soil environments, although methanogen phylogenetic diversity was relatively low. Despite affecting substrate availability and microbial activity, horticultural peat mining and restoration did not consistently affect bacterial or archaeal diversity indices or community composition, and land-use differences were small compared to those between peat samples from the same site incubated under either oxic or anoxic conditions. Across all samples analyzed regardless of land use, diversity and community structure did correlate with peat substrate and nutrient properties; however, the relationships were not the same under oxic and anoxic conditions and there were no consistent relationships between community structure and activity. Our findings imply that characterizing the bacterial and archaeal community structure might not help understand functional impacts of mining and restoration, as different taxa exhibit functional redundancy and/or the same taxa function at very different rates when exposed to different peat substrates. In contrast to other earlier work focusing on fungal communities across similar mined and restored peatlands, bacterial and archaeal communities appear to be more resistant or resilient to substrate changes brought about by mining and restoration.

ACKNOWLEDGMENTS

Funding came from the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants Program, the German Academic Exchange Service (DAAD),

and the German Research Foundation (DFG-Deutsche Forschungsgemeinschaft). Nina Ringleff provided laboratory assistance. Premier Horticulture and Sun Gro Horticulture kindly allowed site and sample access.

REFERENCES

- Andersen, R., Chapman, S. J., and Artz, R. R. E. (2013). Microbial communities in natural and disturbed peatlands: a review. *Soil Biol. Biochem.* 57, 979–994. doi: 10.1016/j.soilbio.2012.10.003
- Andersen, R., Francez, A. J., and Rochefort, L. (2006). The physicochemical and microbiological status of a restored bog in Québec: identification of relevant criteria to monitor success. *Soil Biol. Biochem.* 38, 1375–1387. doi: 10.1016/j.soilbio.2005.10.012
- Artz, R. R. E., Anderson, I. C., Chapman, S. J., Hagn, A., Schloter, M., Potts, J. M., et al. (2007). Changes in fungal community composition in response to vegetational succession during the natural regeneration of cutover peatlands. *Microbiol. Ecol.* 54, 508–522. doi: 10.1007/s00248-007-9220-7
- Artz, R. R. E., Chapman, S. J., Siegenthaler, A., Mitchell, E. A. D., Buttler, A., Bortoluzzi, E., et al. (2008). Functional microbial diversity in regenerating cutover peatlands responds to vegetation succession. *Appl. Soil Ecol.* 45, 1799–1809. doi: 10.1111/j.1365-2664.2008.01573.x
- Ausec, L., Kraigher, B., and Mandic-Mulec, I. (2009). Differences in the activity and bacterial community structure of drained grassland and forest peat soils. *Soil Biol. Biochem.* 41, 1874–1881. doi: 10.1016/j.soilbio.2009.06.010
- Baker, B. J., Tyson, G. W., Webb, R. I., Flanagan, J., Hugenholtz, P., Allen, E. A., et al. (2006). Lineages of acidophilic archaea revealed by community genomic analysis. *Science* 314, 1933–1935. doi: 10.1126/science.1132690
- Basiliko, N., Blodau, C., Roehm, C., Bengtson, P., and Moore, T. R. (2007). Regulation of decomposition and methane dynamics across natural, commercially mined, and restored northern peatlands. *Ecosystems* 10, 1148–1165. doi: 10.1007/s10021-007-9083-2
- Basiliko, N., Moore, T. R., Lafleur, P. M., and Roulet, N. T. (2005). Seasonal and inter-annual decomposition, microbial biomass and nitrogen dynamics in a Canadian bog. *Soil Sci.* 170, 902–905. doi: 10.1097/01.ss.0000196765.59412.14
- Basiliko, N., Yavitt, J. B., Dees, P. M., and Merkel, S. M. (2003). Methane biogeochemistry and methanogen communities in two northern peatlands, New York State. *Geomicrobiol. J.* 20, 563–577. doi: 10.1080/713851165
- Bengtson, P., Basiliko, N., Dumont, M. G., Hillis, M., Murrell, J. C., Roy, R., et al. (2009). Links between methanotroph community composition and methane oxidation in a pine forest soil. *FEMS Microbiol. Ecol.* 70, 356–366. doi: 10.1111/j.1574-6941.2009.00751.x
- Bräuer, S. L., Cadillo-Quiroz, H., Yashiro, E., Yavitt, J. B., and Zinder, S. H. (2006). Isolation of a novel acidiphilic methanogen from an acidic peat bog. *Nature* 443, 192–194. doi: 10.1038/nature04810
- Cadillo-Quiroz, H., Yashiro, E., Yavitt, J. B., and Zinder, Z. H. (2008). Characterization of the archaeal community in a minerotrophic fen and terminal restriction fragment length polymorphism-directed isolation of a novel hydrogenotrophic methanogen. *Appl. Environ. Microbiol.* 74, 2059–2068. doi: 10.1128/AEM.02222-07
- Conrad, R., Klose, M., Noll, M., Kemnitz, D., and Bodelier, P. (2008). Soil type links microbial colonization of rice roots to methane emission. *Glob. Change Biol.* 14, 657–669.
- Croft, M., Rochefort, L., and Beauchamp, C. J. (2001). Vacuum-extraction of peatlands disturbs bacterial population and microbial biomass carbon. *Appl. Soil Ecol.* 18, 1–12. doi: 10.1016/S0929-1393(01)00154-8
- Dedysh, S. N. (2011). Cultivating uncultured bacteria from northern wetlands: knowledge gained and remaining gaps. *Front. Microbiol.* 2, 184. doi: 10.3389/fmicb.2011.00184
- Dedysh, S. N., Pankratov, T. A., Belova, S. E., Kulichevskaya, I. S., and Liesack, W. (2006). Phylogenetic analysis and *in situ* identification of Bacteria community composition in an acidic *Sphagnum* peat bog. *Appl. Environ. Microbiol.* 72, 2110–2117. doi: 10.1128/AEM.72.3.2110-2117.2006
- Drake, H. L., Horn, M. A., and Wüst, P. K. (2009). Intermediary ecosystem metabolism as a main driver of methanogenesis in acidic wetland soil. *Environ. Microbiol. Rep.* 1, 307–318. doi: 10.1111/j.1758-2229.2009.00050.x
- Galand, P. E., Fritze, H., Conrad, R., and Yrjälä, K. (2005a). Pathways for methanogenesis and diversity of methanogenic archaea in three boreal peatland ecosystems. *Appl. Environ. Microbiol.* 71, 2195–2198.
- Galand, P. E., Juottonen, H., Fritze, H., and Yrjälä, K. (2005b). Methanogen communities in a drained bog: effect of ash fertilization. *Microb. Ecol.* 49, 209–217.
- Glatzel, S. N., Basiliko, N., and Moore, T. R. (2004). Carbon dioxide and methane production potentials of peats from natural, harvested and restored sites, eastern Québec, Canada. *Wetlands* 24, 261–267. doi: 10.1672/0277-5212(2004)024[0261:CDAMPP]2.0.CO;2
- Godin, A., McLaughlin, J. W., Webster, K. L., Packalen, M., and Basiliko, N. (2012). Methane and methanogen community dynamics across a boreal peatland nutrient gradient. *Soil Biol. Biochem.* 48, 96–105. doi: 10.1016/j.soilbio.2012.01.018
- Hales, B. A., Edwards, C., Ritchie, D. A., Hall, G., Pickup, R. W., and Saunders, J. R. (1996). Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis. *Appl. Environ. Microbiol.* 62, 668–675.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* 41, 95–98.
- Hines, M. E., Duddleston, K. N., Rooney-Varga, J. N., Fields, D., and Chanton, J. P. (2008). Uncoupling of acetate degradation from methane formation in Alaskan wetlands: connections to vegetation distribution. *Glob. Biogeochem. Cycles* 22, GB2017.
- Juottonen, H., Hynninen, A., Nieminen, M., Tuomivirta, T. T., Tuittila, E. S., Nousiainen, H., et al. (2012). Methane-cycling microbial communities and methane emission in natural and restored peatlands. *Appl. Environ. Microbiol.* 78, 6386–6389. doi: 10.1128/AEM.00261-12
- Juottonen, H., Tuittila, E.-S., Juutinen, S., Fritze, H., and Yrjälä, K. (2008). Seasonality of rDNA- and rRNA-derived archaeal communities and methanogenic potential in a boreal mire. *ISME J.* 2, 1157–1168. doi: 10.1038/ismej.2008.66
- Jurgens, G., and Saano, A. (1999). Diversity of soil archaea in boreal forest before and after clear-cutting and prescribed burning. *FEMS Microbiol. Ecol.* 29, 205–213.
- Kemnitz, D., Kolb, S., and Conrad, R. (2007). High abundance of *Crenarchaeota* in a temperate acidic forest soil. *FEMS Microbiol. Ecol.* 60, 442–448. doi: 10.1111/j.1574-6941.2007.00310.x
- Killham, K., and Prosser, J. I. (2007). “The Prokaryotes,” in *Soil Microbiology, Ecology, and Biochemistry*, 3rd Ed., ed E. A. Paul (Oxford: Academic Press), 119–143.
- Kim, S. Y., Lee, S. H., Freeman, C., Fenner, N., and Kang, H. (2008). Comparative analysis of soil microbial communities and their responses to the short-term drought in bog, fen, and riparian wetlands. *Soil Biol. Biochem.* 40, 2874–2880. doi: 10.1016/j.soilbio.2008.08.004
- Kraigher, B., Sres, B., Hacin, J., Ausec, L., Mahne, I., van Elsas, J. D., et al. (2006). Microbial activity and community structure in two drained fen soils in the Ljubljana Marsh. *Soil Biol. Biochem.* 38, 2762–2771. doi: 10.1016/j.soilbio.2006.04.031
- Krebs, C. J. (1999). *Ecological Methodology*. Menlo Park, CA: Benjamin Cummings Press.
- Kulichevskaya, I. S., Suzina, N. E., Liesack, W., and Dedysh, S. N. (2010). *Bryobacter aggregatus* gen. nov., sp. nov. a peat-inhabiting, aerobic chemo-organotroph from subdivision 3 of the *Acidobacteria*. *Int. J. Syst. Evol. Microbiol.* 60, 301–306.
- Lesaulnier, C., Papamichail, D., McCorkle, S., Ollivier, B., Skiena, S., Taghavi, S., et al. (2008). Elevated atmospheric CO₂ affects soil microbial diversity associated with trembling aspen. *Environ. Microbiol.* 10, 926–941. doi: 10.1111/j.1462-2920.2007.01512.x
- Lin, X., Green, S., Tfaily, M. M., Prakash, O., Konstantinidis, K. T., Corbett, J. E., et al. (2012). Microbial community structure and activity linked to contrasting biogeochemical gradients in

- bog and fen environments of the Glacial Lake Agassiz Peatland. *Appl. Environ. Microbiol.* 78, 7023–7031. doi: 10.1128/AEM.01750-12
- Lu, Y., and Conrad, R. (2005). *In situ* stable isotope probing of methanogenic Archaea in the rice rhizosphere. *Science* 309, 1088–1090. doi: 10.1126/science.1113435
- Lueders, T., Chin, K. J., Conrad, R., Friedrich, M. (2001). Molecular analyses of methyl-coenzyme M reductase alpha-subunit (*mcrA*) genes in rice field soil and enrichment cultures reveal the methanogenic phenotype of a novel archaeal lineage. *Environ. Microbiol.* 3, 194–204. doi: 10.1046/j.1462-2920.2001.00179.x
- Lukow, T., Dunfield, P. F., and Liesack, W. (2000). Use of the T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-transgenic potato plants. *FEMS Microbiol. Ecol.* 32, 241–247. doi: 10.1111/j.1574-6941.2000.tb00717.x
- Marinier, M., Glatzel, S. N., and Moore, T. R. (2004). The role of cotton-grass (*Eriophorum vaginatum*) in the exchange of CO₂ and CH₄ at two restored peatlands, eastern Canada. *Ecoscience* 11, 141–149.
- McNeil, P., and Waddington, J. M. (2003). Moisture controls on Sphagnum growth and CO₂ exchange on a cutover bog. *J. Appl. Ecol.* 40, 354–367. doi: 10.1046/j.1365-2664.2003.00790.x
- Mouquet, N., and Loreau, M. (2002). Coexistence in metacommunities: the regional similarity hypothesis. *Am. Nat.* 159, 420–426. doi: 10.1086/338996
- Myers, B., Webster, K. L., McLaughlin, J. W., and Basiliko, N. (2012). Microbial activity across a boreal peatland nutrient gradient: the role of fungi and bacteria. *Wetlands Ecol. Man.* 20, 77–88. doi: 10.1007/s11273-011-9242-2
- Nicol, G. W., Campbell, C. D., Chapman, C. J., and Prosser, J. I. (2007). Aforestation of moorland leads to changes in crenarchaeal community structure. *FEMS Microbiol. Ecol.* 60, 51–59. doi: 10.1111/j.1574-6941.2006.00258.x
- Noll, M., Matthies, D., Frenzel, P., Derakshani, M., and Liesack, W. (2005). Succession of bacterial community structure and diversity in a paddy soil oxygen gradient. *Environ. Microbiol.* 7, 382–395. doi: 10.1111/j.1462-2920.2005.00700.x
- Nüsslein, K., and Tiedje, J. M. (1998). Characterization of the dominant and rare members of a young Hawaiian soil bacterial community with small-subunit ribosomal DNA amplified from DNA fractionated on the basis of its guanine and cytosine composition. *Appl. Environ. Microbiol.* 64, 1283–1289.
- Preston, M. D., Smemo, K. A., McLaughlin, J. W., and Basiliko, N. (2012). Peatland microbial communities and decomposition processes in the James Bay lowlands, Canada. *Front. Microbiol.* 3:70. doi: 10.3389/fmicb.2012.00070
- Ramakrishnan, B., Lueders, T., Dunfield, P. F., Conrad, R., and Friedrich, M. W. (2001). Archaeal community structures in rice soils from different geographical regions before and after initiation of methane production. *FEMS Microbiol. Ecol.* 37, 175–186. doi: 10.1111/j.1574-6941.2001.tb00865.x
- Robert, E. C., Rochefort, L., and Garneau, M. (1999). Natural revegetation of two block-cut mined peatlands in eastern Canada. *Can. J. Bot.* 77, 447–459.
- Rooney-Varga, J. N., Giewat, M. W., Duddleston, K. N., Chanton, J. P., and Hines, M. E. (2007). Links between archaeal community structure, vegetation type and methanogenic pathway in Alaskan peatlands. *FEMS Microbiol. Ecol.* 60, 240–251. doi: 10.1111/j.1574-6941.2007.00278.x
- Roulet, N. T., Lafleur, P. M., Richard, P. J., Moore, T. R., Hymphreys, E. R., and Bubier, J. B. (2007). Contemporary carbon balance and late Holocene carbon accumulation in a northern peatland. *Glob. Change Biol.* 13, 397–411. doi: 10.1111/j.1365-2486.2006.01292.x
- Rousk, J., Brookes, P. C., and Bååth, E. (2009). Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Appl. Environ. Microbiol.* 75, 1589–1596. doi: 10.1128/AEM.02775-08
- Simpson, E. H. (1949). Measurement of diversity. *Nature* 163, 688. doi: 10.1038/163688a0
- Smemo, K. A., and Yavitt, J. B. (2011). Anaerobic oxidation of methane: an underappreciated aspect of methane cycling in peatland ecosystems. *Biogeosciences* 8, 779–793. doi: 10.5194/bg-8-779-2011
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28, 2731–2739. doi: 10.1093/molbev/msr121
- Trinder, C. J., Johnson, D., and Artz, R. E. (2008). Interactions among fungal community structure, litter decomposition, and depth of water table in a cutover peatland. *FEMS Microbiol. Ecol.* 64, 433–448. doi: 10.1111/j.1574-6941.2008.00487.x
- Trinder, C. J., Johnson, D., and Artz, R. E. (2009). Litter type, but not plant cover, regulates initial litter decomposition and fungal community structure in a recolonising cutover peatland. *Soil Biol. Biochem.* 41, 651–655. doi: 10.1016/j.soilbio.2008.12.006
- Tuittila, E. S., Komulainen, V. M., Vasander, H., and Laine, J. (1999). Restored cut-away peatlands as a sink for atmospheric CO₂. *Oecologia* 120, 563–574. doi: 10.1007/s004420050891
- Van der Gucht, K., Cottenie, K., Muylaert, K., Vloemans, N., Cousin, S., Declerck, S., et al. (2007). The power of species sorting: local factors drive bacterial community composition over a wide range of spatial scales. *Proc. Natl. Acad. Sci. U.S.A.* 104, 20404–20409. doi: 10.1073/pnas.0707200104
- Waddington, J. M., Strack, M., and Greenwood, M. J. (2010). Toward restoring the net carbon sink function of degraded peatlands: short-term response in CO₂ exchange to ecosystem-scale restoration. *J. Geophys. Res. Biogeosci.* 115, G01008. doi: 10.1029/2009JG001090
- Ward, N. L., Challacombe, J. F., Janssen, P. H., Henrissat, B., Coutinho, P. M., Wu, M., et al. (2009). Three Genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. *Appl. Environ. Microbiol.* 75, 2046–2056. doi: 10.1128/AEM.02294-08
- Williams, R. T., and Crawford, R. L. (1984). Methane production in Minnesota peatlands. *Appl. Environ. Microbiol.* 47, 1266–1271.
- Wüst, P. K., Horn, M. A., and Drake, H. L. (2009). Trophic links between fermenters and methanogens in a moderately acidic fen soil. *Environ. Microbiol.* 11, 1395–1409. doi: 10.1111/j.1462-2920.2009.01867.x
- Winsborough, C. L., and Basiliko, N. (2010). Fungal and bacterial activity in northern peatlands. *Geomicrobiol. J.* 27, 315–320. doi: 10.1080/01490450903424432
- Yan, W., Artz, R. R. E., and Johnson, D. (2008). Species-specific effects of plants colonizing cutover peatlands on patterns of carbon source utilization by soil microorganisms. *Soil Biol. Biochem.* 40, 544–549. doi: 10.1016/j.soilbio.2007.09.001
- Zhang, Z., Schwartz, S., Wagner, L., and Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *J. Comput. Biol.* 7, 203–214. doi: 10.1089/10665270050081478
- Zhou, J., Xia, B., Treves, D. S., Wu, L.-Y., Marsh, T. L., O'Neal, R. V., et al. (2002). Spatial and resource factors influencing high microbial diversity in soil. *Appl. Environ. Microbiol.* 68, 326–334.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 25 April 2013; accepted: 10 July 2013; published online: 31 July 2013.

Citation: Basiliko N, Henry K, Gupta V, Moore TR, Driscoll BT and Dunfield PF (2013) Controls on bacterial and archaeal community structure and greenhouse gas production in natural, mined, and restored Canadian peatlands. *Front. Microbiol.* 4:215. doi: 10.3389/fmicb.2013.00215

This article was submitted to *Frontiers in Terrestrial Microbiology*, a specialty of *Frontiers in Microbiology*.

Copyright © 2013 Basiliko, Henry, Gupta, Moore, Driscoll and Dunfield. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Metabolic adaptation and trophic strategies of soil bacteria—C1- metabolism and sulfur chemolithotrophy in *Starkeya novella*

Ulrike Kappler* and Amanda S. Nouwens

School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia

Edited by:

Per Bengtson, Lund University, Sweden

Reviewed by:

Levente Bodrossy, CSIRO Marine and Atmospheric Research, Australia
Yasuyoshi Sakai, Kyoto University, Japan

*Correspondence:

Ulrike Kappler, School of Chemistry and Molecular Biosciences, The University of Queensland, Cooper Rd Bldg 76, St. Lucia, QLD 4072, Australia
e-mail: u.kappler@uq.edu.au

The highly diverse and metabolically versatile microbial communities found in soil environments are major contributors to the global carbon, nitrogen, and sulfur cycles. We have used a combination of genome –based pathway analysis with proteomics and gene expression studies to investigate metabolic adaptation in a representative of these bacteria, *Starkeya novella*, which was originally isolated from agricultural soil. This bacterium was the first facultative sulfur chemolithoautotroph that was isolated and it is also able to grow with methanol and on over 39 substrates as a heterotroph. However, using glucose, fructose, methanol, thiosulfate as well as combinations of the carbon compounds with thiosulfate as growth substrates we have demonstrated here that contrary to the previous classification, *S. novella* is not a facultative sulfur chemolitho- and methylotroph, as the enzyme systems required for these two growth modes are always expressed at high levels. This is typical for key metabolic pathways. In addition enzymes for various pathways of carbon dioxide fixation were always expressed at high levels, even during heterotrophic growth on glucose or fructose, which suggests a role for these pathways beyond the generation of reduced carbon units for cell growth, possibly in redox balancing of metabolism. Our results then indicate that *S. novella*, a representative of the *Xanthobacteraceae* family of methylotrophic soil and freshwater dwelling bacteria, employs a mixotrophic growth strategy under all conditions tested here. As a result the contribution of this bacterium to either carbon sequestration or the release of climate active substances could vary very quickly, which has direct implications for the modeling of such processes if mixotrophy proves to be the main growth strategy for large populations of soil bacteria.

Keywords: carbon metabolism, methylotrophy, methanol, chemolithotrophy, thiosulfate, soil bacteria, *Starkeya novella*

INTRODUCTION

Agriculturally used soil surfaces account for approximately 37% of the Earth's total land area and thus the biogeochemical processes taking place in this vast ecosystem can affect the entire biosphere. Soil environments are also major contributors to global element cycles including the sulfur and carbon cycle both of which are important for agriculture (Kertesz et al., 2007; Kolb, 2009; Yuan et al., 2012).

As a result, the biological processes in soils contribute significantly to the release or consumption of a variety of climate active substances which include volatile sulfur compounds as well as methanol and carbon dioxide, both of which are important in the global carbon cycle and influence atmospheric chemistry (Kolb, 2009; Hunger et al., 2011). Methanol is known to contribute to ozone formation and is primarily produced and released by growing plants or the decay of plant materials. However, only a fraction of the methanol that is produced enters the atmosphere ($\sim 4.9 \times 10^{12}$ mol year⁻¹) while the rest is oxidized to carbon dioxide by methylo- and methanotrophic bacteria before being released (Kolb, 2009). The microbially mediated interconversion

of compounds with a beneficial effect on atmospheric processes into ones with potentially detrimental effects has become a major focus of research into microbial metabolic activities as these processes can significantly influence the composition of atmospheric gases.

At present at least 56 bacterial species isolated from soils have been reported to be capable of degrading methanol, and the majority of these appear to be facultative methylotrophs (Kolb, 2009), suggesting that they are capable of switching to other growth modes which can include (chemolitho)autotrophy and heterotrophy using other reduced carbon compounds.

While historically bacteria have been classified as either autotrophs (i.e., fixing carbon dioxide) or heterotrophs based on their ability to grow on defined media in the laboratory, it has recently been proposed that these clear cut divisions may not accurately reflect natural processes (Eiler, 2006; Kolb, 2009). In nature bacteria likely encounter multiple energy and/or carbon sources at the same time, and in most cases these would not be present in high concentrations (Eiler, 2006; Kolber, 2007). Consequently, a “mixotrophic” growth strategy would increase

the ability of bacteria to draw on multiple sources of energy rather than relying solely on hetero- or autotrophy at any given time (Eiler, 2006). However, little data on these processes are available at present despite their potential to affect microbial activities that contribute to carbon sequestration and/or the release of climate active substances.

In order to enhance understanding of trophic strategies in often highly versatile soil bacteria, we have investigated the differential expression of key metabolic pathway in the soil bacterium *Starkeya novella* (formerly *Thiobacillus novellus*) (Kelly et al., 2000) as a function of available growth substrates. *S. novella* was the first facultative sulfur oxidizing chemolithoautotroph to be isolated but is also capable of utilizing various C1 compounds, including methanol, for growth as well as at least 39 reduced carbon sources including sugars, amino sugars, amino acids, and organic acids (Starkey, 1935; Chandra and Shethna, 1977; Kelly et al., 2000; Kappler et al., 2012). This combination of metabolic traits should allow *S. novella* to contribute to both the biological sulfur and carbon cycles in various ways and depending on the prevailing growth mode its metabolic activities could either enhance carbon sequestration or the release of carbon dioxide. However, it has never been investigated if or how this bacterium makes use of the many possible growth modes that it is able to adopt. The only published studies of the consumption of sulfur compounds and sugars in *S. novella* yielded contradictory results reporting either simultaneous consumption or sequential use of energy sources (Lejohn et al., 1967; Leefeldt and Matin, 1980; Matin et al., 1980; Perez and Matin, 1980). The ability of *S. novella* to grow on C1 compounds also remains largely unexplored, with the only existing data being a report of robust growth on methanol and formate (Chandra and Shethna, 1977) and while *S. novella* sulfur metabolism was studied intensively in the 1960s and 70s (Aleem, 1965; Charles and Suzuki, 1966a,b; Oh and Suzuki, 1977a,b; Katayama Fujimura and Kuraishi, 1980), only some data that included molecular detail have been reported to date. In the last 15 years the presence of a *sox* gene cluster (*soxAX-soxYZBCDxF*) encoding a thiosulfate oxidizing multi-enzyme complex and a gene locus encoding a sulfite oxidizing enzyme (*sigEorf1-sorAB*) have been reported (Kappler et al., 2000, 2001, 2004).

Here we have used a combination of genomic, proteomic and gene expression data to identify key metabolic pathways involved in dissimilatory sulfur oxidation, utilization of C1 and other carbon compounds or growth in the presence of both types of energy sources with the aim of unraveling the relative activities of carbon sequestering and releasing pathways in *S. novella*.

METHODS

STRAINS AND GROWTH CONDITIONS

Starkeya novella DSMZ506^T was routinely grown at 28°C on modified DSMZ69 medium as described elsewhere (Wilson and Kappler, 2009). The DSMZ69 medium base was supplemented with either 100 mM methanol (M, MeOH) or 40 mM thiosulfate (TS) or a combination of the two (TS/M, TS/MeOH). For strain maintenance DSMZ69 –TS agar plates supplemented with 40 µg/ml nalidixic acid were used. For proteomics experiments, liquid cultures were grown under microaerophilic conditions

(100 ml medium in 250 ml shake flasks, 200 rpm, 28°C) to mid-late exponential growth phase, harvested by centrifugation and stored at –80°C until further use. For RNA isolation cultures were grown to mid-exponential growth phase before preservation with RNA protect bacteria reagent (Qiagen).

MOLECULAR METHODS

Standard methods were used throughout (Ausubel, 1995). Routine PCR used GoTaq Mastermix green (Promega) according to the manufacturer's instructions. Genomic DNA was isolated using the DNAzol reagent (Life Technologies). Culture samples for RNA isolation (2 or 3 ml) were preserved in 1 vol of RNA protect bacteria reagent (Qiagen), RNA was isolated using the RNeasy spin mini Kit (GE Healthcare). RNA samples were tested for gDNA contamination using PCR, only samples that failed to produce a product after 34 cycles of amplification were used for cDNA synthesis. cDNA was prepared with Superscript III (Life Technologies) using 0.5 µg of DNA-free RNA. Primer sets for use in qRT-PCR experiments (product size: 100 bp, annealing temperatures >60°C) (Table S1) were designed using Vector NTI Advance 11 (Life Technologies). qRT-PCR experiments were essentially performed as in (Kappler et al., 2005; Kappler and Nouwens, 2013) using the SYBR green Mastermix (Applied Biosystems) and 10 µL reactions. Experiments were carried out at the University of Queensland SCMB realtime PCR facility using an epMotion workstation (Eppendorf) and an Applied Biosystems 7900 realtime PCR machine.

PROTEOMICS TECHNIQUES

Cell pellets for use in MS/MS proteomics experiments were resuspended in 8 M urea, 50 mM ammonium bicarbonate pH 8.0 and disrupted using a French Pressure Cell (Aminco; 3 passes, 12000 psi). Samples were centrifuged (20,000 × g, 10 min) followed by determination of protein concentrations using the 2D Quant Kit (GE Healthcare). Between 1 and 2 mg of protein were then incubated with 5 mM DTT (30 min, 45°C) followed by treatment with 25 mM iodoacetamide (30 min, in the dark, RT). Samples were diluted 1 in 4 with 50 mM ammonium bicarbonate pH 8.0 before Trypsin Gold seq grade (Promega) was added in a 1:100 ratio. After 4 hours at 37°C the same amount of trypsin was added again and samples incubated overnight at 37°C.

Offline 2D LC-MS/MS analyses were used for shotgun proteomics as described in (Kappler and Nouwens, 2013). The equivalent of 500 µg of digested protein was diluted 1:1 with 5% ACN/0.1% TFA and desalted with a C18 Tiptip (Glygen, USA). Desalting used 100% ACN to wet resin (3 × 150 µl), 5% ACN/0.1% TFA (3 × 150 µl) for tip equilibration and wash steps, and 80% ACN/0.1% TFA (2 × 150 µl) for elution. Eluted peptides were concentrated in a SpeedVac and resuspended in 0.5% acetic acid/2% ACN. Peptides (180 µg) were separated on an Agilent 1100 chromatography system using a Zorbax 300-SCX column (5 µm, 4.6 × 50 mm) (Agilent) at 0.5 ml/min (gradient: 0–5 min, 0% buffer B; 5–25 min, 0–50% buffer B; 25–27 min, 50–80% buffer B; 27–32 min, 80% buffer B; 32–34 min 80–0% buffer B, where buffer A = 0.5% acetic acid/2% ACN and buffer B = 0.5% acetic acid/2% ACN/250 mM ammonium

acetate). Fractions (250 μ l) were collected in a microtitre plate before pooling (final no. of pooled fractions: 10) and desalted using ZipTips (Millipore, Massachusetts, USA) followed by separation using reversed-phase chromatography on a Shimadzu Prominence nanoLC system. All fractions were analyzed in triplicate at a flow rate of 30 μ l/min. Samples were first loaded on an Agilent C18 trap (0.3 \times 5 mm, 5 μ m) for 8 min, followed by separation on a Vydac Everest C18 (300 A, 5 μ m, 150 mm \times 150 μ m) column at a flow rate of 1 μ l/min. Peptides were separated on a gradient of 3–40% buffer B over 67 min followed by 40–98% buffer B over 3 min, where buffer A = 1% ACN/0.1% FA and buffer B = 80% ACN/0.1% FA was used. Eluted peptides were directly analyzed on a TripleTof 5600 instrument (ABSciex) using a Nanospray III interface. Gas and voltage settings were adjusted as required. MS-ToF scan across m/z 350–1800 was performed for 0.5 s followed by data-dependent acquisition of 20 peptides with intensity above 100 counts across m/z 40–1800 (0.05 s per spectra) using “high sensitivity” MS/MS and rolling collision energy.

MS data from triplicates of pooled samples were combined and searched using ProteinPilot v4.2 (ABSciex, Forster City CA) with the Paragon Algorithm using fasta formatted protein sequences for the finished *S. novella* genome obtained from JGI. Search parameters included trypsin as enzyme, iodoacetamide as cysteine modification, emphasis on biological modifications and “thorough” search setting. Only proteins with a ProteinPilot confidence score of 95% or better (estimated global FDR 5% or lower) were accepted. Further data analysis used the integrated microbial genomes resource (IMG; img.jgi.doe.gov) (Markowitz et al., 2012).

BIOINFORMATICS

Analysis of the *S. novella* genome to identify pathways and enzyme systems was performed using the KEGG pathways database (www.genome.jp/kegg/pathway.html) (Kanehisa et al., 2012), comparative analyses used biocyc.org (biocyc.org/) (Caspi et al., 2012), and IMG (img.jgi.doe.gov/) (Markowitz et al., 2012). In some cases BLASTP (Altschul et al., 1997) was used to confirm the absence or presence of genes not identified in other database searches

RESULTS

The high degree of metabolic flexibility that is apparent in the large number of growth substrates that can be used by *S. novella* is also very clearly reflected in the pathways encoded in its genome (Kappler et al., 2012). A partial analysis of *S. novella* initial glucose catabolism and its respiratory chain was carried out as part of the original genome analysis and revealed the presence of a pentose phosphate pathway (PPP) as well as an Entner Doudoroff (ED) pathway for the degradation of glucose (Figure 1A) while the absence of phosphofructokinase (EC 2.7.1.146) in the glycolysis pathway indicates that its primary purpose is gluconeogenesis. In addition the presence of multiple oxygen-dependent terminal reductases in the *S. novella* respiratory chain was noted (Kappler et al., 2012). However, there were no previous analyses of any enzymes or pathways involved in pyruvate metabolism, methanol degradation, and only two gene

clusters encoding sulfur oxidizing enzymes had been previously described.

PYRUVATE METABOLISM

The routes by which pyruvate produced by glucose breakdown can be utilized include oxidation to lactate via the action of one of several putative lactate dehydrogenases (Snov_0154, Snov_0198, Snov_1738, Snov_3299, Snov_4339) or to acetyl-CoA by a pyruvate dehydrogenase complex (genes Snov_1789 to Snov_1795).

Acetyl-CoA can then enter the TCA cycle, which in *S. novella* also includes a glyoxylate shunt, for complete oxidation to carbon dioxide or be converted to acetate via the action of phosphate acetyl transferase (Snov_4211) and acetate kinase (Snov_2209) (Figure 1A, Table S2).

COMPOSITION OF THE RESPIRATORY CHAIN

Following the complete oxidation of acetyl-CoA to carbon dioxide the resulting NADH/FADH₂ can enter the respiratory chain (Table 1 and Table S2) which is composed of a complete complex I (NADH: ubiquinol oxidoreductase, EC 1.6.5.3, Snov_1849–Snov_1864), a succinate dehydrogenase (complex II, EC 1.3.5.1, Snov_3317–Snov_3320), and a cytochrome *bc*₁ complex (complex III, EC 1.10.2.2, Snov_2477–Snov_2479) (Figure 1C, Table S2). In addition there are two more loci (Snov_2406–Snov_2408 and Snov_3849–Snov_3850) encoding three or two proteins, respectively, annotated as subunits of complex I, as well as two putative formate dehydrogenases (FDH) (Snov_3504–Snov_3507, put FDH-F type; Snov_3851–Snov_3852, put FDH-O/N type), an arsenite oxidase (Snov_1288–Snov_1289), and a putative LldD type lactate dehydrogenase (Snov_0680) that can also feed electrons into the respiratory chain.

This large number of dehydrogenase enzymes that can feed electrons into the respiratory chain is linked to an array of terminal reductases that can mediate both aerobic and anaerobic respiration (Figure 1C). The final step in respiration can use at least six types of cytochrome or quinol oxidases, including two aa₃ type oxidases (Snov_0584–Snov_0589 and Snov_4240–Snov_4243). The second of these two enzymes, Snov_4240 has similarity to both Cox1 (aa₃ type oxidase) and CyoB oxidases with the similarity to the Cox1 enzymes being slightly higher. Despite this the current annotation classifies the enzyme as a CyoB-type oxidase. Another gene locus (Snov_1015–Snov_1019) also encodes proteins related to the heme Cu oxidases (cyoB-type quinol oxidases) and here one protein (Snov_1016) is a fusion of the subunit I and subunit III cytochrome oxidase domains. This type of cytochrome oxidase appears to be conserved in a variety of α -, β -, and γ -Proteobacteria as well as some Planctomycetes. A *cbb*₃-type oxidase with high oxygen affinity (Snov_4464–Snov_4468) is present as well as two bd-type quinol oxidases (Snov_0619–Snov_0620 and Snov_3535–3536) which are also known for their high affinity to oxygen. Complementing the aerobic respiration are several terminal reductases known to be involved in anaerobic respiration such as nitrate reductase (Nap-type, Snov_1159–Snov_1162, EC 1.7.99.4), a cytochrome *c* dependent nitrite reductase (Snov_1147; EC 1.7.2.1), and a nitric oxide reductase (Snov_1155; EC 1.7.2.4) (Figure 1C). There are also several uncharacterized enzymes of the DMSO reductase

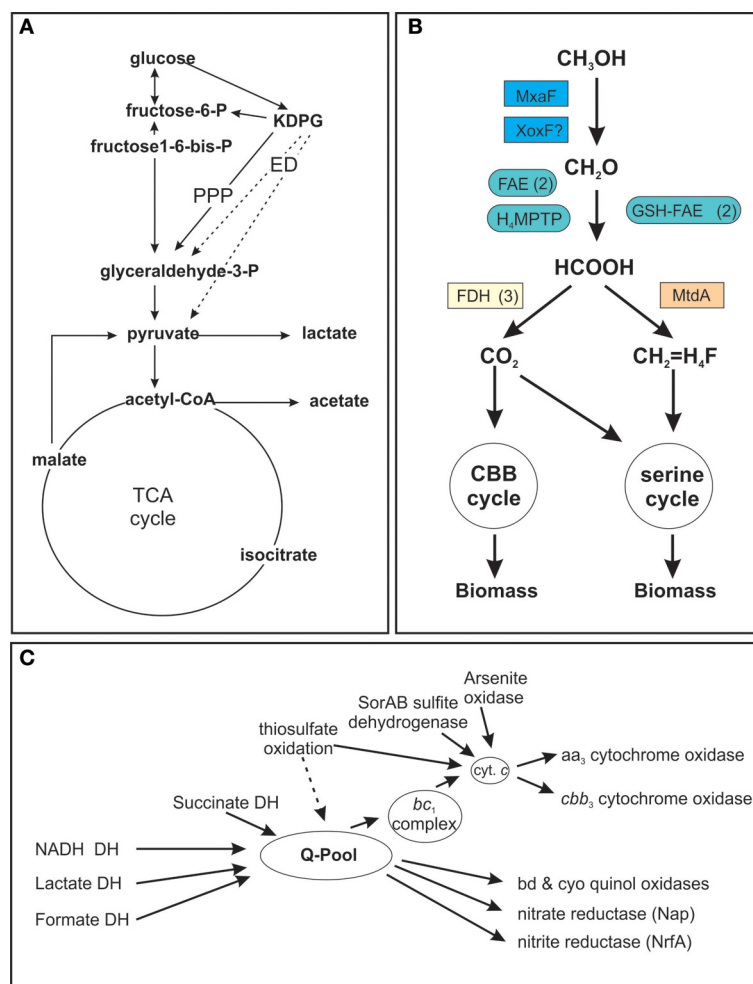


FIGURE 1 | Schematic representation of *S. novella* metabolic pathways.

(A) Glucose catabolism and TCA cycle **(B)** Methanol degradation **(C)** Respiratory chain components. **(A)** Dashed arrows denote reactions of the Entner Doudoroff (ED) pathway, PPP, Pentose Phosphate Pathway; DH, dehydrogenase, KDPG, 2-keto-3-deoxy-6-phosphocluconate. **(B):** H₄MPTP, methylene tetrahydromethanopterin pathway; MtdA, methylene

tetrahydromethanopterin dehydrogenase; FAE, formaldehyde activating enzyme; GSH-FAE, Glutathione dependent formaldehyde activating enzyme; FDH, formate dehydrogenase. **(C)** Q-pool, quinone pool. Gene numbers for all components or the different pathways are listed in the supplementary tables. Carbon metabolism—Table S2; Respiratory chain—Table S3; methanol metabolism—Table S4.

enzyme family encoded in the *S. novella* genome (Kappler and Nouwens, 2013) which may also be linked to the respiratory chain.

METABOLIC PATHWAYS AND GENES INVOLVED IN C1 METABOLISM IN *S. novella*

While it has been known for over 30 years that *S. novella* can use methanol as a growth substrate, the relevant pathways and enzymes had never been studied. Our analyses showed that the ability of *S. novella* to oxidize methanol appears to be based on a combination of pathways and enzymes similar to those of a model methylotroph, *Methylobacterium extorquens* (reviewed in Chistoserdova, 2011). A full operon encoding an MxaF type methanol dehydrogenase (Snov_4185-Snov_4199, *mxkBxHFJGARSACKLDE*) was identified as well as a *xoxF* gene locus (Snov_1035-Snov_1038) encoding a putative methanol

dehydrogenase-homolog (Figure 1B). While XoxF has been suggested to be involved in methanol oxidation in some bacteria, the exact function of this enzyme is still being investigated (Chistoserdova, 2011). Two copies each of genes encoding glutathione dependent (Snov_1125, Snov_1350) and independent putative formaldehyde-activating enzymes (FAEs) (Snov_0740, Snov_1050) (Vorholt et al., 2000; Goenrich et al., 2002) are present in the *S. novella* genome. These enzymes target the formaldehyde produced by the methanol dehydrogenases for further conversion via the tetrahydromethanopterin (TH₄MP) pathway (Chistoserdova et al., 2009; Chistoserdova, 2011). For the assimilation of carbon units into cell biomass *S. novella* contains a complete serine pathway, as well as a tetrahydrofolate (TH₄F) and a tetrahydromethanopterin (TH₄MP) pathway (Tables 1, 3 and Table S2; Figure 1B). A complete Calvin Benson Bassham (CBB)-cycle for carbon dioxide fixation is also present (Table S2).

Table 1 | Presence of proteins involved in carbon dioxide fixation and respiration in *S. novella* cell extracts from chemolithoautotrophic, methylotrophic, heterotrophic, and mixotrophic growth conditions.

	gene	EC	Glc	Glc/TS	Fruc	Fruc/TS	MeOH	MeOH/TS	TS
No. proteins identified			2725	2790	2820	2829	2420	2333	2175
CO₂ FIXATION									
ribulose bis phosphate carboxylase	Snov_0428	4.1.1.3	+++++	+++++	+++++	+++++	+++++	+++++	+++++
	Snov_0429		++++	+++++	++++	+++++	+++++	+++++	+++++
PEP carboxylase	Snov_2431	4.1.1.31	+++++	+++++	+++++	+++++	+++++	+++++	+++++
RESPIRATORY CHAIN									
NADH: ubiquinol dehydrogenase	Snov_1849	1.6.5.3	++	++	+	++	++	++	n.d.
	Snov_1859		+++++	++++	++++	++++	++++	++++	+++
succinate dehydrogenase	Snov_3318	1.3.5.1	+++++	+++++	+++++	+++++	++++	+++++	+++++
cyt b/c1 complex	Snov_2477	1.10.2.2	++++	++++	++++	++++	++	++++	+++
aa3/cyo oxidases	Snov_0584	1.9.3.1	+++	++++	++++	++++	++++	+++++	++++
	Snov_1015		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Snov_4240		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Snov_4241		(+)	n.d.	+	(+)	n.d.	n.d.	n.d.
cbb3 oxidase	Snov_4467		n.d.	n.d.	n.d.	+++	+++	(+)	+
bd-type quinol oxidases	Snov_620	1.10.3.-	n.d.	+	+	++	n.d.	(+)	n.d.
	Snov_3535		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

+, indicate expression levels, with the number of crosses indicating the band in which the protein was detected; + + + + +, top 10% of proteins; + + + +, 10–30%; + + +, 30–50%; ++, 50–70%; +, 70–90%; (+), 90–100%; n.d., not detected. Shading indicates that the protein was in the top 2% of proteins that were detected in the sample.

DISSIMILATORY SULFUR OXIDATION PATHWAYS

Although the ability of *S. novella* to grow as a sulfur chemolithoautotroph was recognized at the time of its isolation (Starkey, 1935), details of the enzymes and pathways involved have only been elucidated recently. A gene region encoding the four core enzymes of a Sox-type sulfur oxidation pathway had been identified previously (Kappler et al., 2001, 2004) and analysis of the genome showed that this gene cluster (Snov_0978–Snov_0965) is more extensive than previously recognized. The full gene cluster contains 15 genes, *soxT(R)S-soxVW-soxAX-soxYZBCDorf1Forf2*, organized in at least four separate transcriptional units as indicated (Figure 2A).

Interestingly, in the current genome annotation the *soxR* gene that encodes a regulator of thiosulfate oxidation is marked as a pseudogene, possibly due to an N-terminal truncation of the encoded protein caused by a frameshift mutation at ~bp58–62 (sequence: CCCC) in the *soxR* gene that leads to a loss of 26 aa at the N-terminus of the protein (assuming translation of the truncated protein would start at the closest ATG codon). Analysis of the genome also revealed the presence of a second, smaller *sox* gene cluster (*soxX₂Y₂Z₂A₂F₂*, genes Snov_1982–1977) which encodes duplicate copies of two of the core components of the Sox system (SoxAX, SoxYZ; the SoxB and SoxCD proteins are missing) (Figure 2A). This operon also contains genes encoding a molybdenum enzyme of the Xanthine Oxidase family (Snov_1975/Snov_1976) that was identified in an analysis

of the molybdoproteome of *S. novella* (Kappler and Nouwens, 2013).

We also analyzed the presence of other enzymes capable of oxidizing a variety of reduced sulfur compounds. A Sox complex independent sulfite oxidizing enzyme, SorAB, had already been identified previously (Kappler et al., 2000) and the enzyme itself has been extensively characterized (Kappler and Bailey, 2005; Kappler et al., 2006, 2012; Rapson et al., 2008; Bailey et al., 2009; Emesh et al., 2009). No evidence was found for the presence of Sox complex independent sulfide oxidizing enzymes such as flavocytochrome *c* and sulfide:quinone reductase. Similarly, despite early observations of a GSH-dependent sulfur oxygenase activity in *S. novella* (Charles and Suzuki, 1966a), no homologs of bacterial sulfur oxygenases were identified using the *Acidithiobacillus caldus* enzyme (acc no ZP_0529337) as the search model.

ADAPTATION OF *S. novella* METABOLISM TO THE PRESENCE OF VARIOUS CARBON AND SULFUR SOURCES

In order to investigate the importance of the enzymes and pathways identified above for metabolic adaptation of *S. novella* we analyzed protein and gene expression in *S. novella* cultures grown under heterotrophic (glucose, fructose), methylotrophic (methanol), and sulfur chemolithoautotrophic (thio-sulfate/carbon dioxide) conditions as well as under mixotrophic conditions where thiosulfate was combined with either a substrate for heterotrophic (glucose or fructose) or methylotrophic growth (methanol).

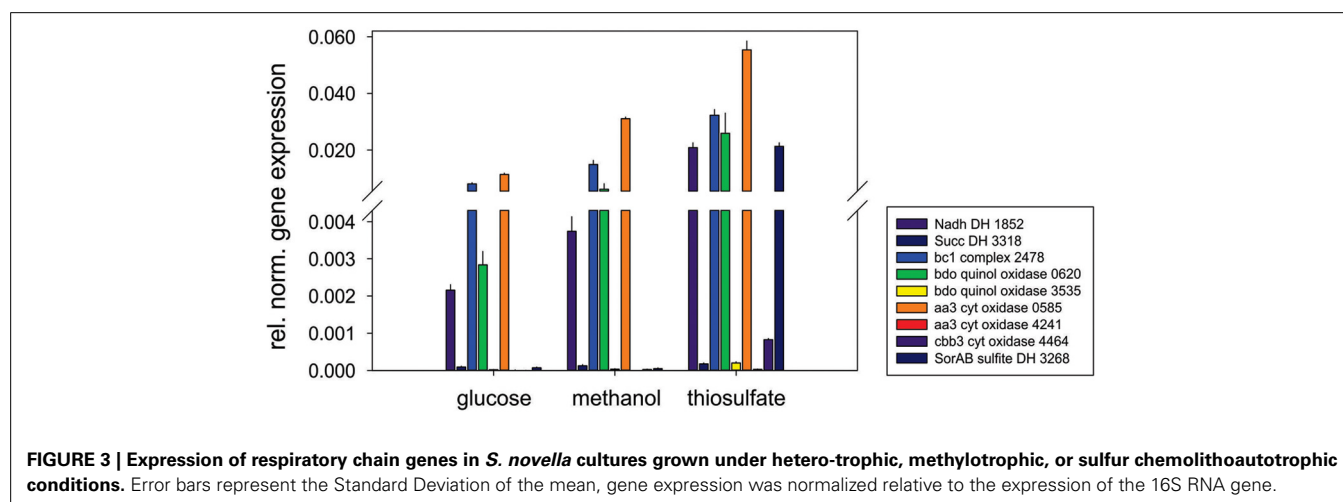
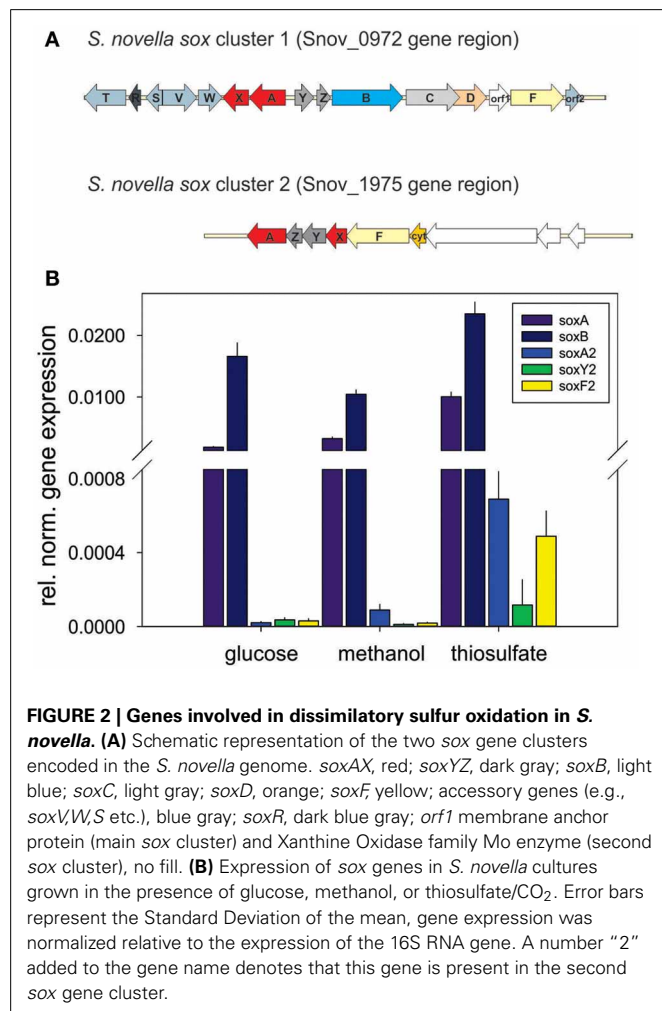
CARBON METABOLISM

As might be expected, enzymes belonging to key pathways of central carbon metabolism were detected under all conditions tested using shotgun proteomics (Table S2). These included the

pentose phosphate and glycolysis pathways (although the latter likely is used for gluconeogenesis rather than glucose oxidation), the pyruvate dehydrogenase complex and all enzymes of the TCA cycle (Table S2). Enzymes of the glyoxylate shunt were also always expressed but its key enzyme, isocitrate lyase (ICL), appeared to undergo some regulation in response to changing carbon sources, for example in the presence of glucose and during growth on thio-sulfate ICL was not detected, and during growth on fructose ICL was in the bottom 5% of proteins detected (Table S2).

Phosphoenolpyruvate carboxylase, an enzyme catalyzing an anaplerotic reaction leading to CO₂ fixation, was always present at high levels (within the top 10% of proteins detected) (Table 1), and in addition to the PPP which appeared to be the main pathway for the degradation of sugars, enzymes specific to the ED pathway were also detected, however, one of the key enzymes, 2-dehydro-3 deoxy-gluconate aldolase was not detected during growth on fructose, methanol or thiosulfate, suggesting that this pathway is not used during growth on these substrates (Table S2).

Respiratory chain complexes also showed very consistent patterns of expression with ATP synthase (Snov_4429-4433), complexes I (Snov_1852-Snov1864 gene region), II (succinate dehydrogenase, Snov_3317-Snov_3320) and the bc₁ complex being present under all conditions tested (Table 1 and Table S3). Considerable variation in expression was, however, present for the six terminal oxidase complexes encoded in the *S. novella* genome. Of the aa₃/cyo type oxidases only the aa₃ -type enzyme encoded by the Snov_0584 gene region was detected at appreciable levels using shotgun proteomics. The enzymes encoded in the Snov_1015 and Snov_4241 gene regions were either completely absent or only present at low levels under specific conditions (Glc and Fruc, bottom 10% and 15% of proteins detected). The core subunits of the cbb₃ terminal oxidase were only present when thiosulfate, methanol, methanol/thiosulfate or fructose/thiosulfate were used as growth substrates, while of the two bd-type quinol oxidases only the enzyme encoded by the Snov_0620 gene region was detected, mostly in samples from mixotrophic growth (Table 1). These results mostly match the qPCR data generated from cDNA of cultures grown on glucose, methanol or thiosulfate medium (Figure 3), with the exception of



expression of the Snov_0620 encoded bd oxidase which showed comparatively high expression levels under the three conditions tested, while only low amounts of the corresponding proteins were detected. A possible explanation for the lower levels of detection for peptides could be the association of the respiratory chain complexes with the cell membrane.

DISSIMILATORY SULFUR METABOLISM

Monitoring of the expression of enzymes for chemolithotrophic growth using sulfur compounds revealed that the previously discovered *sox* operon (Snov_0978-Snov_0968) that encodes a complete Sox-type thiosulfate oxidation enzyme complex is the main operon involved in chemolithotrophic sulfur oxidation in *S. novella* (Table 2, Figure 2). All relevant proteins were detected in all proteome samples analyzed, and several core proteins of the enzyme complex (SoxB, SoxA, and Sox C) were always among the top 100 protein detected in the samples (Table 2). Levels of the flavocytochrome *c*-like SoxF protein were lowest relative to the other complex components (within the top 40–60% of proteins). We also detected peptides for a protein encoding a putative membrane anchor protein (DUF1791 type protein), Snov_0966 or Orf1, which had previously been suggested to act as a potential membrane anchor for the *S. novella* Sox complex (Kappler et al., 2004) which had been postulated by early biochemical studies (Aleem, 1965; Charles and Suzuki, 1966a; Kappler et al., 2004). Using qRT-PCR we also found evidence for the functionality of the *soxR* “pseudogene” for which expression was detected under

all three conditions tested, with the highest levels detected in the presence of thiosulfate (Figure S1).

In contrast the SoxAX and SoxYZ proteins encoded by the second *sox* gene cluster (Snov_1982-Snov_1979) were not detected, although one subunit of the SoxF-like flavocytochrome *c* protein (Snov_1977-Snov_1978) was present at low levels (~bottom 20% of protein detected) throughout except following growth on glucose. A SoxZ-like protein encoded by a single gene (Snov_1060) was detected among the top 25–50% of proteins throughout, but the function of this protein is unclear as it is encoded by a gene that does not appear to form an operon with any of the adjacent genes, and none of these encode a SoxY-like protein.

These results are in agreement with qRT-PCR experiments that also showed high expression for genes encoded in the main *sox* cluster, and very low expression levels for proteins from the second *sox* gene cluster (Figure 2B). Both gene expression and proteomic data clearly showed that the SorAB sulfite dehydrogenase (Snov_3268/3269) is induced by the presence of thiosulfate in the growth medium regardless of the carbon source present as already reported in Kappler and Nouwens (2013).

METABOLISM OF C1-COMPOUNDS

S. novella has been classified as a facultative methylotroph since the discovery of its ability to oxidize methanol, however, similar to what was observed for sulfur chemolithotrophic growth, the MxaF methanol dehydrogenase was one of the five most abundant proteins detected under all conditions tested except when

Table 2 | Presence of proteins involved in sulfur chemolithotrophy in *S. novella* cell extracts from chemolithoautotrophic, methylotrophic, heterotrophic, and mixotrophic growth conditions.

	Gene number	Glc	Glc/TS	Fruc	Fruc/TS	MeOH	MeOH/TS	TS
Total no of proteins detected in cell extracts		2725	2790	2820	2829	2420	2333	2175
MAIN Sox CLUSTER								
SoxF	Snov_965	++	++	+++	+++	+++	+++	++
orf1	Snov_966	+++++	+++++	+++++	+++++	+++++	+++++	+++++
SoxD	Snov_967	+++++	+++++	+++++	+++++	+++++	+++++	+++++
SoxC	Snov_968	+++++	+++++	+++++	+++++	+++++	+++++	+++++
SoxB	Snov_969	+++++	+++++	+++++	+++++	+++++	+++++	+++++
SoxZ	Snov_970	+++++	+++++	+++++	+++++	+++++	+++++	+++++
SoxY	Snov_971	+++++	+++++	+++++	+++++	+++++	+++++	+++++
SoxA	Snov_972	+++++	+++++	+++++	+++++	+++++	+++++	+++++
SoxX	Snov_973	+++++	+++++	+++++	+++++	+++++	+++++	+++++
SECOND Sox CLUSTER								
Flavocyt Su B	Snov_1977	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Flavocyt Su A	Snov_1978	n.d.	(+)	+	(+)	+	+	+
SoxX2	Snov_1979	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SoxY2	Snov_1980	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SoxZ2	Snov_1981	n.d.	n.d.	n.d.	n.d.	n.d.	(+)	n.d.
SoxA2	Snov_1982	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
orphan 'SoxZ'	Snov_1060	+++	+++	+++	+++	+++	+++	+++

+, indicate expression levels, with the number of crosses indicating the band in which the protein was detected; + + + + +, top 10% of proteins; + + + +, 10–30%; + + +, 30–50%; ++, 50–70%; +, 70–90%; (+), 90–100%; n.d., not detected. Shading indicates that the protein was in the top 2% of proteins that were detected in the sample.

fructose was included in the medium where MxaF was among the top 20 proteins detected. This clearly indicates that together with sulfur chemolithotrophy, methylotrophy is another key growth mode for *S. novella* and it is also similar to what was found in *M. extorquens* (Bosch et al., 2008). While all proteins of the *mxo* operon were detected in all samples, a slight increase in protein abundance was observed when methanol was the growth substrate (Table 3 and Table S4). The second possible methanol dehydrogenase, the XoxFGJ protein, was also detected in all samples. XoxF was usually found in the top 100–200 proteins detected, except when methanol or thiosulfate were the growth substrates when XoxF was ranked approx. 70 out of over 2000 detected proteins. This indicates a role for this protein in methylotrophic and possibly also sulfur chemolithotrophic growth.

Other enzymes involved in methylotrophy such as the two glutathione dependent FAEs were either expressed at low levels (~bottom 30%–bottom 5% of proteins detected) or not at all, while of the two GSH - independent FAEs the Snov_0740 enzyme was always among the top 40–50% of protein detected and showed no obvious substrate dependent regulation. In contrast, the Snov_1050 encoded FAE was among the top 10% of proteins detected, and in the presence of methanol and/or thiosulfate the relative abundance of the protein increased (top 3% of proteins detected) clearly linking this enzyme to methylo- and chemolithotrophic growth (Table 3 and Table S4). The qRT-PCR data also showed an increase in Snov_1050 transcripts in the presence of methanol and thiosulfate (Figure 4).

A similar observation was made for the FDHs where the Snov_3851 encoded FDH was clearly induced in the presence of methanol (protein abundance increased from the top 40–50% to the top 5% of proteins), and the Snov_3504 FDH showed increased abundance in thiosulfate and methanol/thiosulfate containing samples. Again, the overall patterns observed for the gene expression data matched the shotgun proteomic data (Figure 4,

Table 3). For further assimilation of the C1 units the serine pathway, the methylene tetrahydromethanopterin pathway and the enzymes of the Calvin cycles were expressed in *S. novella* under all conditions (Figure 3, Table 1 and Table S2), with the abundance of enzymes of the latter pathway increasing in the presence of methanol, thiosulfate or a combination of the two.

DISCUSSION

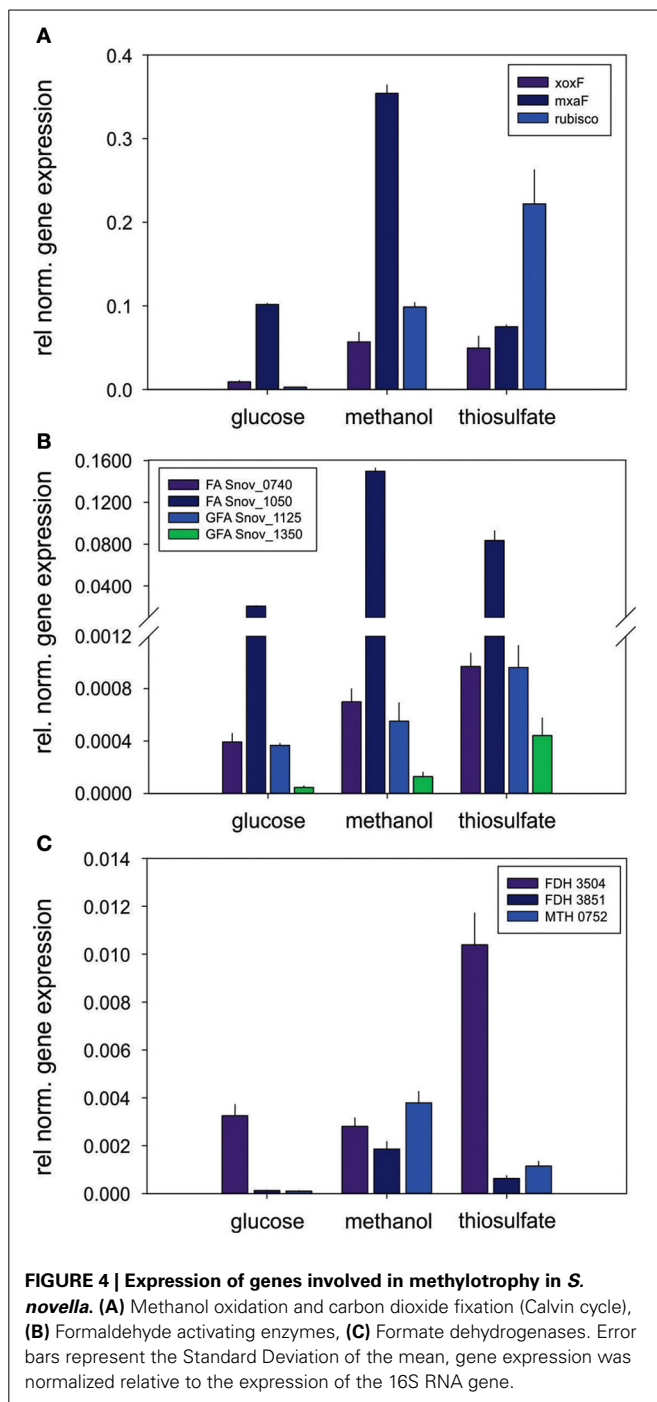
Although several proteomic studies of soil bacteria, their interactions with plants and their responses to a variety of environmental stresses (e.g., heavy metals) have been carried out (Hansmeier et al., 2006; Cheng et al., 2010; Ray et al., 2013; Van Dijk and Hecker, 2013), the interplay between different growth modes associated with carbon and sulfur metabolism have not previously been investigated in a free living soil bacterium despite their significance for global element cycles and the speciation of carbon and sulfur compounds in the environment.

The data presented above clearly demonstrate that in *S. novella*, the pathways associated with sulfur chemolitho- and methylotrophic growth modes are constantly expressed at high levels regardless of the carbon sources present and independent of auto-, mixo- or heterotrophic growth conditions. Some form of substrate dependent regulation appeared to occur for some pathway components, but in general key enzymes were found to be highly abundant. This then indicates that both methylotrophy and sulfur chemolithotrophy are key growth modes for *S. novella* which in turn raises the question whether *S. novella* should be classified as a facultative methylo- and chemolithotroph. It also appears that in the presence of methanol and thiosulfate *S. novella* does employ a mixotrophic growth strategy as cultures grown in the presence of both substrates reached culture densities that were at least twice as high as the maximum values obtained after growth on thiosulfate, indicating that methanol must have been utilized by the bacteria to increase cell growth, and cultures grown

Table 3 | Presence of proteins involved in methylotrophy *S. novella* in cell extracts from chemolithoautotrophic, methylotrophic, heterotrophic and mixotrophic growth conditions.

	Gene number	Glc	Glc/TS	Fruc	Fruc/TS	MeOH	MeOH/TS	TS
No of proteins detected		2725	2790	2820	2829	2420	2333	2175
METHANOL DEHYDROGENASES								
MxaF	Snov_4188	+++++	+++++	+++++	+++++	+++++	+++++	+++++
XoxF	Snov_1035	++++	++++	++++	++++	+++++	+++++	+++++
FORMALDEHYDE ACT. ENZYMES								
FAE1	Snov_0740	+++	+++	+++	++++	+++	++	+
FAE2	Snov_1050	+++++	+++++	+++++	+++++	+++++	+++++	+++++
GSH DEP. FORMALDEHYDE ACT. ENZYMES								
GFA1	Snov_1125	++	+	(+)	+	(+)	++	n.d
GFA2	Snov_1340	++	++	++	+	n.d	n.d	+
FORMATE DEHYDROGENASES								
FDH1	Snov_3504	+++	+++++	+++++	++++	+++	+++++	++++
FDH2	Snov_3851	+++	++	+++	+	+++++	+++++	n.d

+, indicate expression levels, with the number of crosses indicating the band in which the protein was detected; +++++, top 10% of proteins; +++++, 10–30%; +++, 30–50%; ++, 50–70%; +, 70–90%; (+), 90–100%; n.d., not detected. Shading indicates that the protein was in the top 2% of proteins that were detected in the sample.



on methanol and thiosulfate showed a strong thiosulfate dependent respiratory activity in experiments using an oxygen electrode (Kappler, unpublished).

Enzymes for various pathways allowing CO₂ fixation were also expressed under all growth conditions tested, and this included the anaplerotic reaction mediated by PEP carboxylase as well as enzymes of the Calvin and serine cycle, which suggests a role for CO₂ fixation processes in balancing metabolic fluxes and possibly also redox states. Especially the enzymes of the Calvin cycle were highly abundant in *S. novella* under all conditions tested,

indicating that it might be the major pathway for carbon dioxide fixation.

Our results are in contrast to a study by Lejohn et al. (1967) who reported catabolite repression of thiosulfate oxidation in the presence of glucose, lactate, glycerol, ribose, and pyruvate, while several amino acids, including glutamate were reported not to cause any inhibition. The results of our work agree, however, with data of a later study (Perez and Matin, 1980) which found that glucose and thiosulfate were oxidized concurrently to carbon dioxide and sulfate by *S. novella* independent of the relative concentrations of glucose and thiosulfate. As both of these studies as well as our work used the same strain of *S. novella* (DSMZ506^T = ATCC 8093^T) and very similar mineral media for the cultivation of the bacteria it is unclear what caused the observed difference in substrate utilization. Another study that investigated *S. novella* substrate utilization under nutrient limiting conditions in continuous culture (Leefeldt and Matin, 1980) also found a concurrent utilization of substrates.

In this work, proteins involved in central carbon metabolism pathways of *S. novella* were detected under all growth conditions tested, and our data thus agree with enzymatic studies of *S. novella* physiology that indicated that a complete TCA and glyoxylate cycle were present under both auto- and heterotrophic growth conditions (Charles, 1971). The presence of PEP carboxylase and RubisCO under all growth conditions tested also agrees with data from an earlier study (McCarthy and Charles, 1974), although neither the proteomic nor the qRT-PCR data detected the regulatory pattern observed by McCarthy and Charles (1974). McCarthy and Charles (1974) reported that autotrophically grown cells had much higher levels of RubisCO activity and lower levels of PEP carboxylase activity relative to heterotrophically grown cells while our data clearly indicate nearly invariant, high expression levels for both enzymes in the top 10% of proteins detected in each sample. A possible explanation for this difference could be that e.g., the enzymatic activity of RubisCO can be modulated by protein modifications and intracellular inhibitors (Jouanneau and Tabita, 1987; Wang and Tabita, 1992; Tabita, 1999), and thus the amount of protein present in the cell would not necessarily reflect the level of enzyme activity observed.

Another interesting observation is that while there are two gene clusters encoding components of a Sox-type thiosulfate oxidation pathway, only the gene cluster encoding the complete Sox complex was expressed at significant levels. This suggests that in *S. novella* the additional components of the Sox multienzyme system encoded in the second *sox* gene cluster have no functional significance as had been suggested e.g., for modulating substrate specificities through the use of isoenzymes of the SoxAX cytochromes (Frigaard and Dahl, 2008; Gregersen et al., 2011; Kappler and Maher, 2013). As at present not much is known about the roles of duplicate *sox* genes for microbial physiology the results presented here should be followed up by additional work on other bacteria that contain gene loci encoding multiple copies of *sox* genes to confirm the lack of a functional role. It is also possible that the second *sox* gene cluster in *S. novella* is important under growth conditions other than those tested here.

Of additional interest is the putative SoxR regulator encoded in the main *sox* gene cluster. Although classified in the genome

annotation as a pseudogene, qRT-PCR (**Figure S1**) clearly indicated that the gene is functional and would presumably give rise to a functional SoxR protein. Given that regulation of expression of the main *S. novella* *sox* gene cluster happens at a very high level of expression it will be interesting to determine possible functional differences between the truncated SoxR regulator from *S. novella* and the characterized, full length SoxR regulators from *P. pantotrophus* and *Pseudoaminobacter salicylatoxydans* (Rother et al., 2005; Mandal et al., 2007).

Redundancy of genes encoding various elements of the degradation pathway also characterizes the methylotrophy pathways in *S. novella* (**Table 3** and Table S4, **Figure 1**), and based on our data it was possible to clearly assign a role in methylotrophy to some of the redundant systems, such as the MxaF methanol dehydrogenase which appears to be the main methanol oxidizing enzyme, the Snov_1050 encoded FAE and the Snov_3851 encoded FDHs which were clearly methanol inducible.

Overall our results indicate that *S. novella* employs a mixotrophic growth strategy, in which several pathways for “specialized” types of metabolism such as the utilization of C1-compounds and dissimilatory energy generation from sulfur compounds are always expressed at high levels. This is in contrast to studies on other bacteria such as *Paracoccus pantotrophus* where thiosulfate oxidation was induced by the presence of thiosulfate (Robertson and Kuenen, 1983; Chandra and Friedrich, 1986; Rother et al., 2005) but is reminiscent of what has been reported for *M. extorquens*, where methanol and succinate were found to be co-metabolized and enzymes involved in methylotrophy were always expressed at high levels (Bosch et al., 2008; Peyraud et al., 2012).

It would then appear that the classification of *S. novella* as a facultative sulfur chemolithoauto- and methylotroph (Starkey, 1935; Kelly et al., 2000) does not accurately reflect the growth strategy employed by this bacterium. At this stage we can only speculate on the possible advantages inherent in this, but it is obvious that in such a bacterium production or sequestration/degradation of greenhouse active substances such as methanol or carbon dioxide would depend on the exact growth conditions encountered and the net contribution of *S. novella* to these processes might change very quickly in response to a changing environment, and soils are known to undergo significant fluctuations in many environmental parameters including oxygen and nutrient availability. This has direct implications for the modeling of microbially mediated climate relevant processes in soils, and also raises the question whether bacteria

related to *S. novella* might use a similar combination of growth modes.

S. novella is a member of the family *Xanthobacteraceae* and within this family it is most closely related to *Ancylobacter* sp. which have been isolated from soils and waterbodies and are known methylotrophs (Kelly et al., 2000; Xin et al., 2006). No complete genome sequences for *Ancylobacter* sp. are available at present, and the description of most known *Ancylobacter* sp. does not mention whether their ability to oxidize thiosulfate was tested and this trait is not mentioned in the original species description or Bergey's Manual of Systematic Bacteriology (Larkin et al., 1977; Raj, 1989; Garrity et al., 2005; Xin et al., 2006). However, in 1998 several isolates of thiosulfate oxidizing soil bacteria were identified as *Ancylobacter* sp. (Stubner et al., 1998) and a recent description of a new *Ancylobacter* species, *A. dichloromethanicus* mentions growth as a facultative thiosulfate oxidizer as a trait of the species (Firsova et al., 2009). Similarly, *Xanthobacter* species including *X. autotrophicus* are known to be able to derive energy from thiosulfate oxidation, but the trait is not part of the species description, while the utilization of methanol as a growth substrate is recognized as a trait of the species (Padden et al., 1998; Stubner et al., 1998; Garrity et al., 2005).

It would thus appear that the organisms of the family *Xanthobacteraceae* not only share similar habitats (soils, plant root systems, and freshwater, including sediments) but also share many key metabolic traits, and it will be interesting to investigate whether representatives of other species within the family share any of the regulatory features uncovered here for *Starkeya novella*. Data of this type will be of prime importance for understanding mineralization processes in soil environment as well as the impact of these processes on climate relevant processes.

ACKNOWLEDGMENTS

This work was supported by a grant and fellowship by the Australian Research council (DP0878525 and Australian Research Fellowship) to Ulrike Kappler.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://www.frontiersin.org/journal/10.3389/fmicb.2013.00304/abstract>

Figure S1 | Expression of the *S. novella* *soxR* “pseudogene” in cultures grown with glucose, methanol or thiosulfate as energy sources. Error bars represent the standard deviation of the mean. Expression data were normalized relative to the expression of the 16S gene.

REFERENCES

- Aleem, M. I. H. (1965). Thiosulfate oxidation and electron transport in *Thiobacillus novellus*. *J. Bacteriol.* 90, 95–101.
- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., et al. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25, 3389–3402. doi: 10.1093/nar/25.17.3389
- Ausubel, F. (1995). *Short Protocols in Molecular Biology: a Compendium of Methods from Current Protocols in Molecular Biology*. Hoboken, NJ: John Wiley and Sons.
- Bailey, S., Rapson, T., Winters-Johnson, K., Astashkin, A. V., Enemark, J. H., and Kappler, U. (2009). Molecular basis for enzymatic sulfite oxidation - how three conserved active site residues shape enzyme activity. *J. Biol. Chem.* 284, 2053–2063. doi: 10.1074/jbc.M807718200
- Bosch, G., Skovran, E., Xia, Q., Wang, T., Taub, F., Miller, J. A., et al. (2008). Comprehensive proteomics of *Methylobacterium extorquens* AM1 metabolism under single carbon and nonmethylotrophic conditions. *Proteomics* 8, 3494–3505. doi: 10.1002/pmic.200800152
- Caspi, R., Altman, T., Dreher, K., Fulcher, C. A., Subhraveti, P., Keseler, I. M., et al. (2012). The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. *Nucleic Acids Res.* 40, D742–D753. doi: 10.1093/nar/gkr1014
- Chandra, T. S., and Friedrich, C. G. (1986). Tn5-induced mutations affecting sulfur-oxidizing ability (Sox) of *Thiosphaera pantotropha*. *J. Bacteriol.* 166, 446–452.
- Chandra, T. S., and Shethna, Y. I. (1977). Oxalate, formate, formamide, and methanol metabolism

- in *Thiobacillus novellus*. *J. Bacteriol.* 131, 389–398.
- Charles, A. M. (1971). Effect of growth substrate on enzymes of the citric and glyoxylic acid cycles in *Thiobacillus novellus*. *Can. J. Microbiol.* 17, 617–624. doi: 10.1139/m71-101
- Charles, A. M., and Suzuki, I. (1966a). Mechanism of thiosulfate oxidation by *Thiobacillus novellus*. *Biochim. Biophys. Acta* 128, 510–521. doi: 10.1016/0926-6593(66)90012-9
- Charles, A. M., and Suzuki, I. (1966b). Purification and properties of sulfite:cytochrome *c* oxidoreductase from *Thiobacillus novellus*. *Biochim. Biophys. Acta* 128, 522–534. doi: 10.1016/0926-6593(66)90013-0
- Cheng, Z., McConkey, B. J., and Glick, B. R. (2010). Proteomic studies of plant–bacterial interactions. *Soil Biol. Biochem.* 42, 1673–1684. doi: 10.1016/j.soilbio.2010.05.033
- Chistoserdova, L. (2011). Modularity of methylotrophy, revisited. *Env. Microbiol.* 13, 2603–2622. doi: 10.1111/j.1462-2920.2011.02464.x
- Chistoserdova, L., Kalyuzhnaya, M. G., and Lidstrom, M. E. (2009). The expanding world of methylotrophic metabolism. *Annu. Rev. Microbiol.* 63, 477–499. doi: 10.1146/annurev.micro.091208.073600
- Eiler, A. (2006). Evidence for the ubiquity of mixotrophic bacteria in the upper ocean: Implications and consequences. *Appl. Env. Microbiol.* 72, 7431–7437. doi: 10.1128/AEM.01559-06
- Emesh, S., Rapson, T. D., Rajapakshe, A., Kappler, U., Bernhardt, P. V., Tollin, G., et al. (2009). Intramolecular electron transfer in sulfite-oxidizing enzymes: elucidating the role of a conserved active site arginine. *Biochemistry* 48, 2156–2163. doi: 10.1021/bi801553q
- Firsova, J., Doronina, N., Lang, E., Spröer, C., Vuilleumier, S., and Trotsenko, Y. (2009). *Ancylobacter dichloromethanicus* sp. nov. – a new aerobic facultatively methylotrophic bacterium utilizing dichloromethane. *Syst. Appl. Microbiol.* 32, 227–232. doi: 10.1016/j.syapm.2009.02.002
- Frigaard, N. U., and Dahl, C. (2008). “Sulfur metabolism in phototrophic sulfur bacteria,” in *Advances in Microbial Physiology*, ed K. P. Robert (Waltham, MA: Academic Press), 103–200.
- Garrity, G., Bell, J., and Lilburn, T. (2005). “Class I. Alphaproteobacteria class. nov,” in *Bergey’s Manual® of Systematic Bacteriology*, eds D. Brenner, N. Krieg, and J. Staley (New York, NY: Springer), 1–574. doi: 10.1007/0-387-29298-5_1
- Goenrich, M., Bartoschek, S., Hagemeyer, C. H., Griesinger, C., and Vorholt, J. A. (2002). A glutathione-dependent formaldehyde-activating Enzyme (Gfa) from *Paracoccus denitrificans* detected and purified via two-dimensional proton exchange NMR spectroscopy. *J. Biol. Chem.* 277, 3069–3072. doi: 10.1074/jbc.C100579200
- Gregersen, L. H., Bryant, D. A., and Frigaard, N.-U. (2011). Mechanisms and evolution of oxidative sulfur metabolism in green sulfur bacteria. *Front. Microbiol.* 2:116. doi: 10.3389/fmicb.2011.00116
- Hansmeier, N., Chao, T.-C., Pühler, A., Tauch, A., and Kalinowski, J. (2006). The cytosolic, cell surface and extracellular proteomes of the biotechnologically important soil bacterium *Corynebacterium efficiens* YS-314 in comparison to those of *Corynebacterium glutamicum* ATCC 13032. *Proteomics* 6, 233–250. doi: 10.1002/pmic.20050144
- Hunger, S., Schmidt, O., Hilgarth, M., Horn, M. A., Kolb, S., Conrad, R., et al. (2011). Competing formate- and carbon dioxide-utilizing prokaryotes in an anoxic methane-emitting fen soil. *Appl. Env. Microbiol.* 77, 3773–3785. doi: 10.1128/AEM.00282-11
- Jouanneau, Y., and Tabita, F. R. (1987). *In vivo* regulation of form I ribulose 1,5-bisphosphate carboxylase/oxygenase from *Rhodospseudomonas sphaeroides*. *Arch. Biochem. Biophys.* 254, 290–303. doi: 10.1016/0003-9861(87)90105-6
- Kanehisa, M., Goto, S., Sato, Y., Furumichi, M., and Tanabe, M. (2012). KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Res.* 40, D109–D114. doi: 10.1093/nar/gkr988
- Kappler, U., Aguey-Zinsou, K. F., Hanson, G. R., Bernhardt, P. V., and McEwan, A. G. (2004). Cytochrome *c*₅₅₁ from *Starkeya novella*: characterization, spectroscopic properties, and phylogeny of a di-heme protein of the SoxAX family. *J. Biol. Chem.* 279, 6252–6260. doi: 10.1074/jbc.M310644200
- Kappler, U., and Bailey, S. (2005). Molecular basis of intramolecular electron transfer in sulfite-oxidizing enzymes is revealed by high resolution structure of a heterodimeric complex of the catalytic molybdopterin subunit and a *c*-type cytochrome subunit. *J. Biol. Chem.* 280, 24999–25007. doi: 10.1074/jbc.M503237200
- Kappler, U., Bailey, S., Feng, C. J., Honeychurch, M. J., Hanson, G. R., Bernhardt, P. V., et al. (2006). Kinetic and structural evidence for the importance of Tyr236 for the integrity of the Mo active site in a bacterial sulfite dehydrogenase. *Biochemistry* 45, 9696–9705. doi: 10.1021/bi060058b
- Kappler, U., Bennett, B., Rethmeier, J., Schwarz, G., Deutzmann, R., McEwan, A. G., et al. (2000). Sulfite: cytochrome *c* oxidoreductase from *Thiobacillus novellus* – purification, characterization and molecular biology of a heterodimeric member of the sulfite oxidase family. *J. Biol. Chem.* 275, 13202–13212. doi: 10.1074/jbc.275.18.13202
- Kappler, U., Davenport, K., Beatson, S., Lucas, S., Lapidus, A., Copeland, A., et al. (2012). Complete genome sequence of the facultatively chemolithoautotrophic and methylotrophic alpha-Proteobacterium *Starkeya novella* type strain (ATCC 8093T). *Stand. Genomic Sci.* 7, 44–58. doi: 10.4056/signs.3006378
- Kappler, U., Friedrich, C. G., Truper, H. G., and Dahl, C. (2001). Evidence for two pathways of thiosulfate oxidation in *Starkeya novella* (formerly *Thiobacillus novellus*). *Arch. Microbiol.* 175, 102–111. doi: 10.1007/s002030000241
- Kappler, U., and Maher, M. (2013). The bacterial SoxAX cytochromes. *Cell. Mol. Life Sci.* 70, 977–992. doi: 10.1007/s00018-012-1098-y
- Kappler, U., and Nouwens, A. S. (2013). The molybdoproteome of *Starkeya novella* – insights into the diversity and functions of molybdenum containing proteins in response to changing growth conditions. *Metallomics* 5, 325–334. doi: 10.1039/c2mt20230a
- Kappler, U., Sly, L. I., and McEwan, A. G. (2005). Respiratory gene clusters of *Metallosphaera sedula* – differential expression and transcriptional organization. *Microbiology* 151, 35–43. doi: 10.1099/mic.0.27515-0
- Katayama Fujimura, Y., and Kuraishi, H. (1980). Characterization of *Thiobacillus novellus* and its thiosulfate oxidation. *J. Gen. Appl. Microbiol.* 26, 357–367. doi: 10.2323/jgam.26.357
- Kelly, D. P., McDonald, I. R., and Wood, A. P. (2000). Proposal for the reclassification of *Thiobacillus novellus* as *Starkeya novella* gen. nov., comb. nov., in the alpha-subclass of the Proteobacteria. *Int. J. Syst. Evol. Microbiol.* 50, 1797–1802.
- Kertesz, M. A., Fellows, E., Schmalenberger, A., and Allen, I. L. (2007). “Rhizobacteria and plant sulfur supply,” in *Advances in Applied Microbiology*, eds A. I. Laskin, S. Sariaslani and G. M. Gadd (Waltham, MA: Academic Press), 235–268.
- Kolb, S. (2009). Aerobic methanol-oxidizing Bacteria in soil. *FEMS Microbiol. Lett.* 300, 1–10. doi: 10.1111/j.1574-6968.2009.01681.x
- Kolber, Z. (2007). Energy cycle in the ocean: powering the microbial world. *Oceanography* 20, 79–88. doi: 10.5670/oceanog.2007.51
- Larkin, J. M., Williams, P. M., and Taylor, R. (1977). Taxonomy of the genus *Microcyclops* Orskov 1928: reintroduction and emendation of the genus *Spirosoma* Migula 1894 and proposal of a new genus, *Flectobacillus*. *Int. J. Syst. Bacteriol.* 27, 147–156. doi: 10.1099/00207113-27-2-147
- Leefeldt, R. H., and Matin, A. (1980). Growth and physiology of *Thiobacillus novellus* under nutrient-limited mixotrophic conditions. *J. Bacteriol.* 142, 645–650.
- Lejohn, H. B., Van-Caeseele, L., and Lees, H. (1967). Catabolite repression in the facultative chemoautotroph *Thiobacillus novellus*. *J. Bacteriol.* 94, 1484–1491.
- Mandal, S., Chatterjee, S., Dam, B., Roy, P., and Das Gupta, S. K. (2007). The dimeric repressor SoxR binds cooperatively to the promoter(s) regulating expression of the sulfur oxidation (*sox*) operon of *Pseudomonas salicylatoxidans* KCT001. *Microbiology* 153, 80–91. doi: 10.1099/mic.0.29197-0
- Markowitz, V. M., Chen, I.-M. A., Palaniappan, K., Chu, K., Szeto, E., Grechkin, Y., et al. (2012). IMG: the integrated microbial genomes database and comparative analysis system. *Nucleic Acids Res.* 40, D115–D122. doi: 10.1093/nar/gkr1044
- Matin, A., Schleiss, M., and Perez, R. C. (1980). Regulation of glucose transport and metabolism in *Thiobacillus novellus*. *J. Bacteriol.* 142, 639–644.
- McCarthy, J. T., and Charles, A. M. (1974). CO₂ fixation by the facultative autotroph *Thiobacillus novellus* during autotrophy-heterotrophy interconversions. *Can. J. Microbiol.* 20, 1577–1584. doi: 10.1139/m74-244
- Oh, J. K., and Suzuki, I. (1977a). Isolation and characterization of a membrane-associated thiosulphate-oxidising system of *Thiobacillus novellus*. *J. Gen. Microbiol.* 99,

- 397–412. doi: 10.1099/00221287-99-2-397
- Oh, J. K., and Suzuki, I. (1977b). Resolution of a membrane-associated thiosulphate-oxidising complex of *Thiobacillus novellus*. *J. Gen. Microbiol.* 99, 413–423. doi: 10.1099/00221287-99-2-413
- Padden, A. N., Kelly, D. P., and Wood, A. P. (1998). Chemolithoautotrophy and mixotrophy in the thiophene-2-carboxylic acid-utilizing *Xanthobacter tagetidis*. *Arch. Microbiol.* 169, 249–256. doi: 10.1007/s002030050568
- Perez, R. C., and Matin, A. (1980). Growth of *Thiobacillus novellus* on mixed substrates (mixotrophic growth). *J. Bacteriol.* 142, 633–638.
- Peyraud, R., Kiefer, P., Christen, P., Portais, J.-C., and Vorholt, J. A. (2012). Co-Consumption of methanol and succinate by *Methylobacterium extorquens* AM1. *PLoS ONE* 7:e48271. doi: 10.1371/journal.pone.0048271
- Raj, H. D. (1989). Oligotrophic Methylo-trophs: *Ancylobacter* (Basonym, Microcycclus' Ørskov) Raj gen. nov. *Crit. Rev. Microbiol.* 17, 89–106. doi: 10.3109/10408418909105743
- Rapson, T. D., Kappler, U., and Bernhardt, P. V. (2008). Direct catalytic electrochemistry of sulfite dehydrogenase: mechanistic insights and contrasts with related Mo enzymes. *Biochim. Biophys. Acta Bioenerg.* 1777, 1319–1325. doi: 10.1016/j.bbapbio.2008.06.005
- Ray, P., Girard, V., Gault, M., Job, C., Bonneau, M., Mandrand-Berthelot, M.-A., et al. (2013). *Pseudomonas putida* KT2440 response to nickel or cobalt induced stress by quantitative proteomics. *Metallomics* 5, 68–79. doi: 10.1039/c2mt20147j
- Robertson, L. A., and Kuenen, G. J. (1983). *Thiosphaera pantotropha* gen. nov. sp. nov., a facultatively anaerobic, facultatively autotrophic sulphur bacterium. *J. Gen. Microbiol.* 129, 2847–2855.
- Rother, D., Orawski, G., Bardischewsky, F., and Friedrich, C. G. (2005). SoxRS-mediated regulation of chemotrophic sulfur oxidation in *Paracoccus pantotrophus*. *Microbiology* 151, 1707–1716. doi: 10.1099/mic.0.27724-0
- Starkey, R. L. (1935). Isolation of some bacteria which oxidise thiosulfate. *Soil Sci.* 39, 197–219. doi: 10.1097/00010694-193503000-00004
- Stubner, S., Wind, T., and Conrad, R. (1998). Sulfur oxidation in rice field soil: Activity, enumeration, isolation and characterization of thiosulfate-oxidizing bacteria. *Syst. Appl. Microbiol.* 21, 569–578. doi: 10.1016/S0723-2020(98)80069-6
- Tabita, F. R. (1999). Microbial ribulose 1,5-bisphosphate carboxylase/oxygenase: a different perspective. *Photosynth. Res.* 60, 1–28. doi: 10.1023/A:1006211417981
- Van Dijk, J. M., and Hecker, M. (2013). *Bacillus subtilis*: from soil bacterium to super-secreting cell factory. *Microb. Cell Fact.* 12, 3. doi: 10.1186/1475-2859-12-3
- Vorholt, J. A., Marx, C. J., Lidstrom, M. E., and Thauer, R. K. (2000). Novel formaldehyde-activating enzyme in *Methylobacterium extorquens* AM1 required for growth on methanol. *J. Bacteriol.* 182, 6645–6650. doi: 10.1128/JB.182.23.6645-6650.2000
- Wang, X., and Tabita, F. R. (1992). Reversible inactivation and characterization of purified inactivated from I Ribulose-1,5-Bisphosphate Carboxylase Oxygenase of *Rhodobacter sphaeroides*. *J. Bacteriol.* 174, 3593–3600.
- Wilson, J. J., and Kappler, U. (2009). Sulfite oxidation in *Sinorhizobium meliloti*. *Biochim. Biophys. Acta* 1787, 1516–1525. doi: 10.1016/j.bbapbio.2009.07.005
- Xin, Y. H., Zhou, Y. G., and Chen, W. X. (2006). *Ancylobacter polymorphus* sp. nov. and *Ancylobacter vacuolatus* sp. nov. *Int. J. Syst. Evol. Microbiol.* 56, 1185–1188. doi: 10.1099/ijs.0.64118-0
- Yuan, H., Ge, T., Chen, C., O'Donnell, A. G., and Wu, J. (2012). Significant role for microbial autotrophy in the sequestration of soil carbon. *Appl. Env. Microbiol.* 78, 2328–2336. doi: 10.1128/AEM.06881-11

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 20 May 2013; accepted: 23 September 2013; published online: 17 October 2013.

Citation: Kappler U and Nouwens AS (2013) Metabolic adaptation and trophic strategies of soil bacteria—C1-metabolism and sulfur chemolithotrophy in *Starkeya novella*. *Front. Microbiol.* 4:304. doi: 10.3389/fmicb.2013.00304

This article was submitted to *Terrestrial Microbiology*, a section of the journal *Frontiers in Microbiology*.

Copyright © 2013 Kappler and Nouwens. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



A meta-analysis of soil microbial biomass responses to forest disturbances

Sandra R. Holden* and Kathleen K. Treseder

Department of Ecology and Evolutionary Biology, University of California, Irvine, CA, USA

Edited by:

Johannes Rousk, Lund University, Sweden

Reviewed by:

Paul Kardol, Swedish University of Agricultural Sciences, Sweden

Benjamin Sikes, University of Kansas, USA

*Correspondence:

Sandra R. Holden, Department of Ecology and Evolutionary Biology, University of California, Irvine, 321 Steinhaus Hall, Irvine, CA 92697, USA
e-mail: dooleys@uci.edu

Climate warming is likely to increase the frequency and severity of forest disturbances, with uncertain consequences for soil microbial communities and their contribution to ecosystem C dynamics. To address this uncertainty, we conducted a meta-analysis of 139 published soil microbial responses to forest disturbances. These disturbances included abiotic (fire, harvesting, storm) and biotic (insect, pathogen) disturbances. We hypothesized that soil microbial biomass would decline following forest disturbances, but that abiotic disturbances would elicit greater reductions in microbial biomass than biotic disturbances. In support of this hypothesis, across all published studies, disturbances reduced soil microbial biomass by an average of 29.4%. However, microbial responses differed between abiotic and biotic disturbances. Microbial responses were significantly negative following fires, harvest, and storms (48.7, 19.1, and 41.7% reductions in microbial biomass, respectively). In contrast, changes in soil microbial biomass following insect infestation and pathogen-induced tree mortality were non-significant, although biotic disturbances were poorly represented in the literature. When measured separately, fungal and bacterial responses to disturbances mirrored the response of the microbial community as a whole. Changes in microbial abundance following disturbance were significantly positively correlated with changes in microbial respiration. We propose that the differential effect of abiotic and biotic disturbances on microbial biomass may be attributable to differences in soil disruption and organic C removal from forests among disturbance types. Altogether, these results suggest that abiotic forest disturbances may significantly decrease soil microbial abundance, with corresponding consequences for microbial respiration. Further studies are needed on the effect of biotic disturbances on forest soil microbial communities and soil C dynamics.

Keywords: disturbance, fire, forest, harvest, insect, soil microbial biomass, pathogen, storm

INTRODUCTION

Forest ecosystems are a critical component of the global carbon (C) cycle. Boreal, temperate, and tropical forests cover ~30% of the global land surface and store ~1600 Pg C, accounting for up to 45% of global terrestrial carbon (Bonan, 2008). Forests are subject to frequent stand disturbances that can alter the amount of C stored in forests. For example, forest fires burn an average of ~40,000 km² in North American forests (Giglio et al., 2006), and ~2,000 km² in European forests each year (Schelhaas et al., 2003). Likewise, ~50,000 km² of North American forests are harvested annually (Birdsey et al., 2006). Other common forest disturbances include storms, insect outbreaks, and pathogen infection of trees (Goetz et al., 2012). These disturbances can be grouped into abiotic (fire, harvesting, storm) and biotic (insect, pathogen) disturbances. Although already common, some forest disturbances may occur more frequently and severely as a result of climate warming. For example, modeling studies predict that the burned area in Alaskan and Canadian boreal forests will increase 3.5–5.5 times by the end of the century (Balshi et al., 2009). Higher temperatures may also provide more favorable conditions for insects and pathogens, and make forests more susceptible to infestation (Dale et al., 2001). Although, insect outbreaks are not

always directly related to climatic conditions (Kardol et al., 2010). Given the large amount of C stored in forests, it is important to understand how disturbances alter ecosystem C dynamics.

Soil microbial respiration of CO₂, produced as a result of organic matter decomposition in soil, comprises a large flux of C from forest ecosystems to the atmosphere. Classic ecosystem theory predicts that the total amount of CO₂ released by soil microbes increases following forest disturbances (Odum, 1969; Chapin et al., 2002), owing to post-disturbance increases in soil temperature and C availability. Direct *in situ* measurements of microbial respiration following disturbances are scarce (but see Czimczik et al., 2006). Indirect evidence for increased microbial respiration following disturbances is derived primarily from measurements of soil C stocks (Covington, 1981) and from measurements of total soil respiration (Richter et al., 2000). However, despite the central role of microbes in decomposition and C release from soils, the response of soil microbial biomass and community composition to forest disturbances is not accounted for in this classic ecosystem theory.

Abiotic and biotic disturbances change a variety of soil properties in forests, which may in turn alter soil microbial biomass and respiration. For example, abiotic disturbances usually kill

(fire, storm) or remove (harvest) aboveground vegetation. Post-disturbance reductions in aboveground vegetation decrease plant litter inputs and root exudation into soil and thus can result in long-term declines in soil C (Johnson and Curtis, 2001; Wang et al., 2012; Zhou et al., 2013) and total soil nitrogen (Wan et al., 2001). In addition, soil temperatures often increase following abiotic disturbances (Treseder et al., 2004), and this may augment microbial respiration. However, microbes living in post-disturbance soils may also experience greater moisture stress, as higher soil temperatures following abiotic disturbance can lead to soil drying. Biotic disturbances may differ from abiotic disturbances in their effect on soil properties because they less frequently kill aboveground vegetation. Tree defoliation caused by biotic disturbances can result in an influx of dead plant litter into soils (Hicke et al., 2012). Insect biomass and frass deposition following insect defoliation can also increase soil nutrient availability (Lovett et al., 2002). Increases in labile C and nutrient availability following biotic disturbances may stimulate soil microbial growth and respiration. On the other hand, biotic disturbances that kill aboveground vegetation might cause soil C availability to decline. The net effect of these altered soil conditions on soil microbial communities is poorly understood.

Soil microbial responses to forest disturbances are likely to differ as a function of the time since disturbance. Disturbance effects on soil microbial communities may only persist until aboveground vegetation re-grows, as the recovery of aboveground vegetation may reverse changes in soil properties caused by disturbance (Hart et al., 2005). Soil nutrient availability may quickly return to pre-disturbance levels if soil microbes and plants can readily assimilate the pulse of available nutrients. Furthermore, soil microbial communities may have the capacity to quickly recover from disturbances if nearby undisturbed forests or mineral soils serve as a source of microbial inoculum (Grogan et al., 2000; Barker et al., 2013). However, we currently have a limited understanding of changes in soil microbial biomass during forest recovery from a variety of disturbance types.

In a previous meta-analysis we summarized soil microbial biomass responses to fire (Dooley and Treseder, 2012). This work demonstrated that fires reduce soil microbial biomass in forest ecosystems. However, our previous work did not examine other types of forest disturbances besides fire. It is important to consider microbial responses to a variety of disturbances because of their prevalence in forests worldwide and the likelihood that disturbances may occur more frequently as a result of climate warming. Determining the relative impact of different disturbance types will allow us to better predict how climate-linked increases in disturbance frequency will affect soil microbial communities and soil C dynamics. Many studies have documented soil microbial responses to forest disturbances, but the results among these studies are inconsistent. Some studies find increases in microbial abundance following disturbances (Holmes and Zak, 1999; Bogorodskaya et al., 2009), while others report negative microbial responses to disturbance (Arunachalam et al., 1996; Bárcenas-Moreno et al., 2011) and we lack a quantitative synthesis across disturbance types. Here, we build on our previous work by asking how does soil microbial biomass and respiration respond

to disturbance events in forests and how does this response differ across disturbance types? We also highlight forest disturbance types that require further study. We hypothesized that forest disturbances would reduce soil microbial biomass. Second, we expected that abiotic disturbances would lead to greater reductions in microbial biomass than biotic disturbances. Third, we predicted that post-disturbance changes in microbial biomass would diminish over time as forests recover from disturbance. Fourth, we expected that changes in soil microbial biomass would be associated with changes in microbial respiration. We tested these hypotheses separately for studies that measured total soil microbial biomass, and for studies that measured fungal and bacterial abundances separately since these major classes of microbes may have different responses to disturbance. Given previous work suggesting that fungi may be more sensitive to fires than bacteria (Pietikäinen and Fritze, 1995; Dooley and Treseder, 2012), we expected that fungi would have larger responses to disturbance than bacteria.

MATERIALS AND METHODS

LITERATURE SURVEY AND CRITERIA FOR INCLUSION

We searched the published literature for studies that reported microbial abundance measurements in disturbed and undisturbed forest soils. Searches were conducted using the ISI Web of Science database and Google Scholar. We performed our literature searches separately by each type of forest disturbance. Key words for each disturbance type included: burn, forest fire, prescribed fire, wildfire (fire); harvest, logging (forest harvest); insect, insect defoliation, insect outbreak (insect outbreaks); pathogen (pathogen-caused tree mortality); and storm, windthrow (storms). To narrow our search results to studies that focused on soil microbes, we also used the search terms *microb**, *bacteri**, and *fung** in combination with the key words listed above for each disturbance type. Published studies were collected for analysis until 15 January 2013.

Meta-analyses were performed on a subset of studies that met our search criteria (Table A1) following Dooley and Treseder (2012). Importantly, we only included multiple data sets from a single study if the data sets could reasonably be considered independent (e.g., different geographic locations, dominant vegetation).

DATA ACQUISITION

For each study, we recorded the mean, standard deviation (SD), and sample size (*n*) of microbial biomass, fungal abundance, or bacterial abundance in the disturbed area and the undisturbed control. In addition to changes in microbial abundances, we recorded the type of disturbance, the disturbance agent, the time elapsed since disturbance, and the biome in which the study took place. We included studies from boreal forests, temperate forests, tropical forests, and woodlands. Studies in woodlands were primarily from Mediterranean ecosystems and had decreased tree biomass and higher amounts of shrub biomass. We also recorded the method used for measuring microbial abundances in soil. When means and errors were presented in graph form, we digitized the data using PlotDigitizer 2.6.2 (<http://plotdigitizer.sourceforge.net>). If

standard errors (SEs) were presented instead of SDs, they were converted using the formula: $SD = SE (n^{1/2})$. Any unidentified errors bars in graphs were assumed to represent SEs. There were a total of two studies in which error bars were not identified (Chang et al., 1995; Pietikäinen and Fritze, 1995).

INDICES OF MICROBIAL ABUNDANCE

Authors employed a variety of techniques to measure microbial abundances in soil. Microbial biomass in soil was measured through chloroform fumigation and extraction (Brooks et al., 1985), substrate-induced respiration (Anderson and Domsch, 1978), total amounts of phospholipid fatty acids (PLFAs) in soil (Frostegard and Bååth, 1996), total amounts of ATP extracted from soil (Eiland, 1983), and microwave irradiation of soil (Islam and Weil, 1998). Fungal abundance in soil was most commonly determined using fungal specific PLFAs. Additional methods for characterizing fungal abundance included total amounts of ergosterol in soil (Djakirana et al., 1996), microscopy, plating soil and counting colony formation, and quantitative PCR with universal fungal primers (Borneman and Hartin, 2000). Bacterial abundances were determined through bacteria specific PLFAs, dilution plating, and microscopy.

SPECIFIC MICROBIAL GROUPS

A subset of the studies generated from our literature search also reported changes in the abundance of specific groups of bacteria in response to disturbance. We found studies that reported the response of gram-negative bacteria, gram-positive bacteria, and actinomycetes to forest disturbances. The abundance of these bacterial groups was measured using PLFAs or dilution plating.

BASAL RESPIRATION

Where possible, we also recorded changes in soil basal respiration following disturbances. We defined basal respiration as the amount of CO₂ produced during laboratory incubations of soil in the absence of carbon or nutrient additions.

STATISTICS

Meta-analyses were used to determine the significance of microbial abundance responses to disturbance. For each study and group of microorganisms (microbes, fungi, bacteria, gram-negative, gram-positive, actinomycetes), the effect size was calculated at the natural log of the response ratio ("R"). R is calculated as the mean of the disturbed treatment divided by the mean of the control group. Thus, an R of 1 indicates that disturbance had no effect on microbial abundance. Variance within each study (v_{lnR}) is computed using the means, n , and SD of the control and disturbed groups (Hedges et al., 1999).

To determine if disturbances had a significant effect on microbial abundance, we employed a random effects models using MetaWin software (Rosenberg et al., 2000). Bias-corrected bootstrap 95% confidence intervals (CIs) were calculated for each mean R. If the 95% CIs of R do not overlap with 1, then responses were significant at $P < 0.05$. Random effects models allow for comparisons between groups in a framework that is similar to analysis of variance. We applied random effects meta-analyses to

test for differences in R between abiotic and biotic disturbances and disturbance types (fire, harvest, storm, insect, pathogen). Within each disturbance type, we further tested for differences among disturbance agents (e.g., wildfire vs. prescribed fire), biomes, and the method of measurement used to estimate microbial abundances. In addition, we used continuous randomized effects meta-analyses to test for relationships between R and the time since disturbance. Tests for the relationship between R and the time since disturbance were performed separately for each disturbance type and biome. Statistical results reported include: R, 95% CIs for R, and total heterogeneity in R among studies (Q_T). For comparisons among groups, total heterogeneity (Q_T) can be partitioned into the amount of heterogeneity explained by groups (Q_M) and the amount of heterogeneity left unexplained (Q_E). The significance of Q_T and Q_M is tested by comparison to the chi-squared distribution. A significant Q_T value means that the variance among studies is greater than expected due to sampling error. A significant Q_M values indicates that a significant portion of the total heterogeneity among studies can be explained by subdividing the studies into the group of interest (Rosenberg et al., 2000, 2004; Koricheva et al., 2013). We used a Pearson's correlation to analyze the relationship between the R of microbial biomass and the R of basal respiration for studies in which both were reported.

We employed a number of complementary approaches to test for the presence of publication bias in our data. We performed a Kendall's tau rank correlation test and a Spearman rank correlation test (Sokal and Rohlf, 1995) to test for the relationship between replicate number of each study and the standardized effect size. Such a relationship would be indicative of a publication bias in which larger effects of disturbance were more likely to be published than smaller effects. We visually inspected funnel plots of standard error or replicate number versus standardized effect size for the presence of asymmetry (Egger et al., 1997; Sterne and Egger, 2001). Funnel plot asymmetry was formally tested using Egger's regression (Sterne and Egger, 2005). Publication bias was assessed in all data for a given group of microorganisms (microbes, fungi, bacteria) and also for abiotic and biotic data sets within each group of microorganisms.

RESULTS

In this study we focused on five of the most prevalent disturbances in forest ecosystems. Specifically, we focused on three abiotic disturbances (fire, harvest, and storms) and two biotic disturbances (insect infestation and pathogen infection). Each disturbance type was further separated into its causative disturbance agent. Fires were grouped into wildfires, prescribed fires, or slash burns. Harvesting was grouped into clear cutting or partial harvesting (e.g., thinning, selective harvesting). Storms were subdivided into hurricanes, typhoons, and windthrow. We found studies reporting insect infestation by the gypsy moth, hemlock woolly adelgid, pine beetle, and pine lappet. Pathogen infection studies reported the effects of pine wilt disease and *Phellinus weirii* infection. Our literature search produced 88 observations of changes in soil microbial biomass following forest disturbances, collected from a total of 61 published papers. We found 35 reports of fungal abundance responses to disturbance from 24

published studies. Finally, we found 16 observations of changes in bacteria abundance following disturbance from 12 published papers.

TOTAL MICROBIAL BIOMASS

Soil microbial biomass significantly decreased following disturbances, by an average of 29.4% across all studies (**Table 1**). However, disturbance responses were not consistent across studies, as indicated by a significant Q_T value ($Q_T = 110.95$, $P = 0.043$). Microbial biomass responses to disturbance differed significantly between abiotic and biotic disturbances ($Q_M = 14.68$, $Q_E = 99.45$, $P = 0.038$, **Figure 1A**). Fires, harvesting, and storms resulted in significant reductions in microbial biomass (by 48.7, 19.1, and 41.7%, respectively). In contrast, changes in soil microbial biomass following insect attack and pathogen-induced mortality were non-significant (**Figure 1A**).

Fire, harvest, and insect infestation had high enough replication in the literature to further test for differences among groups within each disturbance type. Within fires, microbial biomass response ratios were not significantly different among fire types, biomes, or measurement methods (**Table 1**). Fires in boreal and temperate forests significantly reduced microbial biomass, but woodland fires had non-significant effects.

Following forest harvest, the response of microbial biomass was not significantly different between harvest types, biomes, or measurement methods (**Table 1**). Forest clear cutting elicited a significant negative response from soil microbes. In contrast, partial harvesting did not significantly alter soil microbial biomass. Within insect studies, infestation by pine beetles resulted in a significant reduction in soil microbial biomass, while defoliation by the gypsy moth significantly increased soil microbial biomass (**Table 1**). Studies following storms ($n = 3$) and pathogen infection ($n = 2$) were scarce in the literature and thus we could not perform further comparisons within these disturbance types.

We performed tests for publication bias separately for each group of microorganisms (microbes, fungi, bacteria). Within each group, we also performed tests separately for abiotic and biotic studies. Across all microbe studies, we did not detect significant publication bias with any of the three tests used (**Table A2**). However, when abiotic and biotic disturbances were examined separately, Egger's regression test was significant for biotic disturbance studies (**Table A2**). This indicates a potential bias toward publishing significant results.

FUNGI

Across all studies, disturbances resulted in a 34.0% reduction in fungal abundance (**Table 1**). Abiotic and biotic disturbances had significantly different effects on fungal biomass ($Q_M = 16.45$, $Q_E = 30.93$, $P = 0.008$, **Figure 1B**). Fire and harvest resulted in 55.2 and 26.6% declines in soil fungi, respectively. Responses of fungi to insect infestation were significantly positive (**Figure 1B**). However, it is important to note that insect infestations were only represented by two observations in the literature.

Within fire studies, fungal responses were significantly negative, regardless of fire type, biome, or measurement method (**Table 1**). Within harvest studies, fungal responses were significantly different across biomes. Harvesting in tropical forests led

to greater reductions in fungal biomass than harvesting in either boreal forests or temperate forests. Harvest responses did not differ by harvest type or measurement method. Similar to total soil microbial biomass, clear cutting significantly reduced fungal biomass, but partial harvesting had non-significant effects.

The Kendall's Tau and Spearman rank correlation tests for publication bias were significant for all fungal studies and for fungal studies of abiotic disturbances. However, Egger's regression test detected no significant publication bias for these same studies (**Table A2**). Our data set contained only two observations of changes in fungal abundance in response to biotic disturbances. Thus, we could not test for publication bias within biotic disturbances for fungi using correlation or regression methods.

BACTERIA

Bacterial abundance declined by an average of 15.3% in response to disturbances (**Table 1**). Bacterial responses to disturbance differed significantly between abiotic and biotic disturbances ($Q_M = 29.53$, $Q_E = 66.45$, $P = 0.037$, **Figure 1C**). Fire and harvest reduced bacteria by 33.3% and 13.9%, respectively. In contrast, bacteria increased following insect infestation (**Figure 1C**). Harvesting was the only disturbance type with sufficient replication to further test for differences within harvest studies. Bacteria harvesting responses were significantly different across biomes (**Table 1**). Harvesting in tropical forests significantly reduced bacterial biomass, but responses in temperate forests were non-significant. There were no significant differences in bacterial responses among harvest types and measurement methods. Clear-cutting significantly lowered soil bacterial abundance, but there was no significant effect of partial forest harvest.

A small subset of the studies included in this meta-analysis reported the response of specific groups of bacteria to disturbance (**Table A1**). Across all of these studies, we found that disturbances significantly reduced the abundance of gram-positive ($n = 5$, 95% CI of $R = 0.50 - 0.99$) and gram-negative soil bacteria ($n = 5$, 95% CI of $R = 0.58 - 0.99$). Within the gram-positive bacteria, actinomycete abundance did not change following disturbances ($n = 14$, 95% CI of $R = 0.73 - 1.09$; data not shown).

We found no evidence for publication bias among bacterial studies (**Table A2**). Similar to fungi, we could not use correlation or regression methods to test for publication bias in bacterial studies following biotic disturbance because there were only two observations.

RECOVERY OF MICROBIAL BIOMASS FOLLOWING DISTURBANCES

There was a significant positive relationship between the time since disturbance and the microbial biomass R following boreal forest fires (**Figure 2A**) and boreal forest harvesting (**Figure 2B**). Response ratios significantly increased as the time since fire increased in boreal forests ($n = 21$, $r^2 = 0.793$, $P < 0.0001$). Similarly, microbial response ratios increased with the time since harvest in boreal forests ($n = 32$, $r^2 = 0.201$, $P = 0.010$), and the relationship was linear.

We did not detect a significant relationship between microbial biomass response ratios and the time since disturbance for any other disturbance type and biome (data not shown). In addition, fungal and bacteria response ratios were not significantly related

Table 1 | Results of statistical comparisons among and within groups.

Organism	Group	Sub-group	<i>R</i>	95% CI	Number of studies	<i>Q_M</i>	<i>Q_E</i>	<i>P</i> -value groups ^a
Microbes	All microbe studies*		0.71	0.63–0.80	88			
	Abiotic	All abiotic studies*	0.68	0.61–0.76	80			
	Fire	All fire studies*	0.51	0.38–0.66	28			
	Fire Type	Prescribed fire*	0.65	0.47–0.87	13	2.79	29.86	0.160
		Wildfire*	0.41	0.23–0.60	15			
	Biome	Boreal forest*	0.46	0.35–0.60	7	6.14	26.26	0.110
		Temperate forest*	0.35	0.19–0.57	11			
		Woodland/shrubland	0.79	0.53–1.09	10			
	Measurement	Chloroform fumigation*	0.46	0.31–0.64	21	3.44	27.17	0.303
		PLFA*	0.72	0.65–0.84	3			
		SIR*	1.17	1.06–1.29	2			
	Harvest	All harvest studies*	0.81	0.72–0.88	49			
	Harvest type	Clear cut*	0.78	0.67–0.86	34	1.23	42.01	0.315
		Partial harvest	0.89	0.78–1.02	13			
	Biome	Boreal forest*	0.87	0.81–0.94	20	1.76	46.37	0.434
		Temperate forest*	0.77	0.63–0.90	24			
		Tropical forest*	0.75	0.51–0.97	5			
	Measurement	Chloroform fumigation*	0.79	0.58–0.93	21	2.12	47.85	0.511
		PLFA*	0.90	0.81–0.98	11			
		SIR*	0.79	0.70–0.90	13			
	Storm	All storm studies*	0.58	0.25–0.85	3			
	Biotic	All biotic studies	0.90	0.74–1.30	8			
	Insect	All insect studies	0.87	0.59–1.21	6			
	Insect type	Gypsy moth*	1.46	1.42–1.51	2	28.23	2.51	0.102
		Pine beetle*	0.59	0.37–0.65	3			
	Biome	Boreal forest*	1.46	1.42–1.51	2	7.07	4.08	0.061
		Temperate forest*	0.68	0.44–0.92	4			
	Measurement	Chloroform fumigation*	0.68	0.44–0.92	4	7.07	4.08	0.061
		SIR*	1.46	1.42–1.51	2			
	Pathogen	All pathogen studies	0.93	0.54–1.55	2			
Fungi	All fungi studies*		0.66	0.57–0.76	35			
	Abiotic	All abiotic studies*	0.64	0.56–0.73	33			
	Fire	All fire studies*	0.45	0.36–0.57	13			
	Fire Type	Prescribed fire*	0.41	0.35–0.51	7	0.02	11.89	0.864
		Wildfire*	0.43	0.31–0.56	5			
	Biome	Boreal forest*	0.37	0.31–0.41	4	2.53	10.00	0.241
		Temperate forest*	0.55	0.35–0.78	5			
		Woodland/shrubland*	0.50	0.35–0.61	4			
	Measurement	Dilution plate count*	0.53	0.03–0.63	3	16.04	8.54	0.066
		Ergosterol*	0.36	0.30–0.42	2			
		Microscopy*	0.74	0.60–0.89	3			
		PLFA*	0.37	0.34–0.46	4			
	Harvest	All harvest studies*	0.73	0.62–0.84	20			
	Harvest type	Clear cut*	0.70	0.60–0.80	15	1.44	17.20	0.249
		Partial harvest	0.86	0.60–1.14	5			
	Biome	Boreal forest*	0.84	0.75–0.91	11	22.46	34.39	0.015
		Temperate forest*	0.71	0.52–0.95	7			
		Tropical forest*	0.45	0.45–0.45	2			
	Measurement	Dilution plate count	0.68	0.45–1.01	4	1.18	14.64	0.562
		Microscopy*	0.62	0.47–0.75	3			
		PLFA*	0.79	0.65–0.94	12			
	Biotic	All biotic studies*	1.13	1.07–1.19	2			
	Insect	All insect studies*	1.13	1.07–1.19	2			

(Continued)

Table 1 | Continued

Organism	Group	Sub-group	<i>R</i>	95% CI	Number of studies	<i>Q_M</i>	<i>Q_E</i>	<i>P</i> -value groups ^a
Bacteria	All bacteria studies*		0.85	0.73–0.95	16			
	Abiotic	All abiotic studies*	0.81	0.70–0.92	14			
	Fire	All fire studies*	0.67	0.47–0.82	4			
	Harvest	All harvest studies*	0.86	0.71–0.97	10			
	Harvest type	Clear cut*	0.89	0.70–0.98	8	4.25	58.96	0.369
		Partial harvest	0.74	0.63–1.52	2			
	Biome	Temperate forest	0.99	0.96–1.01	7	132.14	18.96	0.020
		Tropical forest*	0.60	0.57–0.63	2			
	Measurement	Dilution plate count	0.74	0.57–1.00	3	15.69	32.89	0.278
		Microscopy	0.99	0.98–1.01	3			
		PLFA	0.88	0.70–1.52	3			
		All biotic studies*	1.12	1.11–1.13	2			
	Biotic	All biotic studies*	1.12	1.11–1.13	2			
	Insect	All insect studies*	1.12	1.11–1.13	2			

PLFA, phospholipid fatty acid; SIR, substrate induced respiration.

*Significant effect of disturbance on group ($P < 0.05$).

^aOnly groups represented by two or more studies were included in comparisons.

to the time since disturbance for any disturbance type and biome (data not shown).

BASAL RESPIRATION

A subset of the studies included in this meta-analysis reported changes in soil basal respiration following disturbance in addition to changes in microbial biomass measurements ($n = 38$). Across all studies that reported both, there was a significant positive correlation between the R of soil basal respiration and the R of microbial biomass ($r = 0.702$, $P < 0.0001$, **Figure 3**).

DISCUSSION

In this study, we conducted a meta-analysis of changes in soil microbial biomass in response to forest disturbances. We initially hypothesized that forest disturbances would reduce soil microbial biomass. In support of this hypothesis, we found that microbial biomass declined by an average of 29.4% after disturbance events (**Table 1**). The responses of soil fungi and bacteria to disturbance largely mirrored the response of the microbial community as a whole, and provide further support for the hypothesis that forest disturbances reduce soil microbial abundance. Although bacterial and fungal responses were less frequently studied than the response of the microbial community as a whole, these data imply that soil bacteria and fungi are affected by forest disturbances in a similar manner. Our data do not suggest that soil fungi are more sensitive to disturbance events than bacteria. We further hypothesized that abiotic disturbances would lead to greater reductions in microbial biomass than biotic disturbances. In support of this hypothesis, soil microbial responses significantly differed between abiotic and biotic disturbances. Fires, harvesting, and storms caused significant reductions in soil microbial biomass, while changes in microbial biomass following insect infestation and pathogen-induced tree mortality were non-significant (**Figure 1A**). Furthermore, bacterial and fungal

abundances significantly increased following insect infestation (**Figures 1B,C**).

We propose two possible explanations for the differential effect of abiotic and biotic disturbances on soil microbial communities. First, abiotic disturbances typically involve higher levels of soil disruption during the disturbance event than biotic disturbances. For example, harvesting practices involve the use of logging equipment that can result in heavy soil compaction. Soil compaction alters soil pore space, potentially leading to impaired gas exchange, decreased soil drainage, and inhibition of soil microbial growth (Kabzems and Haeussler, 2005; Mariani et al., 2006). Forest fires cause soil disruption in the form of soil combustion and heating of the soil surface. Soil surface temperatures during forest fires can reach up to 600°C (Busse et al., 2005), which is well above the upper thermal limit of most microbial taxa (Debano et al., 1998). Storms cause soil disruption by uprooting trees, which can cause soil mixing and changes in soil microtopography (Ruel, 1995). These direct effects of abiotic disturbances on soil properties may in part explain the observed post-disturbance reductions in microbial biomass. In contrast, biotic disturbances do not typically involve immediate soil physical changes and are likely to have mostly indirect effects on soil properties (Hicke et al., 2012). Lower levels of soil physical disruption during biotic disturbances may in part explain the non-significant effect of these disturbances on soil microbial biomass.

In addition, abiotic and biotic disturbances differ in the amount and type of organic C remaining in ecosystems following the disturbance event and this may have consequences for soil microbial communities. Fires remove large amounts of organic C from ecosystems via the combustion of aboveground vegetation and soil organic matter (Amiro et al., 2001; Van Der Werf et al., 2010). The more labile components of soil organic matter may be preferentially volatilized during fires (González-Pérez et al., 2004; Neff et al., 2005), leaving behind organic C that is more difficult for microbes to decompose. Harvesting also removes large

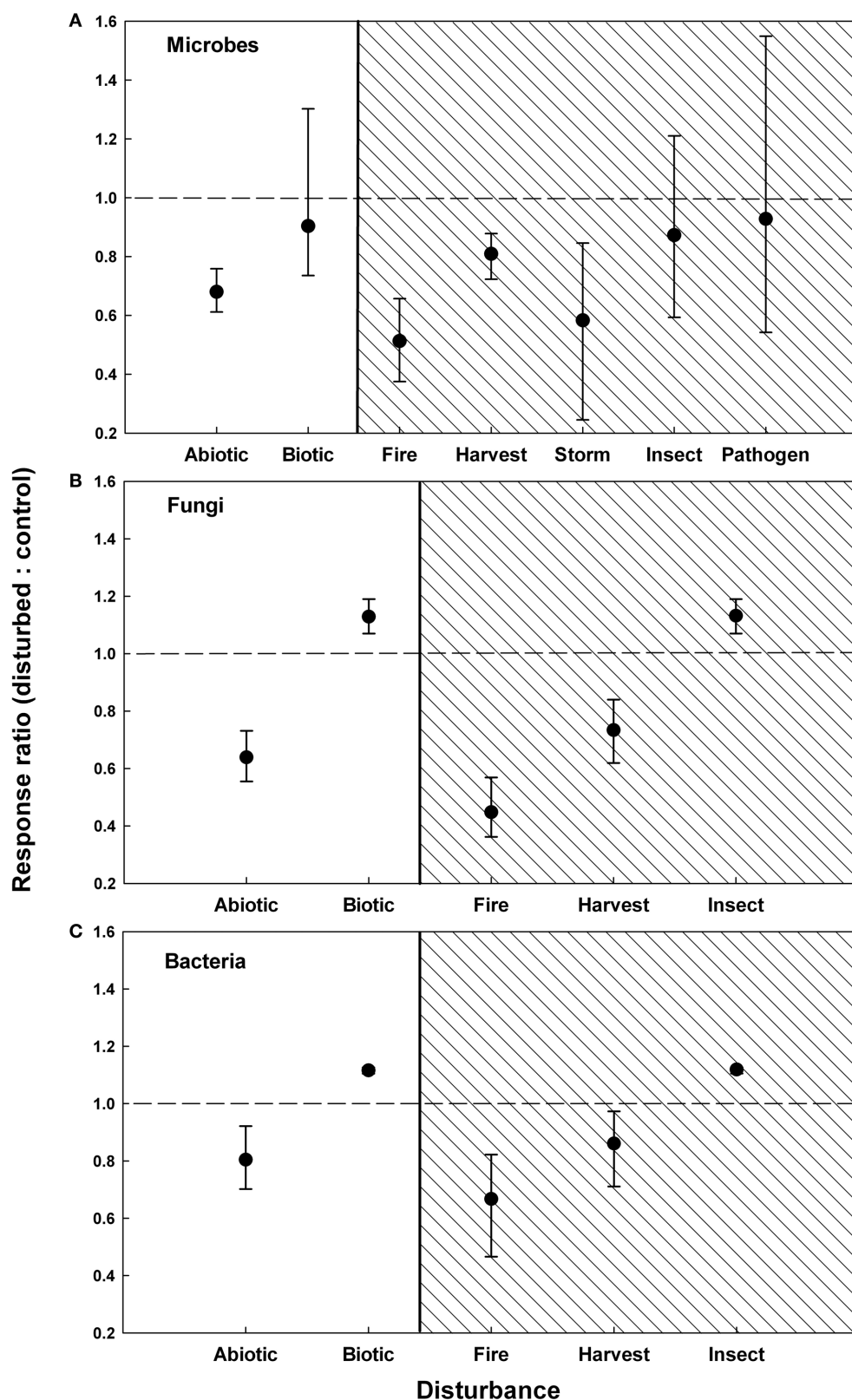
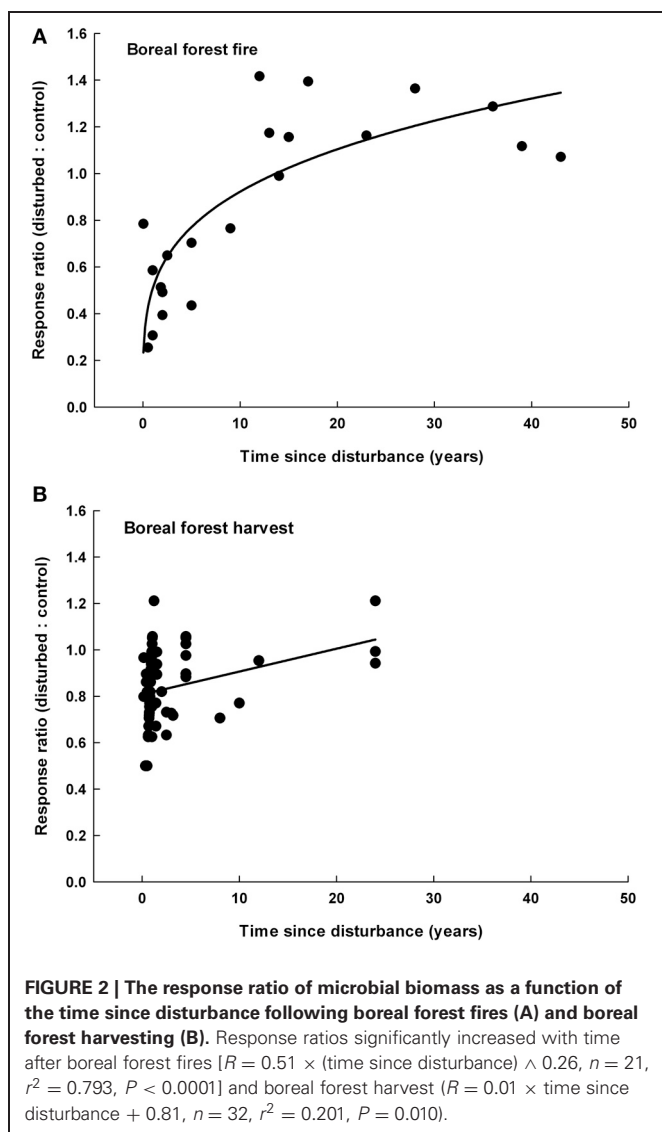


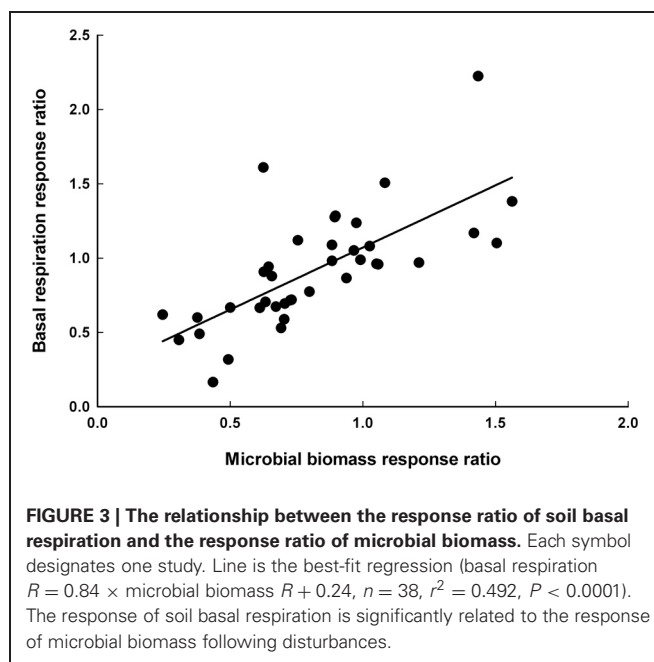
FIGURE 1 | Responses of microbial biomass (A), fungal abundance (B), and bacterial abundance (C) to forest disturbances. Response ratios are grouped by abiotic and biotic disturbances (unshaded) and by disturbance

type (shaded). Symbols are means \pm 95% confidence intervals. A response ratio < 1 indicates that microbial abundances declined following disturbance, a response ratio > 1 indicates an increase in microbial biomass.



amounts of organic C from forests, but can deposit fine woody debris on the soil surface. On the other hand, biotic disturbances are associated with smaller amounts of organic C removal from forests. Although insect or pathogen outbreaks may kill trees, they can also result in an influx of dead plant litter, insect feces, and dead insect biomass to forest soils (Lovett et al., 2002; Yang, 2004; Hicke et al., 2012). Higher amounts of organic C removal from forests during abiotic disturbances may cause C limitation of soil microbial growth, and thus reductions in soil microbial biomass. With our meta-analysis approach, we were unable to evaluate whether differences in soil physical disruption, organic C removal, or a combination of both factors, were responsible for the differential effect of abiotic and biotic disturbances on soil microbial communities. Future studies that are mechanistic rather than observational will make it possible to disentangle the factors that govern microbial responses to disturbance events.

While the mechanisms described above may explain the contrasting effects of abiotic and biotic disturbances that we observed, it is also important to consider that we found evidence



for publication bias in studies of microbial biomass following biotic disturbances and in all fungal studies. The presence of publication bias suggests that the effects of disturbance that are reported in the literature may not be representative of all microbial responses. Moreover, microbial ($n = 8$), fungal ($n = 2$), and bacterial ($n = 2$) biomass responses to biotic disturbances were poorly represented in the literature. Therefore, the differences that we observed between abiotic and biotic disturbances may also be attributable to the scarcity of data on biotic disturbances.

In some cases, contrasts between disturbance agents revealed interesting differences in soil microbial responses. For example, clear cutting consistently reduced microbial abundance, but partial forest harvesting did not result in significant changes in total microbial biomass, fungal abundance, or bacterial abundance (Table 1). In comparison to clear cutting, partial harvesting is associated with lower levels of soil compaction and vegetation removal (Barg and Edmonds, 1999). Together these factors may explain the reduced impact of partial harvesting on below-ground communities (Lindo and Visser, 2003). In addition, we found that gypsy moths and pine beetles had contrasting effects on soil microbial communities (Table 1). Pine beetle infestation reduced microbial biomass (95% CI of $R = 0.367 - 0.646$), while microbial biomass increased following gypsy moth defoliation (95% CI of $R = 1.419 - 1.505$). The differential effect of these insects on soil microbial biomass may be explained by their ecology. Gypsy moths are leaf-feeders that defoliate trees and reduce tree growth. However, gypsy moth feeding does not always kill trees. In contrast, pine beetles do not consume tree needles, but instead feed within the phloem and typically result in tree death (Hicke et al., 2012). Although represented by a limited number of studies, our results suggest that tree defoliating and tree killing insects may have contrasting effects of soil microbial communities and potentially forest C dynamics.

In addition to changing microbial biomass, disturbances may also alter the composition of soil microbial communities. Denaturing gradient gel electrophoresis and phospholipid fatty acid profiles have been used to detect broad changes in microbial community structure following harvesting and forest fires (Siira-Pietikainen et al., 2001; Waldrop and Harden, 2008). Next generation sequencing of environmental samples has made it possible to examine compositional changes in microbial communities following disturbances in greater detail. For example, Hartmann et al. (2012) found that harvesting significantly altered the composition of soil bacterial and fungal communities, with ectomycorrhizal taxa and actinobacteria being most sensitive to harvesting disturbance. Ectomycorrhizal fungi were also sensitive to forest fires in boreal forests, while ascomycete fungi increased in abundance following fire (Holden et al., 2013). These changes in microbial community structure following disturbance suggest that microbial species are differentially affected by disturbance. The functional consequences of compositional changes in soil microbial communities in response to disturbances require further testing. For instance, if plant symbiotic microbes are sensitive to disturbance, the ability of plants to re-establish following disturbances may be hindered. Changes in the composition of soil microbial communities following biotic disturbances have rarely been studied, but would greatly contribute to our knowledge of soil microbial responses to disturbances.

We found a significant positive relationship between the time since disturbance and microbial biomass responses following fire and harvesting in boreal forests (**Figure 2**). These results are consistent with our third hypothesis that post-disturbance changes in microbial biomass would weaken over time. Following both harvesting and fires in boreal forests, microbial responses were typically negative for the first 15 years following disturbance. This finding suggests that forest disturbances can have long-term consequences for belowground communities. Eddy covariance studies and ground-based vegetation surveys have found that primary productivity requires up to 10 years to recover following harvest and fires in boreal forests (Mack et al., 2008; Amiro et al., 2010; Goulden et al., 2011). In addition, post-fire reductions in soil C and soil organic matter can persist for at least 10 years following boreal forest fires (Johnson and Curtis, 2001; Treseder et al., 2004). Thus, the recovery of soil microbial biomass following harvesting and forest fires may be controlled by the recovery of forest primary productivity and soil organic matter accumulation. We found no evidence for a significant relationship between the time since disturbance and microbial abundance responses for any other disturbance type or biome. Although, the majority of the studies used in this meta-analysis assessed microbial responses to disturbance within 1 year of the disturbance event (**Table A1**). The paucity of long-term data may have limited our ability to detect significant relationships between microbial biomass responses and the time since disturbance. Additional long-term studies, especially following insect outbreaks and pathogen infection, are necessary to evaluate the belowground consequences of forest disturbances.

Classic ecosystem theory posits that soil microbial respiration increases following disturbance (Chapin et al., 2002; Harmon et al., 2011). Microbial respiration has long been assumed to

increase following forest disturbance events because soil temperatures usually increase after disturbances and because disturbances can result in the deposition of plant litter and/or woody debris on the soil surface. Instead, we hypothesized that post-disturbance changes in microbial biomass would be associated with concurrent changes in microbial respiration. In support of our hypothesis, we found a significant positive correlation between the response of microbial biomass to disturbance and the response of soil basal respiration (**Figure 3**). Therefore, decreases in soil microbial biomass following abiotic disturbances may be accompanied by reductions in microbial respiration. This finding is in agreement with ecosystem-level studies that have measured microbial respiration following disturbance events and found post-disturbance decreases in microbial respiration (Amiro et al., 2003; Czimczik et al., 2006). Although, the microbial respiration data reported here were measured in the laboratory under standardized conditions. It is therefore possible that differences in soil conditions between disturbed and undisturbed forests may cause differences in microbial respiration in the field. However, any post-disturbance increases in microbial respiration would likely result from increases in mass-specific rates of respiration, since microbial abundance declined by an average of 29.4% following disturbances. Our understanding of changes in microbial respiration following disturbance would benefit from additional studies that combine *in situ* measurements of microbial respiration with detailed microbial community analyses.

In summary, we found that forest disturbances significantly reduced soil microbial biomass, but that responses differed by disturbance type. Microbial biomass responses were consistently negative following abiotic disturbances, but our data suggest that forest disturbances caused by biotic agents may have a neutral or positive effect on microbial abundance in soil. This contrast is potentially attributable to differences in soil physical disruption and organic C removal from forests between abiotic and biotic disturbances. Evidence for publication bias in biotic studies, and the overall paucity of data on soil microbial responses to biotic disturbances, may have also contributed to the patterns we observed. Further studies following biotic disturbances will help clarify their impact on soil microbial communities. We found that changes in soil microbial biomass following disturbances were significantly related to changes in microbial respiration. Disturbances are common in forest ecosystems and one indirect impact of climate warming in terrestrial ecosystems may be an increase in the frequency and severity of disturbance events in forests. Our results imply that these disturbance events can alter soil microbial biomass in forests, with corresponding consequences for microbial respiration and ecosystem C balance.

ACKNOWLEDGMENTS

We thank the authors whose work was included in this meta-analysis. This manuscript was improved by insightful comments from two reviewers. Sandra R. Holden was supported in part by the Department of Energy Office of Science Graduate Fellowship Program (DOE SCGF), made possible in part by the American Recovery and Reinvestment Act of 2009, administered by ORISE-ORAU under contract no. DE-AC05-06OR23100.

REFERENCES

- Amiro, B. D., Barr, A. G., Barr, J. G., Black, T. A., Bracho, R., Brown, M., et al. (2010). Ecosystem carbon dioxide fluxes after disturbance in forests of North America. *J. Geophys. Res.* 115:G00K02. doi: 10.1029/2010JG001390
- Amiro, B. D., Ian Macpherson, J., Desjardins, R. L., Chen, J. M., and Liu, J. (2003). Post-fire carbon dioxide fluxes in the western Canadian boreal forest: evidence from towers, aircraft and remote sensing. *Agric. For. Meteorol.* 115, 91. doi: 10.1016/S0168-1923(02)00170-3
- Amiro, B. D., Todd, J. B., Wotton, B. M., Logan, K. A., Flannigan, M. D., Stocks, B. J., et al. (2001). Direct carbon emissions from Canadian forest fires, 1959–1999. *Can. J. For. Res.* 31, 512–525. doi: 10.1139/x00-197
- Anderson, J., and Domsch, K. (1978). A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biol. Biochem.* 10, 215–221. doi: 10.1016/0038-0717(78)90099-8
- Arunachalam, A., Maithani, K., Pandey, H. N., and Tripathi, R. S. (1996). The impact of disturbance on detrital dynamics and soil microbial biomass of a *Pinus kesiyya* forest in north-east India. *For. Ecol. Manag.* 88, 273–282. doi: 10.1016/S0378-1127(96)03801-7
- Balshi, M. S., McGuire, A. D., Duffy, P., Flannigan, M., Walsh, J., and Melillo, J. (2009). Assessing the response of area burned to changing climate in western boreal North America using a multivariate adaptive regression splines (MARS) approach. *Glob. Change Biol.* 15, 578–600. doi: 10.1111/j.1365-2486.2008.01679.x
- Bárcenas-Moreno, G., García-Orenes, F., Mataix-Solera, J., Mataix-Beneyto, J., and Bååth, E. (2011). Soil microbial recolonisation after a fire in a Mediterranean forest. *Biol. Fertil. Soils* 47, 261. doi: 10.1007/s00374-010-0532-2
- Barg, A. K., and Edmonds, R. L. (1999). Influence of partial cutting on site microclimate, soil nitrogen dynamics, and microbial biomass in douglas-fir stands in western Washington. *Can. J. For. Res.* 29, 705–713. doi: 10.1139/x99-045
- Barker, J. S., Simard, S. W., Jones, M. D., and Durall, D. M. (2013). Ectomycorrhizal fungal community assembly on regenerating douglas-fir after wildfire and clearcut harvesting. *Oecologia*. doi: 10.1007/s00442-012-2562-y. [Epub ahead of print].
- Birdsey, R., Pregitzer, K., and Lucier, A. (2006). Forest carbon management in the United States: 1600–2100. *J. Environ. Qual.* 35, 1461–1469. doi: 10.2134/jeq2005.0162
- Bogorodskaya, A. V., Baranchikov, Y. N., and Ivanova, G. A. (2009). The state of microbial complexes in soils of forest ecosystems after fires and defoliation of stands by gypsy moths. *Eurasian Soil Sci.* 42, 310–317. doi: 10.1134/S1064229309030089
- Bonan, G. B. (2008). Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *Science* 320, 1444–1449. doi: 10.1126/science.1155121
- Borneman, J., and Hartin, J. (2000). PCR primers that amplify fungal rRNA genes from environmental samples. *Appl. Environ. Microbiol.* 66, 4356–4360. doi: 10.1128/AEM.66.10.4356-4360.2000
- Brooks, P., Landman, A., Pruden, G., and Jenkinson, D. (1985). Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.* 17, 837–842. doi: 10.1016/0038-0717(85)90144-0
- Busse, M. D., Hubbert, K. R., Fiddler, G. O., Shestak, C. J., and Powers, R. F. (2005). Lethal soil temperatures during burning of masticated forest residues. *Int. J. Wild. Fire* 14, 267–276. doi: 10.1071/WF04062
- Chang, S. X., Preston, C. M., and Weetman, G. F. (1995). Soil microbial biomass and microbial and mineralizable N in a clear-cut chronosequence on northern Vancouver Island, British Columbia. *Can. J. For. Res.* 25, 1595–1607. doi: 10.1139/x95-174
- Chapin, F. S., Matson, P., and Mooney, H. A. (2002). *Principles of Terrestrial Ecosystem Ecology*. New York, NY: Springer.
- Covington, W. W. (1981). Changes in forest floor organic-matter and nutrient content following clear cutting in northern hardwoods. *Ecology* 62, 41–48. doi: 10.2307/1936666
- Czimczik, C. I., Trumbore, S. E., Carbone, M. S., and Winston, G. C. (2006). Changing sources of soil respiration with time since fire in a boreal forest. *Glob. Change Biol.* 12, 957–971. doi: 10.1111/j.1365-2486.2006.01107.x
- Dale, V. H., Joyce, L. A., McNulty, S., Neilson, R. P., Ayres, M. P., Flannigan, M. D., et al. (2001). Climate change and forest disturbances. *Bioscience* 51, 723–734. doi: 10.1641/0006-3568(2001)051[0723:CCAFD]2.0.CO;2
- Debano, L. F., Neary, D. G., and Ffolliott, P. F. (1998). *Fire's Effects on Ecosystems*. New York, NY: John Wiley and Sons Inc.
- Djakirana, G., Joergensen, R., and Meyer, B. (1996). Ergosterol and microbial biomass relationship in soil. *Biol. Fertil. Soils* 22, 299–304. doi: 10.1007/BF00334573
- Dooley, S. R., and Treseder, K. K. (2012). The effect of fire on microbial biomass: a meta-analysis of field studies. *Biogeochemistry* 109, 49–61. doi: 10.1007/s10533-011-9633-8
- Egger, M., Davey Smith, G., Schneider, M., and Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315, 629–634. doi: 10.1136/bmj.315.7109.629
- Eiland, F. (1983). A simple method for quantitative determination of ATP in soil. *Soil Biol. Biochem.* 15, 665–670. doi: 10.1016/0038-0717(83)90030-5
- Frostegard, A., and Bååth, E. (1996). The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol. Fertil. Soils* 22, 59–65. doi: 10.1007/BF00384433
- Giglio, L., Van Der Werf, G. R., Randerson, J. T., Collatz, G. J., and Kasibhatla, P. (2006). Global estimation of burned area using MODIS active fire observations. *Atmos. Chem. Phys.* 6, 957–974. doi: 10.5194/acp-6-957-2006
- Goetz, S. J., Bond-Lamberty, B., Law, B. E., Hicke, J. A., Huang, C., Houghton, R. A., et al. (2012). Observations and assessment of forest carbon dynamics following disturbance in North America. *J. Geophys. Res. Biogeosci.* 117, 1–17. doi: 10.1029/2011JG001733
- González-Pérez, J. A., González-Vila, F. J., Alendros, G., and Knicker, H. (2004). The effect of fire on soil organic matter—a review. *Environ. Int.* 30, 855–870. doi: 10.1016/j.envint.2004.02.003
- Goulden, M. L., McMillan, A. M. S., Winston, G. C., Rocha, A. V., Manies, K. L., Harden, J. W., et al. (2011). Patterns of NPP, GPP, respiration, and NEP during boreal forest succession. *Glob. Change Biol.* 17, 855–871. doi: 10.1111/j.1365-2486.2010.02274.x
- Grogan, P., Baar, J., and Bruns, T. D. (2000). Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest. *J. Ecol.* 88, 1051–1062. doi: 10.1046/j.1365-2745.2000.00511.x
- Harmon, M. E., Bond-Lamberty, B., Tang, J., and Vargas, R. (2011). Heterotrophic respiration in disturbed forests: a review with examples from North America. *J. Geophys. Res.* 116:G00K04. doi: 10.1029/2010JG001495
- Hart, S. C., Deluca, T. H., Newman, S. G., Mackenzie, M. D., and Boyle, S. I. (2005). Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. *For. Ecol. Manag.* 220, 166–184. doi: 10.1016/j.foreco.2005.08.012
- Hartmann, M., Howes, C. G., Vaninsberghe, D., Yu, H., Bachar, D., Christen, R., et al. (2012). Significant and persistent impact of timber harvesting on soil microbial communities in northern coniferous forests. *ISME J.* 6, 2199–2218. doi: 10.1038/ismej.2012.84
- Hedges, L., Gurevitch, J., and Curtis, P. (1999). The meta-analysis of response ratios in experimental ecology. *Ecology* 80, 1150–1156. doi: 10.1890/0012-9658(1999)080[1150:TMAORR]2.0.CO;2
- Hicke, J. A., Allen, C. D., Desai, A. R., Dietze, M. C., Hall, R. J., Hogg, E. H., et al. (2012). Effects of biotic disturbances on forest carbon cycling in the United States and Canada. *Glob. Change Biol.* 18, 7–34. doi: 10.1111/j.1365-2486.2011.02543.x
- Holden, S. R., Gutierrez, A., and Treseder, K. K. (2013). Changes in Soil fungal communities, extracellular enzyme activities, and litter decomposition across a fire chronosequence in alaskan boreal forests. *Ecosystems* 16, 34–46. doi: 10.1007/s10021-012-9594-3
- Holmes, W. E., and Zak, D. R. (1999). Soil microbial control of nitrogen loss following clearcut harvest in northern hardwood ecosystems. *Ecol. Appl.* 9, 202–215. doi: 10.1890/1051-0761(1999)009[0202:SMCONL]2.0.CO;2
- Islam, K. R., and Weil, R. R. (1998). Microwave irradiation of soil for routine measurements of microbial biomass carbon. *Biol. Fertil. Soils* 27, 408–416. doi: 10.1007/s003740050451
- Johnson, D. W., and Curtis, P. S. (2001). Effects of forest management on soil C and N storage: meta analysis. *For. Ecol. Manag.* 140, 227–238. doi: 10.1016/S0378-1127(00)00282-6
- Kabzems, R., and Haeussler, S. (2005). Soil properties, aspen, and white spruce responses 5 years after organic matter removal and compaction treatments. *Can. J. For. Res.* 35, 2045–2055. doi: 10.1139/x05-175

- Kardol, P., Todd, D. E., Hanson, P. J., and Mulholland, P. J. (2010). Long-term successional forest dynamics: species and community responses to climatic variability. *J. Veg. Sci.* 21, 627–642.
- Koricheva, J., Gurevitch, J., and Mengersen, K. (2013). *Handbook of Meta-Analysis in Ecology and Evolution*. Princeton, NJ: Princeton University Press.
- Lindo, Z., and Visser, S. (2003). Microbial biomass, nitrogen and phosphorus mineralization, and mesofauna in boreal conifer and deciduous forest floors following partial and clear-cut harvesting. *Can. J. For. Res.* 33, 1610–1620. doi: 10.1139/x03-080
- Lovett, G. M., Christenson, L. M., Groffman, P. M., Jones, C. G., Hart, J. E., and Mitchell, M. J. (2002). Insect defoliation and nitrogen cycling in forests. *Bioscience* 52, 335–341. doi: 10.1641/0006-3568(2002)052[0335:IDANCI]2.0.CO;2
- Mack, M. C., Treseder, K. K., Manies, K. L., Harden, J. W., Schuur, E. A. G., Vogel, J. G., et al. (2008). Recovery of aboveground plant biomass and productivity after fire in mesic and dry black spruce forests of interior Alaska. *Ecosystems* 11, 209–225. doi: 10.1007/s10021-007-9117-9
- Mariani, L., Chang, S. X., and Kabzems, R. (2006). Effects of tree harvesting, forest floor removal, and compaction on soil microbial biomass, microbial respiration, and N availability in a boreal aspen forest in British Columbia. *Soil Biol. Biochem.* 38, 1734–1744. doi: 10.1016/j.soilbio.2005.11.029
- Neff, J. C., Harden, J. W., and Gleixner, G. (2005). Fire effects on soil organic matter content, composition, and nutrients in boreal interior Alaska. *Can. J. For. Res.* 35, 2178–2187. doi: 10.1139/x05-154
- Odum, E. P. (1969). The strategy of ecosystem development. *Science* 164, 262–270. doi: 10.1126/science.164.3877.262
- Pietikäinen, J., and Fritze, H. (1995). Clear-cutting and prescribed burning in coniferous forest - comparison of effects on soil fungal and total microbial biomass, respiration activity and nitrification. *Soil Biol. Biochem.* 27, 101–109. doi: 10.1016/0038-0717(94)00125-K
- Richter, D., O'Neill, K., and Kasischke, E. (2000). "Postfire stimulation of microbial decomposition in black spruce (*Picea mariana* L.) forest soils: a hypothesis," in *Fire, Climate Change, and Carbon Cycling in the Boreal Forest*, eds E. Kasischke and B. Stocks. (New York, NY: Springer-Verlag), 197–213. doi: 10.1007/978-0-387-21629-4_11
- Rosenberg, M., Adams, D., and Gurevitch, J. (2000). *MetaWin: Statistical Software for Meta-analysis*. Sunderland: Sinauer Associates.
- Rosenberg, M. S., Garrett, K. A., Su, Z., and Bowden, R. L. (2004). Meta-analysis in plant pathology: synthesizing research results. *Phytopathology* 94, 1013–1017. doi: 10.1094/PHYTO.2004.94.9.1013
- Ruel, J. C. (1995). Understanding windthrow - silvicultural implications. *For. Chron.* 71, 434–445.
- Schelhaas, M. J., Nabuurs, G. J., and Schuck, A. (2003). Natural disturbances in the European forests in the 19th and 20th centuries. *Glob. Change Biol.* 9, 1620–1633. doi: 10.1046/j.1365-2486.2003.00684.x
- Siira-Pietikäinen, A., Pietikäinen, J., Fritze, H., and Haimi, J. (2001). Short-term responses of soil decomposer communities to forest management: clear felling versus alternative forest harvesting methods. *Can. J. For. Res.* 31, 88–99. doi: 10.1139/x00-148
- Sokal, R., and Rohlf, F. (1995). *Biometry*. New York, NY: W.H. Freeman and Company.
- Sterne, J. A., and Egger, M. (2001). Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. *J. Clin. Epidemiol.* 54, 1046–1055. doi: 10.1016/S0895-4356(01)00377-8
- Sterne, J. A. C., and Egger, M. (2005). "Regression methods to detect publication and other bias in meta-analysis," in *Publication Bias in Meta-Analysis: Prevention, Assessment and Adjustments*, eds H. R. Rothstein, A. J. Sutton, and M. Borenstein (San Francisco, CA: Wiley), 99–110.
- Treseder, K. K., Mack, M. C., and Cross, A. (2004). Relationships among fires, fungi, and soil dynamics in Alaskan boreal forests. *Ecol. Appl.* 14, 1826–1838. doi: 10.1890/03-5133
- Van Der Werf, G. R., Randerson, J. T., Giglio, L., Collatz, G. J., Mu, M., Kasibhatla, P. S., et al. (2010). Global fire emissions and the contribution of deforestation, savanna, forest, agricultural, and peat fires (1997–2009). *Atmos. Chem. Phys.* 10, 11707–11735. doi: 10.5194/acp-10-11707-2010
- Waldrop, M. P., and Harden, J. W. (2008). Interactive effects of wildfire and permafrost on microbial communities and soil processes in an Alaskan black spruce forest. *Glob. Change Biol.* 14, 2591–2602.
- Wan, S., Hui, D., and Luo, Y. (2001). Fire effects on nitrogen pools and dynamics in terrestrial ecosystems: a meta-analysis. *Ecol. Appl.* 11, 1349–1365. doi: 10.1890/1051-0761(2001)011[1349:FEONPA]2.0.CO;2
- Wang, Q. K., Zhong, M. C., and Wang, S. L. (2012). A meta-analysis on the response of microbial biomass, dissolved organic matter, respiration, and N mineralization in mineral soil to fire in forest ecosystems. *For. Ecol. Manag.* 271, 91–97. doi: 10.1016/j.foreco.2012.02.006
- Yang, L. H. (2004). Periodical cicadas as resource pulses in North American forests. *Science* 306, 1565–1567. doi: 10.1126/science.1103114
- Zhou, D., Zhao, S. Q., Liu, S., and Oeding, J. (2013). A meta-analysis on the impacts of partial cutting on forest structure and carbon storage. *Biogeosci. Discuss.* 10, 787–813. doi: 10.5194/bgd-10-787-2013

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 07 April 2013; accepted: 01 June 2013; published online: 20 June 2013.

Citation: Holden SR and Treseder KK (2013) A meta-analysis of soil microbial biomass responses to forest disturbances. *Front. Microbiol.* 4:163. doi: 10.3389/fmicb.2013.00163

This article was submitted to *Frontiers in Terrestrial Microbiology*, a specialty of *Frontiers in Microbiology*.

Copyright © 2013 Holden and Treseder. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.

APPENDIX

Table A1 | A list of the studies used in meta-analyses.

Study	Disturbance type	Disturbance agent	Biome	Time since disturbance (Y)	Biomass method	R	lnR
MICROBES							
Bååth et al., 1995	Fire	PF	BF	2.50	PLFA	0.65	−0.43
Bárcenas-Moreno et al., 2011	Fire	WF	TF	2.67	CF	0.38	−0.96
D'Ascoli et al., 2005	Fire	PF	WS	0.02	SIR	1.29	0.26
Dannenmann et al., 2011	Fire	WF	WS	0.50	CF	0.75	−0.29
Dangi et al., 2010	Fire	PF	WS	3.00	PLFA	0.69	−0.38
De Marco et al., 2005	Fire	PF	WS	40	CF	1.43	0.26
Dumontet et al., 1996	Fire	WF	TF	0.08	CF	0.75	−0.29
Fioretto et al., 2005	Fire	PF	WS	0.02	ATP	0.25	−1.39
Fenn et al., 1993	Fire	WF	WS	0.01	SIR	1.06	0.06
Fonturbel et al., 2012	Fire	PF	WS	0.01	SF	0.66	−0.42
Fritze et al., 1993	Fire	PF	BF	0.01	CF	0.78	−0.24
Fritze et al., 1994	Fire	PF	BF	2.00	CF	0.39	−0.93
Gömöryová et al., 2008	Fire	PF	WS	0.01	CF	1.08	0.08
Goberna et al., 2012	Fire	WF	TF	0.96	Micro	0.59	−0.52
Grady and Hart, 2006	Fire	WF	TF	7.00	CF	0.38	−0.98
Hamman et al., 2007	Fire	WF	TF	1.00	PFLA	0.84	−0.18
Kara and Bolat, 2009	Fire	WF	TF	0.17	CF	0.98	−0.02
Leduc and Rothstein, 2007	Fire	WF	TF	4.50	CF	0.61	−0.49
Litton et al., 2003	Fire	WF	TF	13.00	CF	0.44	−0.83
Mabuhay et al., 2006	Fire	WF	TF	0.01	CF	0.04	−3.12
Palese et al., 2004	Fire	PF	WS	1.00	CF	0.37	−1.00
Pietikäinen and Fritze, 1995	Fire	PF	BF	1.00	CF	0.31	−1.18
Prieto-Fernández et al., 1998	Fire	WF	TF	0.01	CF	0.04	−3.14
Rutigliano et al., 2007	Fire	PF	WS	0.02	CF	1.50	0.41
Smith et al., 2008	Fire	WF	BF	0.50	CF	0.25	−1.37
Swallow et al., 2009	Fire	PF	BF	1.83	CF	0.51	−0.67
Waldrop and Harden, 2008	Fire	WF	BF	5.00	CF	0.43	−0.83
Arunachalam et al., 1996	Harvest	CC	TF	1.08	CF	0.19	−1.66
Bååth et al., 1995	Harvest	CC	BF	3.17	PLFA	0.72	−0.33
Barbhuiya et al., 2004	Harvest	CC	TrF	7.00	CF	0.37	−1.00
Barbhuiya et al., 2004	Harvest	PH	TrF	8.00	CF	0.58	−0.54
Barg and Edmonds, 1999	Harvest	CC	TF	3.50	CF	1.07	0.06
Barg and Edmonds, 1999	Harvest	PH	TF	3.50	CF	1.13	0.13
Bradley et al., 2001	Harvest	CC	TF	4.00	SIR	0.67	−0.40
Bradley et al., 2001	Harvest	PH	TF	4.00	SIR	0.70	−0.35
Busse et al., 2006	Harvest	CC	TF	6.00	SIR	0.47	−0.76
Chang et al., 1995	Harvest	CC	TF	3.00	CF	0.63	−0.46
Chatterjee et al., 2008	Harvest	CC	TF	15.00	PLFA	0.83	−0.19
Edmonds et al., 2000	Harvest	CC	TF	3.50	CF	1.19	0.18
Entry et al., 1986	Harvest	CC	TF	2.00	CF	1.02	0.02
Forge and Simard, 2000	Harvest	CC	TF	2.00	CF	0.51	−0.67
Grady and Hart, 2006	Harvest	PH	TF	8.00	CF	0.64	−0.44
Hannam et al., 2006	Harvest	CC	BF	4.50	PLFA	0.88	−0.12
Hannam et al., 2006	Harvest	PH	BF	4.50	PLFA	0.89	−0.11
Hassett and Zak, 2005	Harvest	CC	BF	10.00	PLFA	0.77	−0.26

(Continued)

Table A1 | Continued

Study	Disturbance type	Disturbance agent	Biome	Time since disturbance (Y)	Biomass method	R	lnR
Hazlett et al., 2007	Harvest	CC	BF	2.00	CF	0.82	−0.20
Holmes and Zak, 1999	Harvest	CC	BF	1.00	CF	1.31	0.27
Houston et al., 1998	Harvest	CC	BF	8.00	SIR	0.71	−0.35
Lapointe et al., 2005	Harvest	CC	BF	1.50	SIR	0.94	−0.06
Leduc and Rothstein, 2007	Harvest	CC	TF	4.50	CF	0.69	−0.37
Lindo and Visser, 2003	Harvest	CC	BF	2.50	SIR	0.73	−0.31
Maassen et al., 2006	Harvest	PH	TF	5.00	SIR	1.56	0.45
Moore-Kucera and Dick, 2008	Harvest	CC	TF	8.00	PLFA	0.66	−0.42
Pérez-Batallón et al., 2001	Harvest	CC	TF	1.00	CF	0.99	−0.01
Pietikäinen and Fritze, 1995	Harvest	CC	BF	3.00	CF	0.73	−0.32
Saynes et al., 2012	Harvest	PH	TrF	1.00	CF	0.63	−0.47
Siira-Pietikäinen et al., 2001	Harvest	CC	BF	0.17	SIR	0.97	−0.03
Siira-Pietikäinen et al., 2001	Harvest	PH	BF	0.17	SIR	0.80	−0.22
Smith et al., 2008	Harvest	CC	BF	0.50	CF	0.82	−0.20
Tan et al., 2008	Harvest	PH	BF	24	CF	1.21	0.19
Taylor et al., 1999	Harvest	CC	TF	3.21	Count	0.88	−0.13
Wright and Coleman, 2002	Harvest	CC	TF	0.25	CF	0.97	−0.03
Zhao et al., 2011	Harvest	CC	TrF	0.33	PLFA	1.12	0.11
Zu et al., 2009	Harvest	CC	TF	8.00	CF	1.10	0.09
Gömöryová et al., 2008	Storm	WT	TF	0.96	Micro	0.54	−0.61
Tsai et al., 2007	Storm	TY	TrF	0.01	CF	0.24	−1.41
Wright and Coleman, 2002	Storm	HU	TF	0.25	CF	1.04	0.04
Bogorodskaya et al., 2009	Insect	GM	BF	0.13	SIR	1.41	0.35
Le Mellec and Michalzik, 2008	Insect	PL	TF	0.08	CF	1.03	0.03
Xiong et al., 2011	Insect	PB	TF	2.00	CF	0.60	−0.52
Xiong et al., 2011	Insect	PB	TF	4.00	CF	0.67	−0.41
Cromack et al., 1991	Pathogen	PW	TF	2.00	CF	0.54	−0.61
Mabuhay and Nakagoshi, 2012	Pathogen	PWD	TF	2.00	CF	1.55	0.44
FUNGI							
Bååth et al., 1995	Fire	PF	BF	2.50	PLFA	0.37	−0.99
Bárcenas-Moreno et al., 2011	Fire	WF	TF	2.67	PLFA	0.33	−1.10
Capogna et al., 2009	Fire	PF	WS	0.23	Count	0.42	−0.87
D'Ascoli et al., 2005	Fire	PF	WS	0.02	Microsc	0.60	−0.51
Dangi et al., 2010	Fire	PF	WS	3.00	PLFA	0.34	−1.08
Esquilín et al., 2007	Fire	SB	TF	0.02	Microsc	0.89	−0.12
Fritze et al., 1994	Fire	PF	BF	2.00	Ergosterol	0.42	−0.87
Hamman et al., 2007	Fire	WF	TF	1.00	PLFA	0.53	−0.64
Kara and Bolat, 2009	Fire	WF	TF	0.17	Count	0.62	−0.47
Mabuhay et al., 2006	Fire	WF	TF	0.01	Count	0.03	−3.47
Pietikäinen and Fritze, 1995	Fire	PF	BF	1.00	Ergosterol	0.30	−1.21
Rutigliano et al., 2007	Fire	PF	WS	0.02	Microsc	0.61	−0.50
Waldrop and Harden, 2008	Fire	WF	BF	5.00	qPCR	0.40	−0.93
Bååth et al., 1995	Harvest	CC	BF	3.17	PLFA	0.41	−0.89
Barbhuiya et al., 2004	Harvest	CC	TrF	7.00	Count	0.45	−0.79
Barbhuiya et al., 2004	Harvest	PH	TrF	8.00	Count	0.45	−0.79
Carter et al., 2002	Harvest	CC	TF	0.50	Count	1.00	0.00
Chatterjee et al., 2008	Harvest	CC	TF	15.00	PLFA	0.47	−0.76
Forge and Simard, 2000	Harvest	CC	TF	2.00	Microsc	0.47	−0.76
Hannam et al., 2006	Harvest	CC	BF	4.50	PLFA	0.88	−0.13
Hannam et al., 2006	Harvest	PH	BF	4.50	PLFA	1.00	0.00
Hassett and Zak, 2005	Harvest	CC	BF	10.00	PLFA	0.85	−0.16
Hernesmaa et al., 2008	Harvest	CC	BF	0.75	Count	1.02	0.02

(Continued)

Table A1 | Continued

Study	Disturbance type	Disturbance agent	Biome	Time since disturbance (Y)	Biomass method	R	lnR
Maassen et al., 2006	Harvest	PH	TF	5.00	PLFA	1.6	0.47
Moore-Kucera and Dick, 2008	Harvest	CC	TF	8.00	PLFA	0.49	−0.70
Pietikäinen and Fritze, 1995	Harvest	CC	BF	3.00	Ergosterol	0.68	−0.39
Stadler et al., 2006	Insect	HWA	TF	0.08	Count	1.19	0.17
BACTERIA							
Bååth et al., 1995	Fire	PF	BF	2.50	PLFA	0.73	−0.31
Bárcenas-Moreno et al., 2011	Fire	WF	TF	2.67	PLFA	0.43	−0.85
Esquilín et al., 2007	Fire	SB	TF	0.02	Microsc	0.77	−0.26
Hamman et al., 2007	Fire	WF	TF	1.00	PLFA	0.94	−0.06
Kara and Bolat, 2009	Fire	WF	TF	0.17	Count	5.73	1.75
Bååth et al., 1995	Harvest	CC	BF	3.17	PLFA	0.76	−0.28
Barbhuiya et al., 2004	Harvest	CC	TrF	7.00	Count	0.57	−0.57
Barbhuiya et al., 2004	Harvest	PH	TrF	8.00	Count	0.63	−0.46
Carter et al., 2002	Harvest	CC	TF	0.50	Count	1.00	0.00
Chatterjee et al., 2008	Harvest	CC	TF	15.00	PLFA	0.84	−0.17
Forge and Simard, 2000	Harvest	CC	TF	2.00	Microsc	0.98	−0.02
Maassen et al., 2006	Harvest	PH	TF	5.00	PLFA	1.52	0.42
Moore-Kucera and Dick, 2008	Harvest	CC	TF	8.00	PLFA	0.66	−0.42
Stadler et al., 2006	Insect	HWA	TF	0.08	Count	1.10	0.10
GRAM-NEGATIVE BACTERIA							
Dangi et al., 2010	Fire	PF	WS	3.00	PLFA	0.69	−0.37
Chatterjee et al., 2008	Harvest	CC	TF	15.00	PLFA	0.96	−0.04
Hassett and Zak, 2005	Harvest	CC	BF	10.00	PLFA	1.01	0.01
Moore-Kucera and Dick, 2008	Harvest	CC	TF	8.00	PLFA	0.99	0.00
Mabuhay and Nakagoshi, 2012	Pathogen	PWD	TF	2.00	Count	0.46	−0.77
GRAM-POSITIVE BACTERIA							
Dangi et al., 2010	Fire	PF	WS	3.00	PLFA	0.86	−0.15
Chatterjee et al., 2008	Harvest	CC	TF	15.00	PLFA	0.62	−0.47
Hassett and Zak, 2005	Harvest	CC	BF	10.00	PLFA	1.00	0.00
Moore-Kucera and Dick, 2008	Harvest	CC	TF	8.00	PLFA	1.10	0.10
Mabuhay and Nakagoshi, 2012	Pathogen	PWD	TF	2.00	Count	0.35	−1.04
ACTINOMYCETES							
Bárcenas-Moreno et al., 2011	Fire	WF	TF	2.67	PLFA	2.84	1.04
Dangi et al., 2010	Fire	PF	WS	3.00	PLFA	0.66	−0.42
Carter et al., 2002	Harvest	CC	TF	0.50	Count	1.00	0.00
Chatterjee et al., 2008	Harvest	CC	TF	15.00	PLFA	0.88	−0.13
Hannam et al., 2006	Harvest	CC	BF	4.50	PLFA	1.06	0.06
Hannam et al., 2006	Harvest	PH	BF	4.50	PLFA	1.00	0.00
Hassett and Zak, 2005	Harvest	CC	BF	10.00	PLFA	0.98	−0.03
Maassen et al., 2006	Harvest	PH	TF	5.00	PLFA	1.17	0.15
Moore-Kucera and Dick, 2008	Harvest	CC	TF	8.00	PLFA	1.11	0.11
Mabuhay and Nakagoshi, 2012	Pathogen	PWD	TF	2.00	Count	0.29	−1.23

PF, prescribed fire; SB, slash burn; WF, wildfire; CC, clear cut; PH, partial harvest; HU, hurricane; WT, wind throw; TY, typhoon; GM, gypsy moth; HWA, hemlock woolly adelgid; PB, pine beetle; PL, pine lappet; PV, *Phellinus weirii* infection; PWD, pine wilt disease; BF, boreal forest; TF, temperate forest; TrF, tropical forest; WS, woodland/shrubland, CF, chloroform fumigation; Count, dilution plate count; Micro, microwave irradiation; Microsc, microscopy; PLFA, phospholipid fatty acid; qPCR, quantitative PCR; SIR, substrate-induced respiration.

Table A2 | Outcomes of test for publication bias.

Organism	Group	Kendall's tau rank correlation	Spearman rank correlation	Egger's regression
Microbes	All microbe studies	τ : -0.084 P : 0.249	ρ : -0.099 P : 0.360	Intercept: -5.62 P : 0.136
	All abiotic	τ : -0.038 P : 0.618	ρ : -0.037 P : 0.746	Intercept: -5.99 P : 0.124
	All biotic	τ : -0.512 P : 0.076	ρ : -0.655 P : 0.078	Intercept: -5.71 P: 0.029
Fungi	All fungi studies	τ : -0.314 P: 0.008	ρ : -0.416 P: 0.013	Intercept: -7.23 P : 0.377
	All abiotic	τ : -0.425 P: 0.001	ρ : -0.560 P: 0.001	Intercept: -5.61 P : 0.230
	All biotic	n.a.	n.a.	n.a.
Bacteria	All bacteria studies	τ : 0.033 P : 0.855	ρ : 0.062 P : 0.812	Intercept: -2.67 P : 0.537
	All abiotic	τ : 0.082 P : 0.669	ρ : 0.144 P : 0.608	Intercept: -4.00 P : 0.446
	All biotic	n.a.	n.a.	n.a.

Tests could not be performed on biotic studies within fungi and bacteria because not enough studies were present. Boldface type indicates significance at $P < 0.05$.

REFERENCES

- Arunachalam, A., Maithani, K., Pandey, H. N., and Tripathi, R. S. (1996). The impact of disturbance on detrital dynamics and soil microbial biomass of a *Pinus kesiyia* forest in north-east India. *For. Ecol. Manag.* 88, 273–282. doi: 10.1016/S0378-1127(96)03801-7
- Bååth, E., Frostegård, Å., Pennanen, T., and Fritze, H. (1995). Microbial community structure and pH response in relation to soil organic-matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. *Soil Biol. Biochem.* 27, 229–240. doi: 10.1016/0038-0717(94)00140-V
- Barbhuiya, A. R., Arunachalam, A., Pandey, H. N., Arunachalam, K., Khan, M. L., and Nath, P. C. (2004). Dynamics of soil microbial biomass C, N and P in disturbed and undisturbed stands of a tropical wet-evergreen forest. *Eur. J. Soil Biol.* 40, 113–121. doi: 10.1016/j.ejsobi.2005.02.003
- Bárcenas-Moreno, G., García-Orenes, F., Mataix-Solera, J., Mataix-Beneyto, J., and Bååth, E. (2011). Soil microbial recolonisation after a fire in a Mediterranean forest. *Biol. Fertil. Soils* 47, 261. doi: 10.1007/s00374-010-0532-2
- Barg, A. K., and Edmonds, R. L. (1999). Influence of partial cutting on site microclimate, soil nitrogen dynamics, and microbial biomass in Douglas-fir stands in western Washington. *Can. J. For. Res.* 29, 705–713. doi: 10.1139/x99-045
- Bogorodskaya, A. V., Baranchikov, Y. N., and Ivanova, G. A. (2009). The state of microbial complexes in soils of forest ecosystems after fires and defoliation of stands by gypsy moths. *Eur. Soil Sci.* 42, 310–317. doi: 10.1134/S1064229309030089
- Bradley, R. L., Titus, B. D., and Hogg, K. (2001). Does shelterwood harvesting have less impact on forest floor nutrient availability and microbial properties than clearcutting? *Biol. Fertil. Soils* 34, 162–169. doi: 10.1007/s003740100389
- Busse, M. D., Beattie, S. E., Powers, R. F., Sanchez, F. G., and Tiarks, A. E. (2006). Microbial community responses in forest mineral soil to compaction, organic matter removal, and vegetation control. *Can. J. For. Res.* 36, 577–588. doi: 10.1139/x05-294
- Capogna, F., Persiani, A., Maggi, O., Dowling, G., Puppi, G., and Manes, F. (2009). Effects of different fire intensities on chemical and biological soil components and related feedbacks on a mediterranean shrub (*Phillyrea angustifolia* L.). *Plant Ecol.* 204, 155–171. doi: 10.1007/s11258-009-9579-2
- Carter, M. C., Dean, T. J., Zhou, M. Y., Messina, M. G., and Wang, Z. Y. (2002). Short-term changes in soil C, N, and biota following harvesting and regeneration of loblolly pine (*Pinus taeda* L.). *For. Ecol. Manag.* 164, 67–88. doi: 10.1016/S0378-1127(01)00590-4
- Chang, S. X., Preston, C. I., and Weetman, G. F. (1995). Soil microbial biomass and microbial and mineralizable N in a clear-cut chronosequence on northern Vancouver-island, british-columbia. *Can. J. For. Res.* 25, 1595–1607. doi: 10.1139/x95-174
- Chatterjee, A., Vance, G. F., Pendall, E., and Stahl, P. D. (2008). Timber harvesting alters soil carbon mineralization and microbial community structure in coniferous forests. *Soil Biol. Biochem.* 40, 1901–1907. doi: 10.1016/j.soilbio.2008.03.018
- Cromack, K., Entry, J. A., and Savage, T. (1991). The effect of disturbance by *Phellinus weirii* on decomposition and nutrient mineralization in a tuga mertensiana forest. *Biol. Fertil. Soils* 11, 245–249. doi: 10.1007/BF00335842
- D'Ascoli, R., Rutigliano, F. A., De Pascale, R. A., Gentile, A., and De Santo, A. V. (2005). Functional diversity of the microbial community in Mediterranean maquis soils as affected by fires. *Int. J. Wildland Fire* 14, 355–363. doi: 10.1071/WF05032
- Dangi, S. R., Stahl, P. D., Pendall, E., Cleary, M. B. and Buyer, J. S. (2010). Recovery of soil microbial community structure after fire in a sagebrush-grassland ecosystem. *Land Degrad. Develop.* 21, 423–432. doi: 10.1002/ldr.975
- Dannenmann, M., Willibald, G., Sippel, S., and Butterbach-Bahl, K. (2011). Nitrogen dynamics at undisturbed and burned Mediterranean shrublands of Salento Peninsula, Southern Italy. *Plant Soil* 343, 5–15. doi: 10.1007/s11104-010-0541-9
- De Marco, A., Gentile, A. E., Arena, C., and De Santo, A. V. (2005). Organic matter, nutrient content and biological activity in burned and unburned soils of a Mediterranean maquis area of southern Italy. *Int. J. Wildland Fire* 14, 365–377. doi: 10.1071/WF05030
- Dumontet, S., Dinel, H., Scopa, A., Mazzatura, A., and Saracino, A. (1996). Post-fire soil microbial biomass and nutrient content of a pine forest soil from a dunal Mediterranean environment. *Soil Biol. Biochem.* 28, 1467–1475. doi: 10.1016/S0038-0717(96)00160-5
- Edmonds, R. L., Marra, J. L., Barg, A. K., and Sparks, G. B. (2000). *Influence of forest harvesting on soil organisms and decomposition in Western Washington*. USDA Forest Service Gen. Tech. Rep. PSW-GTR-178.
- Entry, J. A., Stark, N. M., and Loewenstein, H. (1986). Effect of timber harvesting on microbial biomass fluxes in a northern rocky-mountain forest soil. *Can. J. For. Res.* 16, 1076–1081. doi: 10.1139/x86-186
- Esquirlín, A. E. J., Stromberger, M. E., Massman, W. J., Frank, J. M., and Shepperd, W. D. (2007). Microbial community structure and activity in a Colorado Rocky Mountain forest soil scarred by slash pile burning. *Soil Biol. Biochem.* 39, 1111–1120. doi: 10.1016/j.soilbio.2006.12.020
- Fenn, M. E., Poth, M. A., Dunn, P. H., and Barro, S. C. (1993). Microbial N and biomass, respiration and N-Mineralization in soils beneath 2 chaparral species along a fire-induced age gradient. *Soil Biol. Biochem.* 25, 457–466. doi: 10.1016/0038-0717(93)90071-I
- Fiochetto, A., Papa, S., and Pellegrino, A. (2005). Effects of fire on soil respiration, ATP content and enzyme activities in Mediterranean maquis. *Appl. Veg. Sci.* 8, 13–20. doi: 10.1111/j.1654-109X.2005.tb00624.x
- Fonturbel, M. T., Barreiro, A., Vega, J. A., Martin, A., Jimenez, E., Carballas, T., et al. (2012). Effects of an experimental fire and post-fire stabilization treatments on soil microbial communities. *Geoderma* 191, 51–60. doi: 10.1016/j.geoderma.2012.01.037
- Forge, T. A., and Simard, S. W. (2000). Trophic structure of nematode communities, microbial biomass, and nitrogen mineralization in soils of forests and clearcuts in the southern interior of British Columbia. *Can. J. Soil Sci.* 80, 401–410. doi: 10.4141/S99-112
- Fritze, H., Pennanen, T., and Pietikainen, J. (1993). Recovery of soil microbial biomass and activity from prescribed burning. *Can. J. For. Res.* 23, 1286–1290. doi: 10.1139/x93-164
- Fritze, H., Smolander, A., Levula, T., Kitunen, V., and Malkonen, E. (1994). Wood-ash fertilization and fire treatments in a scots pine forest stand - effects on the organic layer, microbial biomass, and microbial activity. *Biol. Fertil. Soils* 17, 57–63. doi: 10.1007/BF00418673
- Goberna, M., Garcia, C., Insam, H., Hernandez, M., and Verdu, M. (2012). Burning fire-prone mediterranean shrublands: immediate changes in soil microbial community structure and ecosystem functions. *Microb. Ecol.* 64, 242–255. doi: 10.1007/s00248-011-9995-4
- Gömöryová, E., Střelcová, K., Škvarenina, J., Bebej, J., and Gömöry, D. (2008). The impact of windthrow and fire disturbances on selected soil properties in the Tatra National Park. *Soil Water Res.* 3, S74–S80.
- Grady, K. C., and Hart, S. C. (2006). Influences of thinning, prescribed burning, and wildfire on soil processes and properties in southwestern ponderosa pine forests: a retrospective study. *For. Ecol. Manag.* 234, 123–135. doi: 10.1016/j.foreco.2006.06.031
- Hamman, S. T., Burke, I. C., and Stromberger, M. E. (2007). Relationships between microbial community structure and soil environmental conditions in a recently burned system. *Soil Biol. Biochem.* 39, 1703–1711. doi: 10.1016/j.soilbio.2007.01.018
- Hannam, K. D., Quideau, S. A., and Kishchuk, B. E. (2006). Forest floor microbial communities in relation to stand composition and timber harvesting in northern Alberta. *Soil Biol. Biochem.* 38, 2565–2575. doi: 10.1016/j.soilbio.2006.03.015
- Hassett, J. E., and Zak, D. R. (2005). Aspen harvest intensity decreases microbial biomass, extracellular enzyme activity, and soil nitrogen cycling. *Soil Sci. Soc. Am. J.* 69, 227–235. doi: 10.2136/sssaj2005.0227
- Hazlett, P. W., Gordon, A. M., Voroney, R. P., and Sibley, P. K. (2007). Impact of harvesting and logging slash on nitrogen and carbon dynamics in soils from upland spruce forests in northeastern Ontario. *Soil Biol. Biochem.* 39, 43–57. doi: 10.1016/j.soilbio.2006.06.008
- Hernesmaa, A., Bjorklof, K., Jorgensen, K. S., Haahtela, K., and Romantschuk, M. (2008). Potential impacts of clear-felling on microbial activities in boreal humus and mineral soil layers. *Boreal Environ. Res.* 13, 525–538.
- Holmes, W. E., and Zak, D. R. (1999). Soil microbial control of nitrogen loss following clearcut harvest in northern

- hardwood ecosystems. *Ecol. Appl.* 9, 202–215. doi: 10.1890/1051-0761(1999)009[0202:SMCONL]2.0.CO;2
- Houston, A. P. C., Visser, S., and Lautenschlager, R. A. (1998). Microbial processes and fungal community structure in soils from clear-cut and unharvested areas of two mixedwood forests. *Can. J. Bot.* 76, 630–640. doi: 10.1139/cjb-76-4-630
- Kara, O., and Bolat, I. (2009). Short-term effects of wildfire on microbial biomass and abundance in black pine plantation soils in Turkey. *Ecol. Indic.* 9, 1151–1155. doi: 10.1016/j.ecolind.2009.01.002
- Lapointe, B., Bradley, R. L., and Shipley, B. (2005). Mineral nitrogen and microbial dynamics in the forest floor of clearcut or partially harvested successional boreal forest stands. *Plant Soil* 271, 27–37. doi: 10.1007/s11104-004-1830-y
- Le Mellec, A., and Michalzik, B. (2008). Impact of a pine lappet (*Dendrolimus pini*) mass outbreak on C and N fluxes to the forest floor and soil microbial properties in a Scots pine forest in Germany. *Can. J. For. Res.* 38, 1829–1841. doi: 10.1139/X08-045
- Leduc, S. D., and Rothstein, D. E. (2007). Initial recovery of soil carbon and nitrogen pools and dynamics following disturbance in jack pine forests: a comparison of wildfire and clearcut harvesting. *Soil Biol. Biochem.* 39, 2865–2876. doi: 10.1016/j.soilbio.2007.05.029
- Lindo, Z., and Visser, S. (2003). Microbial biomass, nitrogen and phosphorus mineralization, and mesofauna in boreal conifer and deciduous forest floors following partial and clear-cut harvesting. *Can. J. For. Res.* 33, 1610–1620. doi: 10.1139/x03-080
- Litton, C. M., Ryan, M. G., Knight, D. H., and Stahl, P. D. (2003). Soil-surface carbon dioxide efflux and microbial biomass in relation to tree density 13 years after a stand replacing fire in a lodgepole pine ecosystem. *Glob. Change Biol.* 9, 680–696. doi: 10.1046/j.1365-2486.2003.00626.x
- Maassen, S., Fritze, H., and Wirth, S. (2006). Response of soil microbial biomass, activities, and community structure at a pine stand in northeastern Germany 5 years after thinning. *Can. J. For. Res.* 36, 1427–1434. doi: 10.1139/x06-039
- Mabuhay, J., Isagi, Y., and Nakagoshi, N. (2006). Wildfire effects on microbial biomass and diversity in pine forests at three topographic positions. *Ecol. Res.* 21, 54–63. doi: 10.1007/s11284-005-0094-1
- Mabuhay, J. A., and Nakagoshi, N. (2012). Response of soil microbial communities to changes in a forest ecosystem brought about by pine wilt disease. *Landsc. Ecol. Eng.* 8, 189–196. doi: 10.1007/s11355-011-0165-0
- Moore-Kucera, J., and Dick, R. P. (2008). PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. *Microb. Ecol.* 55, 500–511. doi: 10.1007/s00248-007-9295-1
- Palese, A. M., Giovannini, G., Lucchesi, S., Dumontet, S., and Perucci, P. (2004). Effect of fire on soil C, N and microbial biomass. *Agronomie* 24, 47–53. doi: 10.1051/agro:2003061
- Pérez-Batallón, P., Ouro, G., Macías, F., and Merino, A. (2001). Initial mineralization of organic matter in a forest plantation soil following different logging residue management techniques. *Ann. For. Sci.* 58, 807–818. doi: 10.1051/forest:2001164
- Pietikäinen, J., and Fritze, H. (1995). Clear-cutting and prescribed burning in coniferous forest - comparison of effects on soil fungal and total microbial biomass, respiration activity and nitrification. *Soil Biol. Biochem.* 27, 101–109. doi: 10.1016/0038-0717(94)00125-K
- Prieto-Fernández, A., Acea, M. J., and Carballas, T. (1998). Soil microbial and extractable C and N after wildfire. *Biol. Fertil. Soils* 27, 132–142. doi: 10.1007/s003740050411
- Rutigliano, F. A., De Marco, A., D'Ascoli, R., Castaldi, S., Gentile, A., and De Santo, A. V. (2007). Impact of fire on fungal abundance and microbial efficiency in C assimilation and mineralisation in a Mediterranean maquis soil. *Biol. Fertil. Soils* 44, 377–381. doi: 10.1007/s00374-007-0214-x
- Saynes, V., Etchevers, J. D., Galicia, L., Hidalgo, C., and Campo, J. (2012). Soil carbon dynamics in high-elevation temperate forests of Oaxaca (Mexico): thinning and rainfall effects. *Bosque* 33, 3–11.
- Siira-Pietikäinen, A., Pietikäinen, J., Fritze, H., and Haimi, J. (2001). Short-term responses of soil decomposer communities to forest management: clear felling versus alternative forest harvesting methods. *Can. J. For. Res.* 31, 88–99. doi: 10.1139/x00-148
- Smith, N. R., Kishchuk, B. E., and Mohn, W. W. (2008). Effects of wildfire and harvest disturbances on forest soil bacterial communities. *Appl. Environ. Microbiol.* 74, 216–224. doi: 10.1128/AEM.01355-07
- Stadler, B., Muller, T., and Orwig, D. (2006). The ecology of energy and nutrient fluxes in hemlock forests invaded by hemlock woolly adelgid. *Ecology* 87, 1792–1804. doi: 10.1890/0012-9658(2006)87[1792:TEOEAN]2.0.CO;2
- Swallow, M., Quideau, S. A., Mackenzie, M. D., and Kishchuk, B. E. (2009). Microbial community structure and function: the effect of silvicultural burning and topographic variability in northern Alberta. *Soil Biol. Biochem.* 41, 770–777. doi: 10.1016/j.soilbio.2009.01.014
- Tan, X., Chang, S. X., Comeau, P. G., and Wang, Y. H. (2008). Thinning effects on microbial biomass, N mineralization, and tree growth in a mid-rotation fire-origin lodgepole pine stand in the lower foothills of Alberta. *Can. For. Sci.* 54, 465–474.
- Taylor, L. A., Arthur, M. A., and Yanai, R. D. (1999). Forest floor microbial biomass across a northern hardwood successional sequence. *Soil Biol. Biochem.* 31, 431–439. doi: 10.1016/S0038-0717(98)00148-5
- Tsai, C.-C., Liao, J.-H., Wang, H.-H., and Hseu, Z.-Y. (2007). Effects of typhoon disturbances on soil microbial activities in an uplifted coral reef tropical forest, southern taiwan. *Taiwan J. F. Sci.* 22, 265–279.
- Waldrop, M. P., and Harden, J. W. (2008). Interactive effects of wildfire and permafrost on microbial communities and soil processes in an Alaskan black spruce forest. *Glob. Change Biol.* 14, 2591–2602.
- Wright, C. J., and Coleman, D. C. (2002). Responses of soil microbial biomass, nematode trophic groups, N-mineralization, and litter decomposition to disturbance events in the southern Appalachians. *Soil Biol. Biochem.* 34, 13–25. doi: 10.1016/S0038-0717(01)00128-6
- Xiong, Y. M., D'Atri, J. J., Fu, S. L., Xia, H. P., and Seastedt, T. R. (2011). Rapid soil organic matter loss from forest dieback in a subalpine coniferous ecosystem. *Soil Biol. Biochem.* 43, 2450–2456. doi: 10.1016/j.soilbio.2011.08.013
- Zhao, J., Wang, X. L., Shao, Y. H., Xu, G. L., and Fu, S. L. (2011). Effects of vegetation removal on soil properties and decomposer organisms. *Soil Biol. Biochem.* 43, 954–960. doi: 10.1016/j.soilbio.2011.01.010
- Zu, Y. G., Wang, W. J., Wang, H. M., Liu, W., Cui, S., and Koike, T. (2009). Soil CO₂ efflux, carbon dynamics, and change in thermal conditions from contrasting clear-cut sites during natural restoration and uncut larch forests in north-eastern China. *Clim. Change* 96, 137–159. doi: 10.1007/s10584-009-9601-7



Microbial responses to multi-factor climate change: effects on soil enzymes

J. Megan Steinweg^{1,2†}, Jeffrey S. Dukes^{3,4}, Eldor A. Paul^{1,5} and Matthew D. Wallenstein^{1,2,6*}

¹ Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO, USA

² Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO, USA

³ Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN, USA

⁴ Department of Biological Sciences, Purdue University, West Lafayette, IN, USA

⁵ Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO, USA

⁶ Department of Ecosystem Science and Sustainability, Colorado State University, Fort Collins, CO, USA

Edited by:

Johannes Rousk, Lund University, Sweden

Reviewed by:

David Fernández Calviño, University of Vigo, Spain

Stephanie N. Kivlin, University of Texas at Austin, USA

*Correspondence:

Matthew D. Wallenstein, Natural Resource Ecology Laboratory, Colorado State University, Campus Delivery 1499, Fort Collins, CO 80523-1499, USA
e-mail: matthew.wallenstein@colostate.edu

† Present address:

J. Megan Steinweg, Oak Ridge National Laboratory, Oak Ridge, USA

The activities of extracellular enzymes, the proximate agents of decomposition in soils, are known to depend strongly on temperature, but less is known about how they respond to changes in precipitation patterns, and the interaction of these two components of climate change. Both enzyme production and turnover can be affected by changes in temperature and soil moisture, thus it is difficult to predict how enzyme pool size may respond to altered climate. Soils from the Boston-Area Climate Experiment (BACE), which is located in an old field (on abandoned farmland), were used to examine how climate variables affect enzyme activities and microbial biomass carbon (MBC) in different seasons and in soils exposed to a combination of three levels of precipitation treatments (ambient, 150% of ambient during growing season, and 50% of ambient year-round) and four levels of warming treatments (unwarmed to ~4°C above ambient) over the course of a year. Warming, precipitation and season had very little effect on potential enzyme activity. Most models assume that enzyme dynamics follow microbial biomass, because enzyme production should be directly controlled by the size and activity of microbial biomass. We observed differences among seasons and treatments in mass-specific potential enzyme activity, suggesting that this assumption is invalid. In June 2009, mass-specific potential enzyme activity, using chloroform fumigation-extraction MBC, increased with temperature, peaking under medium warming and then declining under the highest warming. This finding suggests that either enzyme production increased with temperature or turnover rates decreased. Increased maintenance costs associated with warming may have resulted in increased mass-specific enzyme activities due to increased nutrient demand. Our research suggests that allocation of resources to enzyme production could be affected by climate-induced changes in microbial efficiency and maintenance costs.

Keywords: enzymes, carbon, nitrogen, precipitation, temperature, decomposition, microbial biomass

INTRODUCTION

The rate at which soil organic matter (SOM) is decomposed is strongly affected by temperature and moisture, and thus should be sensitive to climate change (Davidson et al., 1998; Schimel and Gullledge, 1998). While heterotrophic respiration is widely used as a proxy for decomposition, the relationship between abiotic drivers and decomposition rates is driven by a series of underlying microbially mediated processes (Ekschmitt et al., 2005). For example, enzymatic depolymerization of SOM controls the rate at which assimilable dissolved organic matter (DOM) is produced (Conant et al., 2011), and has been hypothesized to be the rate-limiting step in decomposition (Schimel and Bennett, 2004; Bengtson and Bengtsson, 2007). Thus, it is important to examine the response of enzyme activities to climate change in order to improve our ability to predict carbon fluxes under future climate regimes.

The rate of *in situ* enzyme activity is directly responsive to temperature and moisture (Trasar-Cepeda et al., 2007;

Allison and Treseder, 2008; Wallenstein and Weintraub, 2008; Steinweg et al., 2012) but is also controlled by enzyme pool size. Enzyme pool size is controlled by the rate at which enzymes are produced by microbes relative to the rate at which they are degraded in the environment. Both enzyme production and turnover rates may be affected by temperature and moisture, and thus may vary seasonally and be affected by climate change.

What controls enzyme production by microbes? The production of enzymes incurs a cost to microbes in terms of both energy and nutrients. Thus, the production of enzymes should be governed by the economics of the amount of resources gained for each enzyme produced (Allison et al., 2011). To maintain the stoichiometry of their biomass (driven by the fixed stoichiometry of cellular components) (Cleveland and Liptzin, 2007), microbes produce enzymes targeting specific compounds that are rich in either carbon (C), nitrogen (N) or phosphorus (P) (Sinsabaugh et al., 2008, 2009). However, enzyme production declines for

many substrates when substrate concentration is low (German et al., 2011).

Temperature and moisture can affect both the overall rate of enzyme production as well as the relative rate of production of different enzymes due to effects on enzyme efficiency, substrate availability, and microbial efficiency. Thus, changes in the soil microclimate, whether they occur within hours, weeks, seasonally, or over decades in response to climate change, will affect enzyme pool sizes. In response to increased activity of the extant enzyme pool as soil temperatures increase, given available substrate, microbes may allocate fewer resources to enzyme production if microbial biomass remains unchanged (Allison and Vitousek, 2005). Several studies have found that N-degrading enzymes have lower temperature sensitivities than C-degrading enzymes (Wallenstein et al., 2009, 2012; Stone et al., 2012). This could result in increasing N limitation as soils warm, spurring microbes to increase the production of N-degrading enzymes and decrease the production of C-degrading enzymes. Soil moisture affects the diffusion of substrates, enzymes and the products of enzyme activity, and thus drought conditions could impose diffusion limitations on enzymes and substrates (Stark and Firestone, 1995; Allison, 2005). In oxic soils, drought could decrease enzyme production as biomass declines, or increase production to satisfy nutrient requirements of the biomass (Allison and Vitousek, 2005; Sardans and Peñuelas, 2005; Sowerby et al., 2005).

Our objective was to assess the response of hydrolytic enzyme activities to a multi-factor climate change experiment. To separate the influences of soil warming and moisture on enzyme activity from seasonal effects, we measured the activity and stoichiometry of six enzymes involved in C, N, and P cycling four times over the course of a year, in soils from the Boston-Area Climate Experiment (BACE). The BACE is a multifactorial climate change manipulation in an old-field ecosystem, providing three levels of precipitation and four levels of warming. Because of the multifactorial design, the BACE allowed us to compare twelve different climate years simultaneously during the 1 year of soil sampling. Potential enzyme activity is a metric for soil microbial function response to disturbance (Henry et al., 2005) and indicates shifts in metabolic requirements (Caldwell, 2005). We hypothesized that climate affects enzyme activity by altering microbial biomass and through abiotic controls on enzyme turnover and stabilization. We predicted that: (1) drought would reduce microbial biomass, decreasing potential enzyme activity, (2) warming in the field would decrease potential enzyme activity measured in the laboratory, because enzymes produced at higher temperatures would have higher reaction efficiency, resulting in decreased microbial enzyme production rates, and (3) N and P enzyme activities would be greater in the growing season compared to the winter due to increased C availability.

MATERIALS AND METHODS

STUDY SITE

The BACE is located in an old field in Waltham, Massachusetts at the University of Massachusetts' Suburban Experiment Station (42° 23' 3"N, 71° 12' 52" W; "old fields" are typically abandoned agricultural fields dominated by perennial grasses and forbs; they are kept from returning to their pre-agricultural forested state by

regular mowing or grazing). Mean annual precipitation and temperature in the area are 1194 mm yr⁻¹ and 9.5°C (Hoeppepner and Dukes, 2012). The soil is a mesic Typic Dystrudept, and the upper 30 cm is loam (45% sand, 46% silt, and 9% clay), with an average pH of 5.5. The site, a former apple orchard, has harbored old-field vegetation for more than 40 years. Recent surveys identified 42 grass and forb species, most of which have been introduced (Hoeppepner and Dukes, 2012).

FIELD EXPERIMENTAL DESIGN

The BACE exposed 36 square, 4 m² plots to three precipitation treatments and four warming levels in a full-factorial design, with three replicates of each treatment. The precipitation treatments included an "ambient" control, a "wet" treatment that received a 50% increase in precipitation during the growing season only, and a "drought" treatment in which 50% of ambient precipitation was excluded across all seasons. These treatments were chosen such that a year with average precipitation would result in "wet" and "drought" treatments that fell within the extremes of a 75-year historical record for the area. Above the drought plots, clear partial roofs excluded half of incoming precipitation, and this water was immediately diverted to wet plots from May to October. The roofs continued to function from November to April, but during these colder months diverted water was not added to the wet plots. Drought treatments began in January 2007, and wet treatments began in June 2008.

The warming treatments (unwarmed ambient, low, medium, and high) were implemented such that warming of the canopy in the high treatment was limited to a maximum of 4°C. This temperature limit was determined by logistical and financial constraints. Warming was achieved using ceramic infrared heaters, which were mounted 1 m above each corner of each plot. An unwarmed treatment had four dummy heaters (providing similar shade as heaters, but no warming), and individual heaters above the low, medium, and high treatments were rated at 200, 600, and 1000 W, respectively. Warming treatments were nested within precipitation treatments; within each area receiving a given precipitation treatment, a group of four plots was arranged linearly, from unwarmed to high. Canopy temperature was monitored every 10 s in the unwarmed and high plots in each group, using infrared radiometers (IRR-PN; Apogee Instruments, Logan, UT, USA). All heaters in each group of four plots were controlled by the same circuit, and the system was programmed to adjust power to the circuit to maintain a target difference of 4°C between the "high" and "unwarmed" plots in each group. Warming treatments began on July 1, 2008.

Soil moisture was measured weekly during the non-freezing months, usually beginning in April and ending in December, using time-domain reflectometry (waveguides were installed across 0–10 and 0–30 cm depths). Dataloggers recorded soil temperature near the center of each plot every 30 min throughout the year, as measured by linear temperature sensors positioned at 2 and 10 cm depths. Field measurements of heterotrophic soil respiration were taken using a LI-COR 6400-09 soil CO₂ flux chamber attached to a 6400 portable photosynthetic system. Once a month, CO₂ flux was measured within a 25 cm diameter PVC collar installed in each plot. Collars extended to 30 cm depth, and

had been installed in November 2007. All plants were removed from the collar shortly after installation, and collars were subsequently covered with a weed-blocking cloth to prevent new plants from colonizing the soil [for details see Suseela et al. (2012)].

SOIL SAMPLING AND PROCESSING

Soils were first collected from all plots in June 2008, 1 year after precipitation manipulations began, but before the start of the warming treatments. Additionally, soil samples were taken three times (August 2008, January 2009, and June 2009) following the initiation of the warming treatment. Three cores (5 cm diameter) were collected from each plot at 0–5 and 5–15 cm depths. Soils were packaged on ice and shipped to the laboratory overnight, where the cores from each plot were sieved (2 mm), picked free of rocks and roots, homogenized and frozen at -10°C until analysis.

SOIL CHARACTERIZATION

Subsamples from each plot were taken for determination of percent soil moisture, pH, and total C and N concentrations. Soil moisture was determined after field-moist soils were weighed and dried for 48 h at 60°C and then reweighed. Soil pH was determined using the supernatant of soil mixed with water (1:5 by volume). Soil subsamples were dried at 60°C and ground to measure total C and N concentrations on a LECO CHN-1000 autoanalyzer (LECO Corporation, St. Joseph, MI, USA).

MICROBIAL BIOMASS

Substrate-induced respiration (SIR) and chloroform fumigation extraction (CFE) were used to estimate microbial biomass carbon (MBC) (Anderson and Domsch, 1978; Vance et al., 1987). SIR-MBC is an estimation of the active microbial biomass whereas CFE-MBC is an estimation of the total microbial biomass.

SIR-MBC

SIR-MBC was measured using a deep-well microplate-based technique called MicroResp™ (Aberdeen, UK) (Campbell et al., 2003). Soils from all sampling dates were removed from the freezer and a 20 g subsample was thawed to about 20°C within 3 h. Since soil moisture varied by date, we brought all soils to 55% water holding capacity through wetting or drying, for optimum microbial activity and to eliminate substrate diffusion constraints. The August 2008 and June 2009 samples were initially below 55% water holding capacity, so after thawing, all August and June samples had water added. Samples were then covered for 1 h, homogenized and added to wells in the 96-well deep-well plates. For the January 2009 sampling, all samples were over 55% water holding capacity. In this case, 20 g subsamples were dried to 55% water holding capacity at 4°C , over 6–36 h. Following drying, the January 2009 samples were homogenized and weighed into 96-well deep-well plates. Three wells on a plate were used per sample, with about 0.2–0.3 g of moist soil added to each well, using the MicroResp manufacturer's protocol. After samples were added to the deep-well plate, they were covered with sealing film and placed at 4°C for about 18 h prior to addition of glucose.

Following the 18 h incubation at 4°C , $25\ \mu\text{l}$ of 1 M glucose solution was added (this concentration had been determined to saturate demand in preliminary assays), and samples were then incubated at 25°C for 6 h. The CO_2 indicator plates were read on

a Tecan Infinite M500 microplate reader at 625 nm prior to being placed on deep-well plates. The indicator plate and deep-well plate were attached to one another using the MicroResp apparatus and allowed to incubate. Following the 6 h incubation the indicator plates were removed from the deep-well plates and read again on the Tecan microplate reader at 625 nm.

Indicator plates (containing cresol red, sodium bicarbonate and potassium chloride) were made 1 week in advance of the assay according to the manufacturer's guidelines. Standard curves were generated by incubating indicator plates in jars filled with known concentrations of CO_2 . The amount of CO_2 produced from the water addition wells was subtracted from the respiration in the glucose addition wells to account for stimulation of respiration due to changes in soil water content. MBC was calculated from respiration produced from the glucose amended wells at 25°C and using the following equation from Anderson and Domsch (1978):

$$\text{mg MBC } 100\ \text{g}^{-1}\text{ soil} = 40.04y + 0.37$$

where y is the amount of CO_2 produced under glucose amendment.

CFE-MBC

CFE-MBC was measured using the method of Vance et al. (1987). Briefly, 10 g of field-moist soil from each plot was thawed and placed in a fumigation chamber and fumigated over the course of 5 days with chloroform. Following fumigation, the soils were shaken with 40 mL of 0.5 M K_2SO_4 for 2 h and then filtered through a Whatman 1 filter. Additionally, another 10 g sample from each plot was shaken for 2 h with 0.5 M K_2SO_4 and then filtered through a Whatman 1 filter. The filtrates were stored frozen until analysis. The organic carbon in the filtrates from both procedures was measured on a Shimadzu TOC analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). The fumigated sample contained dissolved organic carbon and MBC, the non-fumigated sample contained dissolved organic carbon. Soils were frozen prior to DOC and MBC extraction, which may have resulted in cell lysis for both the DOC and MBC extracts leading to an overall reduction in estimated MBC.

ENZYME ASSAYS

Enzyme assays were performed on samples from all plots at each collection date. Each sample was assayed for the potential activity of six different hydrolytic enzymes involved in C, N, and P acquisition (Table 1). The assay protocol was modified from (Saiya-Cork et al., 2002) to include a standard curve for each sample and to minimize quenching effects. The assays for 12 soil samples were incubated for 3 h at 25°C using one deep-well 96-well plate. Two additional plates were used to create standard curves for each sample at 25°C . The reference standard for the leucine amino peptidase assay was 7-amino-4-methylcoumarin (MUC), and for the remaining substrates it was 4-methylumbelliferone (MUB). The standard curve plates had a column for each of the 12 samples and different concentrations of MUB or MUC standards in each well, 0, 2.5, 5, 10, 25, 50, and $100\ \mu\text{M}$.

After soils were removed from the freezer, a 2.75 g subsample was taken and warmed to about 20°C . The subsample was

Table 1 | Enzymes assayed in this study, their abbreviations used in the text, nutrient cycles they are involved in, and their target substrates.

Enzyme name	Abbreviation	Nutrient cycle	Enzyme function
β -glucosidase	BG	C	Hydrolysis of terminal β -D-glucosyl residues
Cellobiohydrolase	CB	C	Hydrolysis of β -D-glucosyl linkages
Xylosidase	XYL	C	Hydrolysis of β -D-xylose residues
Acid phosphomonoesterase	PHOS	P	Hydrolysis of phosphate monoester
N-acetyl glucosaminidase	NAG	N	Hydrolysis of chitin N-acetyl- β -D-glucosaminide
Leucine-amino peptidase	LAP	N	Hydrolysis of N-terminus amino acid leucine

homogenized with 91 mL of 50 mM sodium acetate (pH 5.5) for 1 min on high in a Waring blender. Each column on the deep-well 96-well plates corresponded to one sample. After homogenization, 800 μ L of soil slurry was aliquoted into six wells of a column on all three plates, one assay plate and two standard plates. Following addition of twelve samples into their respective columns the MUB substrates were added. Each substrate was added to one well in each column, so that all twelve samples received each of the six substrates once.

The plates were incubated for 3 h at 25°C and then centrifuged for 3 min at 350 \times g. Afterwards, 250 μ L of supernatant from each well was placed into the corresponding well on a 96-well black plate. Fluorescence was measured immediately following 5 μ L addition of NaOH to each well to terminate the reaction. A Tecan Infinite M500 spectrofluorometer was used to measure fluorescence with wavelengths set at 365 nm and 450 nm for excitation and emission, respectively. The plates with the standards were used to calculate a linear standard curve and determine potential enzyme activity for each sample as nmol g⁻¹ dry soil h⁻¹ and nmol g⁻¹ C h⁻¹.

CALCULATIONS AND STATISTICAL ANALYSIS

Mass-specific enzyme activity was calculated by dividing the potential enzyme activity by the MBC estimated from CFE (Hassett and Zak, 2005). There was no calculation of mass-specific enzyme activity for June 2008 samples because we did not have enough soil to estimate MBC. We included June 2008 samples for all other analyses because it was our pre-warming treatment time point. Ratios for C and N cycling enzymes were calculated as BG:(NAG+LAP) and C:P cycling enzyme ratios as BG:PHOS using potential activity for each sample in nmol g⁻¹ C h⁻¹ (Sinsabaugh et al., 2009). The ratio of potential activity for different enzymes is a metric for understanding microbial nutrient demand.

Potential enzyme activities were log transformed in order to normalize the variance prior to analysis using SAS PROC GLIMMIX with Tukey's adjustment, $\alpha = 0.05$ (SAS Institute, Cary, NC). Block and season were selected as random effects, depth, temperature and precipitation treatments were selected as fixed effects and potential enzyme activities, mass-specific enzyme activities, and enzyme stoichiometric ratios were designated as dependent variables. PROC GLIMMIX was used to determine significant field treatment effects within each season and to identify differences among treatments, seasons or depth. MBC estimates were compared within each season using Tukey's comparisons for all treatments.

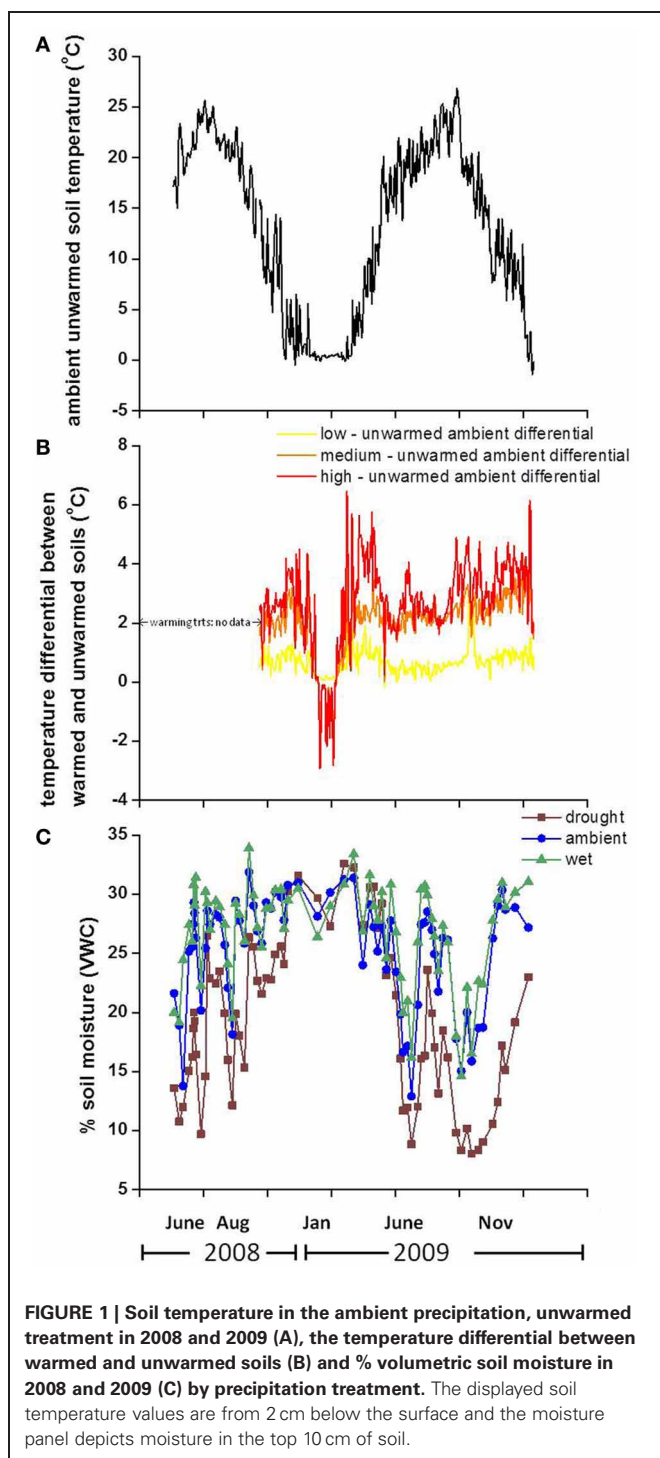
RESULTS

EXPERIMENTAL CLIMATE EFFECTS

Warming treatments increased the soil temperature on average by 0.70, 2.05, and 2.70°C above unwarmed soil temperatures at 2 cm below the surface in both years for the low, medium and high treatments, respectively (Figures 1A,B). There were no soil temperature data available for treatment plots in August 2008. In January 2009, immediately preceding sampling, soil temperatures were 0.37, 1.02, and 1.25°C greater in low, medium, and high temperature plots, respectively, compared to unwarmed plots. However, following the January 2009 sampling, the medium and high temperature treatments soils were cooler than unwarmed soils for the remainder of the month. This counterintuitive pattern resulted in decreased snowpack; by clearing the snow, the warming treatments exposed the soils to freezing air temperature and cooled the soils compared to the unwarmed treatment. Preceding sampling in June 2009, soil temperature increased by 0.43, 2.2, and 2.9°C in low, medium, and high temperature treatments, respectively, compared to the unwarmed treatment. Warming increased the soil temperature in drought and ambient precipitation plots, with the largest soil warming occurring in the drought + high temperature treatment, where soils were 4.0 and 3.5°C warmer than the “unwarmed, ambient” treatment soils during the growing season in 2008 and 2009, respectively.

Precipitation treatments altered soil moisture substantially and soil moisture varied by month at 0–10 cm. Drought treatments resulted in the largest drop in soil moisture during the growing season (Figure 1C). Immediately preceding sampling the soil moisture in drought plots was 20 and 45% lower than ambient soil moisture on the August 2008 and June 2009 sampling dates, respectively. There was no difference in soil moisture among precipitation treatments immediately prior to the January 2009 sampling date. The additional water treatment had no effect on the soil moisture of wet plots in these shallow soil layers, which were already at field capacity in this freely draining, well-structured soil. Soil moisture was further reduced by warming in the drought and ambient precipitation plots in 2008 and 2009.

There was no measurable change in total soil C, N, or C:N ratio due to treatment or seasonal effects. The average total soil C and N values were 57 \pm 2.03 and 4.7 \pm 0.14 mg g⁻¹ dry soil, respectively, for 0–5 cm, and 42 \pm 0.67 and 3.6 \pm 0.09 mg g dry soil⁻¹ for 5–15 cm below the soil surface. Dissolved organic carbon (DOC) was greater in the drought treatments (365.8 \pm 25.5) compared to ambient or wet precipitation treatments (217 \pm 7.8 and 247 \pm 18.11, respectively) at 0–5 cm in June 2009, there



was no difference in DOC between treatments on other sampling dates.

POTENTIAL ENZYME ACTIVITIES

In all treatments, PHOS and BG had the highest potential activities, and the remaining enzymes exhibited similar activities, usually under 200 nmol activity g^{-1} dry soil h^{-1} (Table 2). At 0–5 cm, warming manipulations individually did not affect potential

enzyme activity when calculated per g dry soil. Precipitation treatments also had little overall effect on enzyme activities, but there was a trend toward increased potential activity in drought only plots in June 2009, which was significant for PHOS at 0–5 cm ($P < 0.05$). There was a significant interaction of precipitation \times warming treatments on LAP in January 2009 and CB in June 2009 ($P < 0.05$), which always resulted in decreased activity in drought and warmed plots relative to ambient, unwarmed plots.

In deeper soils (5–15 cm), warming alone tended to decrease potential activity for all enzymes in the medium-warmed plots (data not shown; $P = 0.2$) compared to the “unwarmed, ambient” plots, and this effect was significant for XYL and LAP ($P < 0.01$). Potential enzyme activities were significantly lower in January 2009 at 5–15 cm below the surface for NAG, XYL, and LAP compared to August 2008 (data not shown; $P < 0.05$).

MICROBIAL BIOMASS

In August 2008, warming alone resulted in slightly higher SIR-MBC estimates compared to the “unwarmed ambient” plots in August 2008, but in the drought and wet treatments warming had no effect on SIR-MBC (Figure 2A). In January 2009 there was no consistent effect of individual or combined field treatments on SIR-MBC (Figure 2B). CFE-MBC was affected by precipitation in August 2008 and January 2009, but there was no effect of either climate manipulation in June 2009 (Figures 2D–F). Although SIR-MBC and CFE-MBC were similar in August 2008 and June 2009, SIR was lower than CFE in January 2009 (Figure 2; $P < 0.005$).

MASS SPECIFIC ENZYME ACTIVITY

The climate manipulations did not affect mass-specific enzyme activity in August 2008 or January 2009 (nmol activity $\text{h}^{-1} \mu\text{g}^{-1}$ MBC; calculated using CFE-MBC estimates; data not shown). In June 2009, the mass specific enzyme activity for all enzymes was affected by warming alone, with mass-specific enzyme activity increasing under low- and medium-warmed treatments (Figure 3; $P < 0.01$). Additionally, in June 2009 precipitation had a significant effect on PHOS and CB, with drought only plots having higher mass specific enzyme activity than wet and ambient precipitation plots (Figures 3B,D; $P < 0.05$).

ENZYME STOICHIOMETRY

Season affected the ratio of the potential activities of C- to N-acquiring enzymes at 5–15 cm below the surface, with a significant increase in the ratio in winter 2009 compared to the two June samples ($P < 0.01$, Figure 4A). The C:N enzyme activity ratio increased from June 2008 to January 2009 and then declined in June 2009, whereas C- to P-acquiring enzyme ratios showed no seasonality (Figure 4B). There was also a significant depth effect in the C:N enzyme activity ratio for June 2008 and January 2009, with soils from the 5–15 cm depth having a higher ratio than those from 0 to 5 cm ($P < 0.05$).

DISCUSSION

PRECIPITATION AND WARMING EFFECTS

Previous studies of climate change effects on enzyme activities, decomposition, and heterotrophic respiration have focused

Table 2 | Potential enzyme activity in 0–5 cm soils, mean \pm SE.

Date	Treatment	β -glucosidase	Cellobiohydrolase	Xylosidase	Phosphatase	N-acetyl glucoaminidase	Leucine-amino peptidase
Jun-08	A + U	320 \pm 82	121 \pm 27	73 \pm 19	65 \pm 132	145 \pm 43	196 \pm 28
	D + U	323 \pm 45	120 \pm 17	99 \pm 18	740 \pm 86	350 \pm 79	173 \pm 18
	W + U	347 \pm 90	138 \pm 52	79 \pm 12	667 \pm 102	236 \pm 55	169 \pm 26
	A + L	309 \pm 92	119 \pm 31	61 \pm 10	617 \pm 128	101 \pm 26	174 \pm 33
	A + M	410 \pm 138	171 \pm 57	89 \pm 27	715 \pm 184	204 \pm 63	184 \pm 45
	A + H	244 \pm 45	101 \pm 19	44 \pm 15	474 \pm 92	111 \pm 29	164 \pm 27
	D + L	219 \pm 21	88 \pm 12	61 \pm 8	497 \pm 49	172 \pm 5	153 \pm 26
	D + M	283 \pm 91	110 \pm 30	80 \pm 28	572 \pm 145	189 \pm 58	157 \pm 13
	D + H	286 \pm 72	108 \pm 21	69 \pm 13	626 \pm 150	182 \pm 60	162 \pm 48
	W + L	281 \pm 30	106 \pm 9	75 \pm 8	652 \pm 56	180 \pm 25	162 \pm 13
	W + M	345 \pm 103	137 \pm 44	81 \pm 18	746 \pm 120	229 \pm 45	163 \pm 4
	W + H	269 \pm 35	110 \pm 12	65 \pm 7	623 \pm 74	146 \pm 20	172 \pm 9
Aug-08	A + U	176 \pm 75	96 \pm 11	158 \pm 109	277 \pm 125	118 \pm 1	34 \pm 19
	D + U	400 \pm 176	174 \pm 84	152 \pm 58	578 \pm 384	301 \pm 119	70 \pm 14
	W + U	235 \pm 60	158 \pm 53	75 \pm 2	479 \pm 68	187 \pm 33	74 \pm 18
	A + L	250 \pm 34	96 \pm 9	56 \pm 3	416 \pm 71	101 \pm 17	55 \pm 24
	A + M	188 \pm 91	426 \pm 362	235 \pm 174	298 \pm 148	262 \pm 167	41 \pm 9
	A + H	339 \pm 133	178 \pm 65	170 \pm 106	1159 \pm 748	686 \pm 567	57 \pm 17
	D + L	122 \pm 60	88 \pm *	151 \pm 59	226 \pm 129	181 \pm *	48 \pm 16
	D + M	250 \pm 108	112 \pm 72	124 \pm 79	430 \pm 172	158 \pm 65	49 \pm 9
	D + H	1031 \pm 933	57 \pm 30	68 \pm 36	178 \pm 51	66 \pm 19	37 \pm 12
	W + L	278 \pm 23	252 \pm 73	147 \pm 46	506 \pm 56	335 \pm 103	51 \pm 12
	W + M	169 \pm 76	173 \pm 41	123 \pm 41	273 \pm 130	248 \pm 100	84 \pm 9
	W + H	198 \pm 54	66 \pm 44	70 \pm 33	303 \pm 120	77 \pm 38	56 \pm 14
Jan-09	A + U	820 \pm 221	265 \pm 88	144 \pm 50	1588 \pm 542	386 \pm 100	120 \pm 38
	D + U	275 \pm 62	144 \pm 18	134 \pm 26	1083 \pm 158	240 \pm 25	134 \pm 38
	W + U	1067 \pm 302	410 \pm 124	263 \pm 83	2352 \pm 566	521 \pm 152	140 \pm 41
	A + L	501 \pm 143	178 \pm 47	84 \pm 18	973 \pm 181	219 \pm 60	53 \pm 7
	A + M	800 \pm 204	308 \pm 84	164 \pm 38	1537 \pm 511	301 \pm 57	93 \pm 16
	A + H	687 \pm 315	264 \pm 125	114 \pm 45	1234 \pm 443	249 \pm 125	93 \pm 52
	D + L	365 \pm 35	137 \pm 3	88 \pm 16	799 \pm 124	156 \pm 29	92 \pm 29
	D + M	549 \pm 254	210 \pm 104	114 \pm 20	998 \pm 344	244 \pm 76	85 \pm 21
	D + H	273 \pm 13	98 \pm 2	56 \pm 6	543 \pm 36	119 \pm 11	42 \pm 9
	W + L	506 \pm 54	200 \pm 31	133 \pm 37	1227 \pm 176	212 \pm 32	72 \pm 10
	W + M	549 \pm 109	228 \pm 59	182 \pm 54	1736 \pm 612	391 \pm 133	106 \pm 40
	W + H	699 \pm 372	276 \pm 168	165 \pm 99	1673 \pm 851	310 \pm 171	60 \pm 44
Jun-09	A + U	226 \pm 27	82 \pm 19	58 \pm 9	416 \pm 63	117 \pm 22	96 \pm 54
	D + U	341 \pm 44	150 \pm 39	130 \pm 58	647 \pm 701	264 \pm 78	154 \pm 63
	W + U	264 \pm 17	101 \pm 10	77 \pm 13	474 \pm 46	118 \pm 18	74 \pm 31
	A + L	174 \pm 31	66 \pm 13	38 \pm 6	262 \pm 71	68 \pm 18	95 \pm 56
	A + M	223 \pm 29	99 \pm 11	66 \pm 12	411 \pm 30	87 \pm 10	76 \pm 21
	A + H	234 \pm 32	95 \pm 13	45 \pm 9	371 \pm 48	106 \pm 35	69 \pm 28
	D + L	291 \pm 48	125 \pm 32	86 \pm 25	496 \pm 99	180 \pm 51	168 \pm 52
	D + M	232 \pm 51	78 \pm 16	46 \pm 7	307 \pm 28	111 \pm 19	121 \pm 47
	D + H	261 \pm 59	95 \pm 26	74 \pm 24	368 \pm 83	155 \pm 24	130 \pm 54
	W + L	526 \pm 252	104 \pm 16	51 \pm 25	410 \pm 108	93 \pm 48	79 \pm 24
	W + M	217 \pm 40	88 \pm 20	58 \pm 14	400 \pm 32	100 \pm 20	111 \pm 47
	W + H	364 \pm 102	166 \pm 44	122 \pm 36	639 \pm 140	203 \pm 73	166 \pm 37

Treatment abbreviations are noted by precipitation and temperature manipulations, A, ambient precipitation; D, drought; W, 150% ambient precipitation; U, unwarmed; L, low warming; M, medium warming; H, high warming. * $n = 1$.

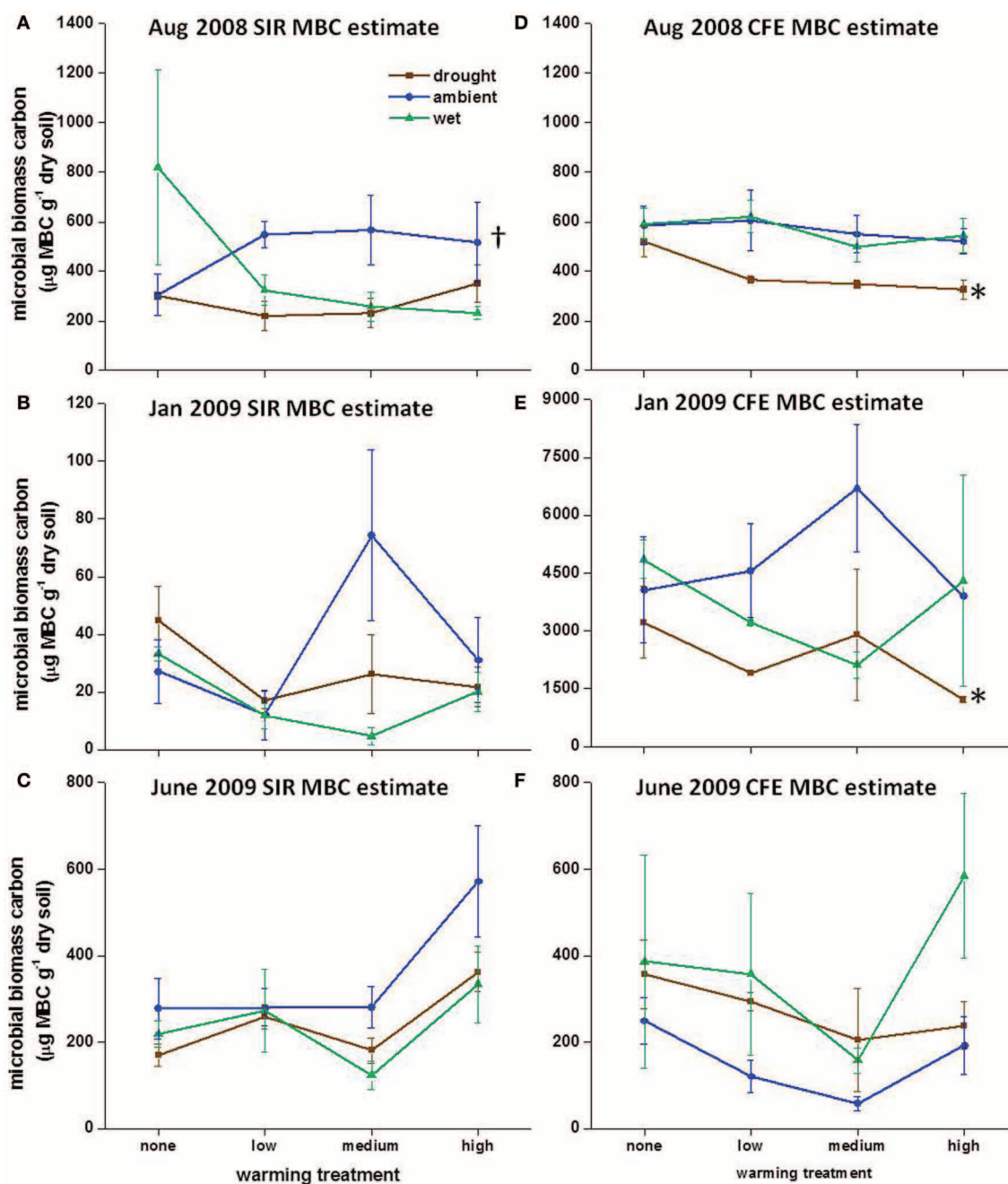


FIGURE 2 | Microbial biomass carbon (MBC) calculated using substrate-induced respiration (SIR) in (A) August 2008, (B) January 2009, and (C) June 2009 and using chloroform fumigation extraction (CFE) in (D) August 2008, (E) January 2009, and (F) June 2009. Symbols show averages with standard error bars, $n = 3$.

Crosses indicate significant differences in MBC between temperature treatments under ambient precipitation and asterisks indicate a significant difference in MBC between precipitation treatments without warming ($P < 0.05$). Note different y-axes in January 2009 for SIR and CFE estimated MBC.

primarily on warming effects. Climate effects on soil enzyme activities involve not only short-term changes in activity driven by thermodynamics (Trasar-Cepeda et al., 2007) but also long-term changes in enzyme pools due to direct and indirect effects on microbial production of enzymes and on turnover rates (Sowerby et al., 2005; Schimel et al., 2007).

A recent study pointed out that there is an even greater uncertainty associated with the effects of altered soil moisture [through both warming and altered precipitation patterns (Falloon and Betts, 2010)]. Here, we assessed the interactive effects of warming and precipitation on soil enzymes relative to MBC.

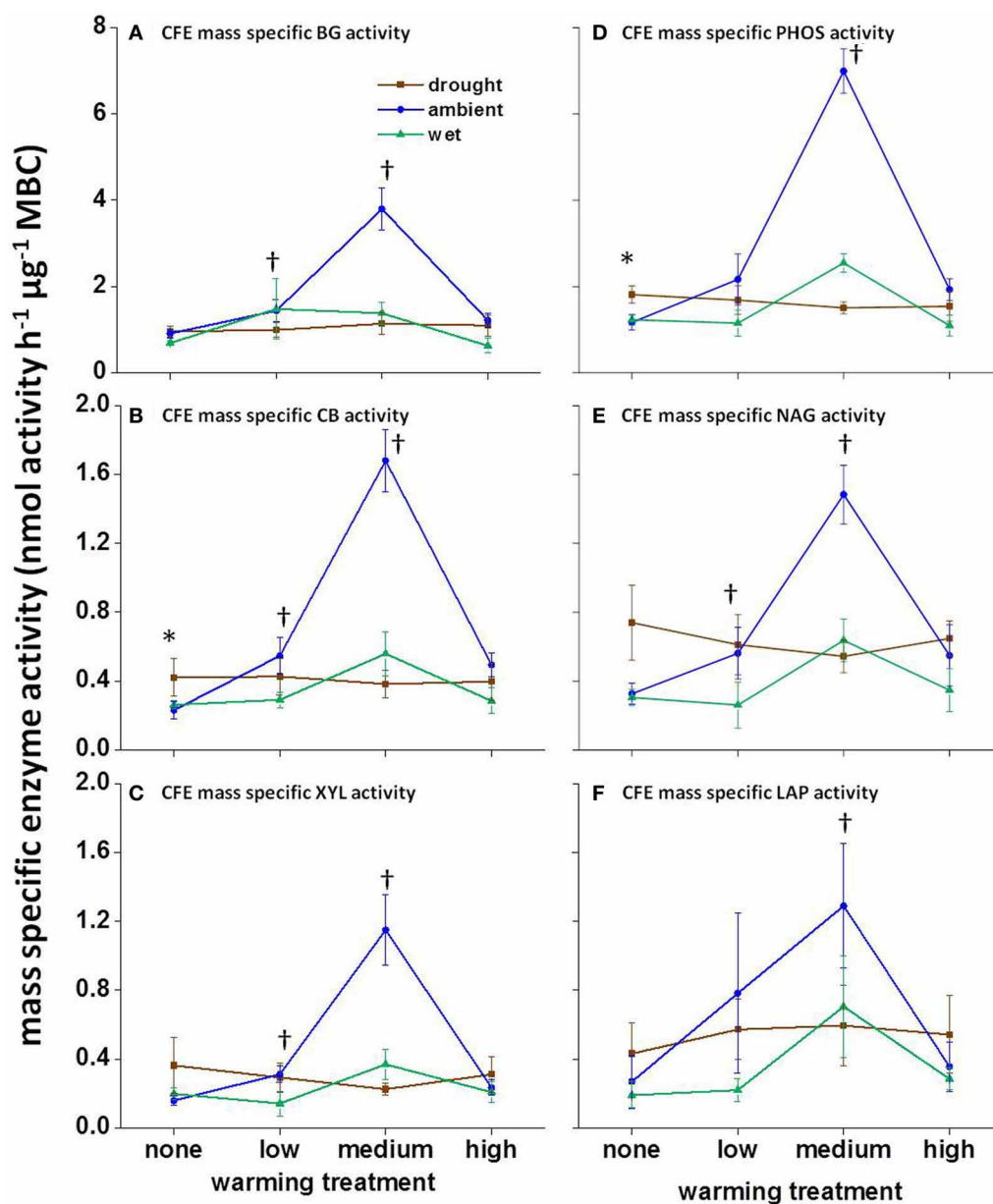


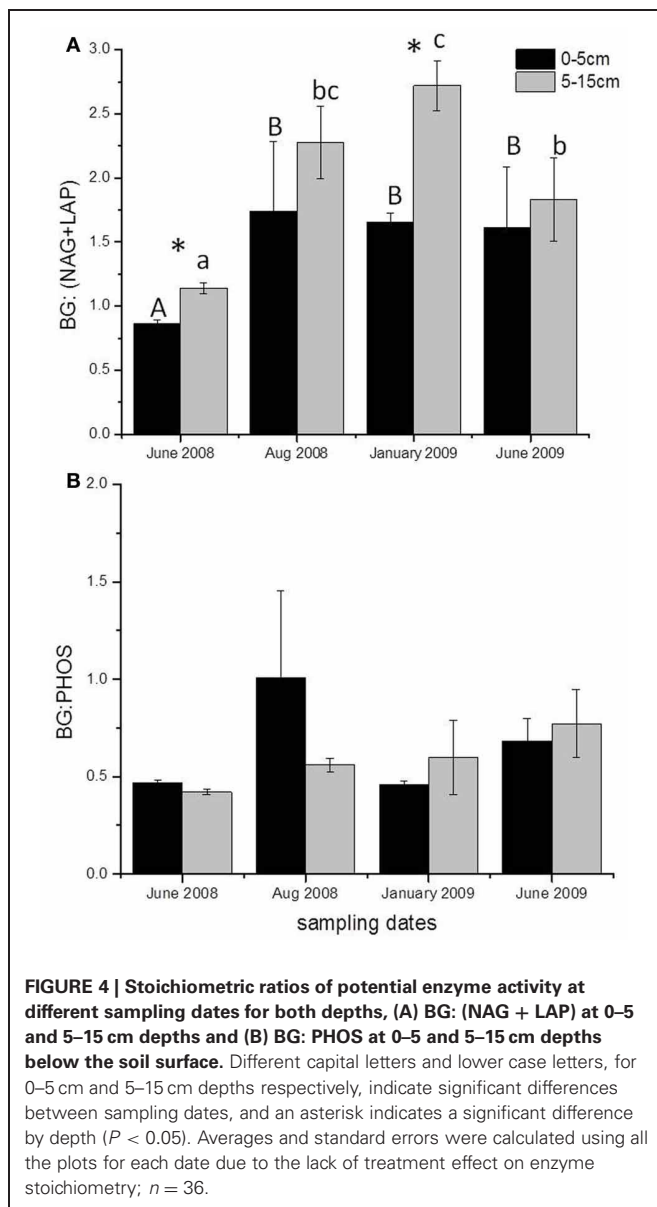
FIGURE 3 | Mass-specific potential enzyme activity in June 2009 calculated using CFE estimated biomass. CFE estimated mass specific enzyme activity for (A) BG, (B) CB, (C) XYL, (D) PHOS, (E) NAG, and (F) LAP. For each substrate, asterisks indicate significant differences in mass-specific enzyme activity ($P < 0.05$) between the

precipitation treatments without warming. Crosses indicate significant differences in mass-specific enzyme activity ($P < 0.01$) between temperature treatments under ambient precipitation. Substrate abbreviations are noted in Table 1. Averages and standard errors, $n = 3$. Note different y-axis scales in panels.

We predicted that potential enzyme activities would decrease in response to drought due to decreased microbial biomass and enzyme production. However, we observed no change in the potential activities of any of the enzymes involved in C and N cycling in any precipitation treatment, and a small increase in phosphatase under drought, whereas both total microbial biomass and field respiration declined under drought (Suseela et al., 2012). Several other studies in oxic soils have detected declines in hydrolytic and oxidative potential enzyme activities under drought conditions (Sardans et al., 2008; Toberman

et al., 2008; Sardans and Penuelas, 2010). Most of these studies have been in Mediterranean systems, which are drought-prone, whereas drought is a less frequent condition at the BACE location. The stable enzyme pool under drought could indicate that either mass-specific enzyme production was higher under drought, or more likely that enzyme turnover decreased in dry soils.

Although we measured stable potential enzyme activities under drought, this assay does not necessarily indicate that these enzymes were active *in situ* in dry soils. Suseela et al. (2012) measured a 21% reduction in heterotrophic respiration



under drought at BACE, suggesting that decomposition rates are lower in drought plots despite potential enzyme activities similar to ambient conditions. This may be due either to reduced enzyme activities in dry soils or because of reduced microbial uptake of assimilable DOM. Potential enzyme activity as measured in laboratory assays does not necessarily directly correlate with *in situ* activity under field conditions (Wallenstein and Weintraub, 2008). Using a combination of empirical data and modeling, we previously determined that *in situ* enzyme activity at the BACE is significantly reduced by drought, such that despite a stable pool of enzymes, the actual activity is constrained by a lack of moisture and diffusion (Steinweg et al., 2012). Under low soil moisture conditions, the diffusion of enzymes and substrates is limited to thin water films and pockets of moisture with low connectivity (Stark and Firestone, 1995). As substrates are concentrated in these hotspots, enzyme activities could continue

even in relatively dry soils, resulting in the production of assimilable DOM. The accumulation of DOM under drought would indicate that microbial uptake is more sensitive to soil moisture than enzyme activity. Consistent with this mechanism, we observed higher concentrations of DOC in drought plots compared to ambient or wet plots in June 2009, when drought treatment soils were 50% drier than ambient and wet soils. If enzyme activities persist in dry soils but microbial uptake is suppressed, declines in microbial respiration would mask the continuation of decomposition in dry soils. This mechanism could also explain the pulse of respiration that often accompanies rewetting in laboratory incubations and field studies (Fierer and Schimel, 2003; Schimel et al., 2007), as accumulated DOM is rapidly metabolized by microbes upon rewetting.

Warming increased mass-specific potential enzyme activity under low- and medium-warmed treatments, such that more enzymes were present per unit of MBC in June 2009. Enzyme reactions are temperature-sensitive, and we had expected that *in situ* enzymatic reaction rates would increase with field warming, reducing the number of enzymes needed to perform the same number of reactions. However, warming not only affects extracellular enzyme reaction rates, but also affects the reactions occurring within the microbial cell. Maintenance costs also increase with temperature (Joergensen et al., 1990; Alvarez et al., 1995), causing an increased nutrient demand to maintain cellular function. Several models suggest that microorganisms increase allocation of nutrients to enzyme production in order to acquire the nutrients needed to sustain increased maintenance costs with warming (Schimel and Weintraub, 2003; Wang and Post, 2012; Wang et al., 2013).

SEASONAL TRENDS

SIR-MBC and CFE-MBC were similar during the growing season. However, in winter SIR was lower than CFE. SIR-MBC is often interpreted to indicate the size of the active microbial biomass pool whereas CFE-MBC indicates the total microbial biomass pool (Wardle and Parkinson, 1991; Lipson et al., 1999). During the growing season it appears that the total microbial community was active, whereas in winter a very small subset was active at the BACE site. The increase in CFE-MBC during winter is similar to the results from Lipson et al. (1999), however, they measured similar increases during winter in their SIR-MBC estimates as well. Our use of a consistent incubation temperature, 25°C, for SIR-MBC may have underestimated MBC during the winter if the community was better adapted to colder temperatures at that time, however, other work indicates that bacterial growth rate in temperate regions are higher than 25°C (Rousk et al., 2012).

The most striking response of enzymes to season was a change in the stoichiometry of potential enzyme acquisition activities. Sinsabaugh et al. (2009) reported an average enzyme C:N acquisition ratio (BG activity: NAG + LAP activities) close to 1.41 for soils from 40 ecosystems. The average enzyme C:N acquisition activity ratio in BACE soils was 1.74, driven primarily by the high stoichiometric ratio at 5–15 cm depth. The increase in enzyme C:N acquisition activity from June 2008 to January 2009 was driven by both an increase in C-acquiring enzymes and a decrease in the potential activity of N-acquiring

enzymes in the winter. Maintenance costs continue and may increase with freezing events (Methe et al., 2005), resulting in a continual need for C substrates, without a corresponding increase in N demand. In addition, increased C mineralization:N mineralization was measured during winter at the BACE site (Auyeung et al., 2012), indicating increased microbial C utilization relative to N transformation. The decline in potential activity of N-acquiring enzymes in winter relative to C-acquiring enzymes indicated a reduction in organic N degradation in the winter compared to the growing season. The reduction in organic N acquiring enzymes could possibly be due to increased dissolved N (Chróst, 1991), which was measured in the winter at the BACE (data not shown). The average BG:PHOS ratios at the BACE, 0.73, were similar to the reported average of 0.62 for soils (Sinsabaugh et al., 2009). The stability of enzymatic C:P activity ratios through time suggests a consistent P requirement over the year. Even though there may be consistent potential enzyme activities in the winter and summer for some enzymes, it is unlikely that *in situ* activity is the same (Bell et al., 2010). Low soil temperatures would result in slower reaction rates and frozen soils would limit diffusion of substrates resulting in reduced *in situ* activities.

CONCLUSION

Our findings from 1 year of climate manipulations suggest that neither experimental warming nor moisture manipulation consistently affected potential enzyme activities. The stable enzyme pool under drought could indicate that either mass-specific enzyme production was higher under drought, or more likely that enzyme turnover decreased in dry soils. Experimental warming did impact mass-specific enzyme activities through small

decreases in MBC and small increases in potential enzyme activity, indicating increased allocation to enzyme production. Seasonal shifts in C:N acquisition enzyme activity ratios, resulting in increased potential for acquisition for processing C during the winter, could be due to increased maintenance costs associated with freezing events. The shifts in mass-specific enzyme activity and enzyme stoichiometry indicate increased microbial allocation to enzymes during periods when maintenance costs were likely to be high due to high temperatures, similar to results predicted in microbial enzyme-mediated models (Schimel and Weintraub, 2003; Wang et al., 2013). Our results highlight the need to elucidate how abiotic and biotic factors affect the relationship between maintenance costs and enzyme activities to better predict microbial responses to future climate regimes.

ACKNOWLEDGMENTS

We thank Carol Goranson for sampling and shipping soils and maintaining the BACE, and we thank Vidya Suseela for providing heterotrophic respiration data. We also thank Carol Goranson and Hollie Emery for taking soil moisture measurements, and Caroline Melle and Sarah Berg for their help with laboratory analyses. This research was supported by grants to Matthew D. Wallenstein and Jeffrey S. Dukes from the U.S. Department of Energy's Office of Science (BER), through the Northeastern Regional Center of the National Institute for Climate Change Research, and from NSF (Division of Environmental Biology to Jeffrey S. Dukes). Mary Stromberger and Rich Conant provided valuable comments on an earlier version of this manuscript. We would like to thank the reviewers for their helpful comments which improved the manuscript.

REFERENCES

- Allison, S. D. (2005). Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. *Ecol. Lett.* 8, 626–635. doi: 10.1111/j.1461-0248.2005.00756.x
- Allison, S. D., and Treseder, K. K. (2008). Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Glob. Chang. Biol.* 14, 2898–2909. doi: 10.1111/j.1365-2486.2008.01716.x
- Allison, S. D., and Vitousek, P. M. (2005). Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biol. Biochem.* 37, 937–944. doi: 10.1016/j.soilbio.2004.09.014
- Allison, S. D., Weintraub, M. N., Gartner, T. B., and Waldrop, M. P. (2011). "Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function," in *Soil Enzymology*, eds G. Shukla and A. Varma. (Berlin: Springer-Verlag), 229–243.
- Alvarez, R., Santanoglia, O. J., and Garcia, R. (1995). Effect of temperature on soil microbial biomass and its metabolic quotient *in-situ* under different tillage systems. *Biol. Fertil. Soils* 19, 227–230. doi: 10.1007/BF00336164
- Anderson, J. P. E., and Domsch, K. H. (1978). Physiological method for quantitative measurement of microbial biomass in soils. *Soil Biol. Biochem.* 10, 215–221. doi: 10.1016/0038-0717(78)90099-8
- Auyeung, D. S. N., Suseela, V., and Dukes, J. S. (2012). Warming and drought reduce temperature sensitivity of nitrogen transformations. *Glob. Chang. Biol.* doi: 10.1111/gcb.12063
- Bell, T. H., Klironomos, J. N., and Henry, H. A. L. (2010). Seasonal responses of extracellular enzyme activity and microbial biomass to warming and nitrogen addition. *Soil Sci. Soc. Am. J.* 74, 820–828. doi: 10.2136/sssaj2009.0036
- Bengtson, P., and Bengtsson, G. (2007). Rapid turnover of DOC in temperate forests accounts for increased CO₂ production at elevated temperatures. *Ecol. Lett.* 10, 783–790. doi: 10.1111/j.1461-0248.2007.01072.x
- Caldwell, B. A. (2005). Enzyme activities as a component of soil biodiversity: a review. *Pedobiologia* 49, 637–644. doi: 10.1016/j.pedobi.2005.06.003
- Campbell, C. D., Chapman, S. J., Cameron, C. M., Davidson, M. S., and Potts, J. M. (2003). A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. *Appl. Environ. Microbiol.* 69, 3593–3599. doi: 10.1128/AEM.69.6.3593-3599.2003
- Chróst, R. J. (1991). "Environmental control of the synthesis and activity of aquatic microbial ectoenzymes," in *Microbial Enzymes in Aquatic Environments*, ed R. J. Chróst (New York, NY: Springer-Verlag), 29–59.
- Cleveland, C. C., and Liptzin, D. (2007). C: N: P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry* 85, 235–252. doi: 10.1007/s10533-007-9132-0
- Conant, R. T., Ryan, M. G., Ågren, G. I., Birge, H. E., Davidson, E. A., Eliasson, P. E., et al. (2011). Temperature and soil organic matter decomposition rates synthesis of current knowledge and a way forward. *Glob. Chang. Biol.* 17, 3392–3404. doi: 10.1111/j.1365-2486.2011.02496.x
- Davidson, E. A., Belk, E., and Boone, R. D. (1998). Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. *Glob. Chang. Biol.* 4, 217–227. doi: 10.1046/j.1365-2486.1998.00128.x
- Ekschmitt, K., Liu, M. Q., Vetter, S., Fox, O., and Wolters, V. (2005). Strategies used by soil biota to overcome soil organic matter stability - why is dead organic matter left over in the soil? *Geoderma* 128, 167–176. doi: 10.1016/j.geoderma.2004.12.024
- Falloon, P., and Betts, R. (2010). Climate impacts on European agriculture and water management in the context of adaptation and mitigation—the importance of an integrated approach. *Sci. Total Environ.* 408, 5667–5687. doi: 10.1016/j.scitotenv.2009.05.002
- Fierer, N., and Schimel, J. P. (2003). A proposed mechanism for the pulse in carbon dioxide production

- commonly observed following the rapid rewetting of a dry soil. *Soil Sci. Soc. Am. J.* 67, 798–805. doi: 10.2136/sssaj2003.7980
- German, D. P., Chacon, S. S., and Allison, S. D. (2011). Substrate concentration and enzyme allocation can affect rates of microbial decomposition. *Ecology* 92, 1471–1480. doi: 10.1890/10-2028.1
- Hassett, J. E., and Zak, D. R. (2005). Aspen harvest intensity decreases microbial biomass, extracellular enzyme activity, and soil nitrogen cycling. *Soil Sci. Soc. Am. J.* 69, 227–235. doi: 10.2136/sssaj2005.0227
- Henry, H. A. L., Juarez, J. D., Field, C. B., and Vitousek, P. M. (2005). Interactive effects of elevated CO₂, N deposition and climate change on extracellular enzyme activity and soil density fractionation in a California annual grassland. *Glob. Chang. Biol.* 11, 1808–1815. doi: 10.1111/j.1365-2486.2005.001007.x
- Hoepfner, S. S., and Dukes, J. S. (2012). Interactive responses of old-field plant growth and composition to warming and precipitation. *Glob. Chang. Biol.* 18, 1754–1768. doi: 10.1111/j.1365-2486.2011.02626.x
- Joergensen, R. G., Brookes, P. C., and Jenkinson, D. S. (1990). Survival of the soil microbial biomass at elevated temperatures. *Soil Biol. Biochem.* 22, 1129–1136. doi: 10.1016/0038-0717(90)90039-3
- Lipson, D. A., Schmidt, S. K., and Monson, R. K. (1999). Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. *Ecology* 80, 1623–1631. doi: 10.1890/0012-9658(1999)080[1623:LBPMDA]2.0.CO;2
- Methe, B. A., Nelson, K. E., Deming, J. W., Momen, B., Melamud, E., Zhang, X. J., et al. (2005). The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. *Proc. Natl. Acad. Sci. U.S.A.* 102, 10913–10918. doi: 10.1073/pnas.0504766102
- Rousk, J., Frey, S. D., and Bååth, E. (2012). Temperature adaptation of bacterial communities in experimentally warmed forest soils. *Glob. Chang. Biol.* 18, 3252–3258. doi: 10.1111/j.1365-2486.2012.02764.x
- Saiya-Cork, K. R., Sinsabaugh, R. L., and Zak, D. R. (2002). The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biol. Biochem.* 34, 1309–1315. doi: 10.1016/S0038-0717(02)00074-3
- Sardans, J., and Peñuelas, J. (2005). Drought decreases soil enzyme activity in a Mediterranean *Quercus ilex* L. forest. *Soil Biol. Biochem.* 37, 455–461. doi: 10.1016/j.soilbio.2004.08.004
- Sardans, J., and Penuelas, J. (2010). Soil enzyme activity in a Mediterranean forest after six years of drought. *Soil Sci. Soc. Am. J.* 74, 838–851. doi: 10.2136/sssaj2009.0225
- Sardans, J., Peñuelas, J., and Estiarte, M. (2008). Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. *Appl. Soil Ecol.* 39, 223–235. doi: 10.1016/j.apsoil.2007.12.011
- Schimel, J., Balser, T. C., and Wallenstein, M. (2007). Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88, 1386–1394. doi: 10.1890/06-0219
- Schimel, J. P., and Bennett, J. (2004). Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85, 591–602. doi: 10.1890/03-8002
- Schimel, J. P., and Gullledge, J. (1998). Microbial community structure and global trace gases. *Glob. Chang. Biol.* 4, 745–758. doi: 10.1046/j.1365-2486.1998.00195.x
- Schimel, J. P., and Weintraub, M. N. (2003). The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biol. Biochem.* 35, 549–563. doi: 10.1016/S0038-0717(03)00015-4
- Sinsabaugh, R. L., Hill, B. H., and Shah, J. J. F. (2009). Ecosystem stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature* 462, 795–798. doi: 10.1038/nature08632
- Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., Allison, S. D., Crenshaw, C., et al. (2008). Stoichiometry of soil enzyme activity at global scale. *Ecol. Lett.* 11, 1252–1264. doi: 10.1111/j.1461-0248.2008.01245.x
- Sowerby, A., Emmett, B., Beier, C., Tietema, A., Penuelas, J., Estiarte, M., et al. (2005). Microbial community changes in heathland soil communities along a geographical gradient: interaction with climate change manipulations. *Soil Biol. Biochem.* 37, 1805–1813. doi: 10.1016/j.soilbio.2005.02.023
- Stark, J. M., and Firestone, M. K. (1995). Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Appl. Environ. Microbiol.* 61, 218–221.
- Steinweg, J. M., Dukes, J. S., and Wallenstein, M. D. (2012). Modeling the effects of temperature and moisture on soil enzyme activity: linking laboratory assays to continuous field data. *Soil Biol. Biochem.* 55, 85–92. doi: 10.1016/j.soilbio.2012.06.015
- Stone, M. M., Weiss, M. S., Goodale, C. L., Adams, M. B., Fernandez, I. J., German, D. P., et al. (2012). Temperature sensitivity of soil enzyme kinetics under N-fertilization in two temperate forests. *Glob. Chang. Biol.* 18, 1173–1184. doi: 10.1111/j.1365-2486.2011.02545.x
- Suseela, V., Conant, R. T., Wallenstein, M. D., and Dukes, J. S. (2012). Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment. *Glob. Chang. Biol.* 18, 336–348. doi: 10.1111/j.1365-2486.2011.02516.x
- Toberman, H., Evans, C. D., Freeman, C., Fenner, N., White, M., Emmett, B. A., et al. (2008). Summer drought effects upon soil and litter extracellular phenol oxidase activity and soluble carbon release in an upland Calluna heathland. *Soil Biol. Biochem.* 40, 1519–1532. doi: 10.1016/j.soilbio.2008.01.004
- Trasar-Cepeda, C., Gil-Sotres, F., and Leiros, M. C. (2007). Thermodynamic parameters of enzymes in grassland soils from Galicia, NW Spain. *Soil Biol. Biochem.* 39, 311–319. doi: 10.1016/j.soilbio.2006.08.002
- Vance, E. D., Brookes, P. C., and Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass-C. *Soil Biol. Biochem.* 19, 703–707. doi: 10.1016/0038-0717(87)90052-6
- Wallenstein, M. D., Haddix, M. L., Lee, D. D., Conant, R. T., and Paul, E. A. (2012). A litter-slurry technique elucidates the key role of enzyme production and microbial dynamics in temperature sensitivity of organic matter decomposition. *Soil Biol. Biochem.* 47, 18–26. doi: 10.1016/j.soilbio.2011.12.009
- Wallenstein, M. D., McMahon, S. K., and Schimel, J. P. (2009). Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. *Glob. Chang. Biol.* 15, 1631–1639. doi: 10.1111/j.1365-2486.2008.01819.x
- Wallenstein, M. D., and Weintraub, M. N. (2008). Emerging tools for measuring and modeling the *in situ* activity of soil extracellular enzymes. *Soil Biol. Biochem.* 40, 2098–2106. doi: 10.1016/j.soilbio.2008.01.024
- Wang, G., and Post, W. M. (2012). A theoretical reassessment of microbial maintenance and implications for microbial ecology modeling. *FEMS Microbiol. Ecol.* 81, 610–617. doi: 10.1111/j.1574-6941.2012.01389.x
- Wang, G., Post, W. M., and Mayes, M. A. (2013). Development of microbial-enzyme-mediated decomposition model parameters through steady-state and dynamic analyses. *Ecol. Appl.* 23, 255–272. doi: 10.1890/12-0681.1
- Wardle, D. A., and Parkinson, D. (1991). A statistical evaluation of equations for predicting total microbial biomass carbon using physiological and biochemical methods. *Agric. Ecosyst. Environ.* 34, 75–86. doi: 10.1016/0167-8809(91)90095-F

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 22 February 2013; accepted: 24 May 2013; published online: 11 June 2013.

Citation: Steinweg JM, Dukes JS, Paul EA and Wallenstein MD (2013) Microbial responses to multi-factor climate change: effects on soil enzymes. *Front. Microbiol.* 4:146. doi: 10.3389/fmicb.2013.00146

This article was submitted to *Frontiers in Terrestrial Microbiology*, a specialty of *Frontiers in Microbiology*.

Copyright © 2013 Steinweg, Dukes, Paul and Wallenstein. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.



Thermal adaptation of decomposer communities in warming soils

Mark A. Bradford*

School of Forestry and Environmental Studies, Yale University, New Haven, CT, USA

Edited by:

Johannes Rousk, Lund University, Sweden

Reviewed by:

Johannes Rousk, Lund University, Sweden

Riikka Rinnan, University of Copenhagen, Denmark
Göran Ågren, Swedish University of Agricultural Sciences, Sweden

***Correspondence:**

Mark A. Bradford, School of Forestry and Environmental Studies, Yale University, New Haven, CT 06511, USA

e-mail: mark.bradford@yale.edu

Temperature regulates the rate of biogeochemical cycles. One way it does so is through control of microbial metabolism. Warming effects on metabolism change with time as physiology adjusts to the new temperature. I here propose that such thermal adaptation is observed in soil microbial respiration and growth, as the result of universal evolutionary trade-offs between the structure and function of both enzymes and membranes. I review the basis for these trade-offs and show that they, like substrate depletion, are plausible mechanisms explaining soil respiration responses to warming. I argue that controversies over whether soil microbes adapt to warming stem from disregarding the evolutionary physiology of cellular metabolism, and confusion arising from the term thermal acclimation to represent phenomena at the organism- and ecosystem-levels with different underlying mechanisms. Measurable physiological adjustments of the soil microbial biomass reflect shifts from colder- to warmer-adapted taxa. Hypothesized declines in the growth efficiency of soil microbial biomass under warming are controversial given limited data and a weak theoretical basis. I suggest that energy spilling (aka waste metabolism) is a more plausible mechanism for efficiency declines than the commonly invoked increase in maintenance-energy demands. Energy spilling has many fitness benefits for microbes and its response to climate warming is uncertain. Modeled responses of soil carbon to warming are sensitive to microbial growth efficiency, but declines in efficiency mitigate warming-induced carbon losses in microbial models and exacerbate them in conventional models. Both modeling structures assume that microbes regulate soil carbon turnover, highlighting the need for a third structure where microbes are not regulators. I conclude that microbial physiology must be considered if we are to have confidence in projected feedbacks between soil carbon stocks, atmospheric CO₂, and climate change.

Keywords: carbon use efficiency, climate warming, microbial growth, modeling, soil respiration, review, soil organic matter, thermal acclimation

INTRODUCTION

CLIMATE-CARBON CYCLE FEEDBACKS

Respiration emits ~120 Pg C-CO₂ per year from a terrestrial biosphere store of >2,000 Pg C to an atmospheric store of ~750 Pg C-CO₂ (Steffen et al., 1998; Falkowski et al., 2000; Jobbágy and Jackson, 2000; Denman et al., 2007). This respiratory efflux is balanced annually by CO₂-fixation by land plants (Steffen et al., 1998; Denman et al., 2007). This balance may be destabilized by climate warming because respiration rates respond more positively to increasing temperature than photosynthetic rates (Ise et al., 2010; Mahecha et al., 2010; Yvon-Durocher et al., 2010; Smith and Dukes, 2013). The net effect of this imbalance under warming is presumed to be a redistribution of organic carbon stored in the biosphere to carbon stored as CO₂ in the atmosphere (Denman et al., 2007). This redistribution might initiate a positive feedback (i.e., self-reinforcing) cycle, where elevated respiration rates enhance the rate of increase in atmospheric CO₂ concentrations, which in turn warms climate, enhancing respiration and so on to cause runaway greenhouse warming. This presumed feedback is captured in the coupled climate-carbon cycle models used by the Intergovernmental Panel on Climate Change (IPCC), and leads to

an additional, global mean annual warming of ~2°C by the year 2100 (Denman et al., 2007). In the IPCC models, the carbon lost from the biosphere to atmosphere derives from mineralization of soil organic matter (SOM), a carbon store to 3-m depth that is approximately triple the size of the atmospheric store (Jobbágy and Jackson, 2000) and so has huge potential to warm climate if converted to CO₂.

Uncertainty about the strength of the positive feedback between warming, SOM mineralization and atmospheric CO₂ concentrations (Melillo et al., 2002; Denman et al., 2007; Luo, 2007; Allison et al., 2010) has motivated the study of how SOM decomposition responds to elevated temperature. Much of the research involves investigating how temperature affects the activity (primarily respiration) of decomposers (Conant et al., 2011). A key question has been whether decomposer communities actively down-regulate their metabolism (i.e., acclimate) under sustained warming, and hence contribute to the diminishing effect over time of experimental warming on soil and ecosystem respiration rates (Oechel et al., 2000; Luo et al., 2001; Melillo et al., 2002; Bradford et al., 2008a; Reich, 2010; **Figure 1**). Numerical models demonstrate that physiological acclimation does not

need to be invoked to explain the ephemeral augmentation of soil respiration in response to a fixed and sustained increase in temperature above ambient (Allison and Martiny, 2008; Kirschbaum, 2004; Eliasson et al., 2005; Knorr et al., 2005; Allison et al., 2010). This has prompted people to question why and by what mechanisms soil decomposer communities would down-regulate their physiological rates when “temperature limitations” are alleviated (e.g., Hartley et al., 2008). These seem fair questions because soil decomposers are poorly represented in the vast literature on how plants, animals and microorganisms physiologically adapt to temperature change (Crowther and Bradford, 2013). This poor representation is likely because it is difficult to study (a) cryptic organisms in an opaque environment and (b) organisms that are challenging to isolate and culture under laboratory conditions. These difficulties preclude soil decomposers from being subjected to the detailed physiological work on individual responses to temperature that is the hallmark of so many plant and animal studies (Hochachka and Somero, 2002; Atkin and Tjoelker, 2003).

The paucity of data on the physiological response of soil decomposer communities to warming is gradually being redressed (e.g., Bradford et al., 2008a; Bárcenas-Moreno et al., 2009; Brzostek and Finzi, 2011; German et al., 2012; Rousk et al., 2012; Crowther and Bradford, 2013; Tucker et al., 2013). Explicitly representing these physiological responses in the new class of microbial SOM models (e.g., Lawrence et al., 2009; Allison et al., 2010) predicts a short-lived increase in soil respiration under sustained warming (Figure 1). That is, the same respiration response as projected by the traditional SOM models, where decomposers are implicit in the model frameworks (Parton et al., 1988; Schimel, 2001; Eliasson et al., 2005; Bonan et al., 2013). Yet the projections for SOM stocks under warming contrast markedly between the microbial and traditional SOM models. The traditional models project large SOM stock losses, but the microbial models project little change in SOM stocks and hence no feedback to climate warming (Eliasson et al., 2005; Knorr et al., 2005; Kirschbaum, 2006; Allison et al., 2010).

PURPOSE AND FRAMEWORK OF REVIEW

Explicitly representing microbes in SOM models, and then embedding them in land-ecosystem and hence Earth System Models (ESMs), faces a number of challenges (Schimel, 2001; Bradford and Fierer, 2012; Todd-Brown et al., 2012; Treseder et al., 2012). One of these challenges is establishing a common conceptual framework through which researchers in a diverse set of fields, including physiology, microbial ecology and ecosystem ecology, can productively interact. I aim to help provide this common framework by:

Section 2 – Clarifying the meaning of the terms thermal acclimation and adaptation

Section 3 – Describing mechanisms underlying soil respiration responses to warming that are independent of direct temperature effects on microbial physiology

Section 4 – Reviewing direct responses of microbial physiology to warming

Section 5 – Discussing theoretical challenges to incorporating microbes into SOM, ecosystem and Earth System Models.

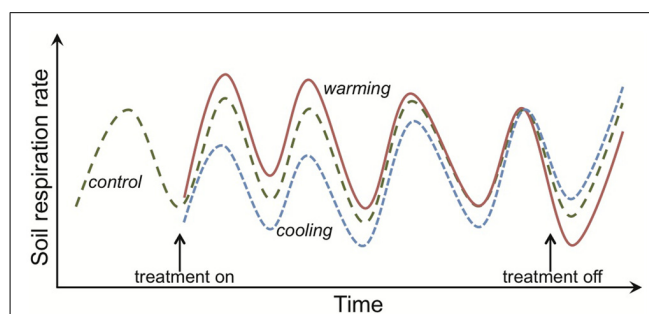
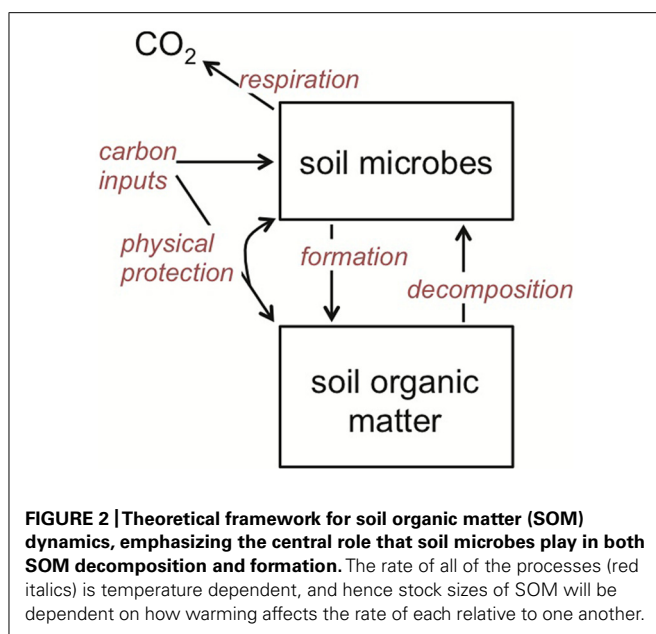


FIGURE 1 | Response of soil respiration to experimental warming or cooling across time in a mesic, temperate system. The rates shown are not observations for any one experiment, but instead are intended to capture characteristic dynamics in respiration responses to warming. The hatched green line depicts respiration from control plots, where each unimodal cycle represents the expected increase in respiration rates across the growing season and then decline as plants senesce. Rates represent both autotrophic and heterotrophic respiration, and so higher rates in the growing season result from multiple mechanisms, including direct temperature effects on respiration and indirect effects such as higher plant-carbon supply to heterotrophic microbes. Similar dynamics would be expected for only heterotrophic respiration given that temperatures are higher during the growing season and the common assumption of a constant substrate supply, making it hard to disentangle the mechanisms underlying apparent thermal acclimation (see main text). Initiation of the warming treatment (e.g., a 5°C increase above ambient; depicted by the red solid line) stimulates soil respiration but this augmentation is ephemeral, with rates in the warming plots being equivalent to the controls by the fourth treatment year. This apparent thermal acclimation could arise through physiological adaptation of the organisms and/or changes in the environment, such as depletion of substrate that supports microbial activity. Both types of mechanism result in respiration rates in warmed plots that are lower than in controls if the treatment is discontinued. The opposite respiration response is observed for experimental cooling (blue dotted line).

Much of my review focuses on respiratory processes because (a) at the ecosystem-level for soil responses to warming the literature focuses primarily on respiration; and (b) a substantial proportion of physiological work on thermal adaptation has focused on respiration. The caveat, however, is that thermal adaptation refers to a suite of phenomena (Hazel, 1995; Hochachka and Somero, 2002; Angilletta, 2009) and I devote considerable discussion to microbial growth because it has marked potential to affect how global and local SOM stocks respond to warming.

I concentrate on soil microorganisms that decompose organic matter using extracellular enzymes and/or assimilate low molecular weight organic compounds from the soil environment. These organisms include free-living, heterotrophic microbes in the litter, bulk soil and rhizosphere, as well as those that are plant mutualists, such as ectomycorrhizal fungi. What they have in common is that together they are the primary biotic agents in terrestrial systems regulating the breakdown of organic matter and its eventual mineralization and hence return to the atmosphere as CO₂ (Figure 2). These organisms are also primary agents of SOM formation (Grandy and Neff, 2008; Schmidt et al., 2011; Miltner et al., 2012; Bradford et al., 2013; Clemmensen et al., 2013), suggesting that it is the balance of their changing catabolic and anabolic activities under warming that together determine SOM stocks (Figure 2).



WHAT IS THERMAL ACCLIMATION?

DEFINITIONS

The scientific literature is burdened with a variety of uses for the same term. The confusion created hinders discourse across disciplines, presenting an obstacle to the interdisciplinary science demanded by environmental problem solving (National_Research_Council, 2009). The terms thermal acclimation, acclimatization and adaptation are all variously used to represent direct and indirect effects of temperature on soil microbial activity. More than a half-century ago, Bullock (1955) decided not to perpetuate the multifarious uses of these terms and, following his lead, I define here the terms I use but do not expect others necessarily to adopt them. I follow Hochachka and Somero (2002) by using ‘thermal adaptation’ as an integrative term that captures *direct* organism responses to temperature across immediate to multi-generational time-scales that manifest as physiological change. This then permits me to discuss thermal adaptation in soil communities without pretending to know the precise mechanisms underlying the adaptive response, because we simply do not yet know which mechanisms contribute most to thermal adaptation in soil microbial activity. These mechanisms operate across three distinct timescales.

The initial adaptations involve changes in active biochemical systems within cells, such as the availability of intracellular carbohydrates whose depletion limits cellular respiration rates (Tjoelker et al., 2008). Over days to a few weeks intermediate timescale adaptations occur, which modify preexisting biochemical systems through synthesis of new or different quantities of cellular machinery (e.g., enzymes). Such intermediate timescale physiological adjustments within individuals are commonly referred to as acclimation or acclimatization (Hochachka and Somero, 2002). Longer timescale adaptations involve evolutionary change but can span few to many generations. For example, species/genotype turnover might occur across few generations, where temperature acts on existing genetic variation among organisms to select

those best adapted to grow at the new environmental temperature. In contrast, selection of beneficial *de novo* mutations could take many generations (Hochachka and Somero, 2002). Later in this review I present arguments that adaptations that influence the activities of soil decomposer communities at ecosystem-scales at management-relevant timescales (i.e., <30 years), in response to warming at a location, likely arise through species/genotype turnover.

I use the term “apparent thermal acclimation” (e.g., as in Tucker et al., 2013) to connote an ephemeral augmentation in soil and ecosystem respiration rates to prolonged warming that result from indirect effects of temperature on microbial activity such as, for example, reductions in SOM or moisture availability. This definition and the one I use for thermal adaptation are then consistent with expected responses of respiration to prolonged warming at both organism- and ecosystem-levels. That is, for an initial increase in respiration under warming to diminish or for recovery of an initial decrease in respiration under cooling (Figure 1).

MEASURING THERMAL ADAPTATION OF RESPIRATION

Thermal adaptation of respiration involves dampening in the response of mass-specific respiration rates to temperature change. Mass-specific respiration (R_{mass}) rates are calculated as respiration per unit biomass, making the measurement of individual, population and/or community biomass essential for calculating and discussing thermal adaptation of respiration.

Adaptation of R_{mass} to warming is exhibited through a dampening of Q_{10} (type I adaptation) and/or a change in absolute R_{mass} rates at any one temperature (type II adaptation; Atkin and Tjoelker, 2003). The metric “ Q_{10} ” is commonly used to estimate temperature sensitivity, where for example a value of 2 means that respiration rates double per 10°C rise. Type I and II adaptation patterns (Figures 3A,B) are achieved at the cell level through, for example, changes in the inherent properties of enzymes that determine the temperature sensitivity or absolute magnitude of their catalytic rates, respectively. Type II adaptation dampens respiration responses to a sustained temperature change without adjustment of temperature sensitivity. Bradford et al. (2008a) define a third class of adaptation (type III), where a shift from a cold- to warm-adapted community or vice versa leads to a fundamental change in the temperature response of R_{mass} (Figure 3C). Such community shifts have the potential to generate seemingly paradoxical Q_{10} values; where over the same temperature range warm-adapted communities have elevated (as opposed to dampened) Q_{10} values. These elevated Q_{10} values could arise because measured Q_{10} typically decreases across the temperature range over which respiration is active (Davidson and Janssens, 2006), and so a warm- vs. cool-adapted community falls at an earlier part of its active range at intermediate temperatures (Figure 3C). I am not aware of this phenomenon of elevated Q_{10} values being shown for soil microbial respiration, but the same mechanism might explain why soil communities adapted to warmer temperatures have higher Q_{10} values for growth (Rousk et al., 2012). The commonality in pattern across the type I to III R_{mass} responses is that the temperature optima for respiration of warm-adapted enzymes, individuals, populations or communities are shifted right of those

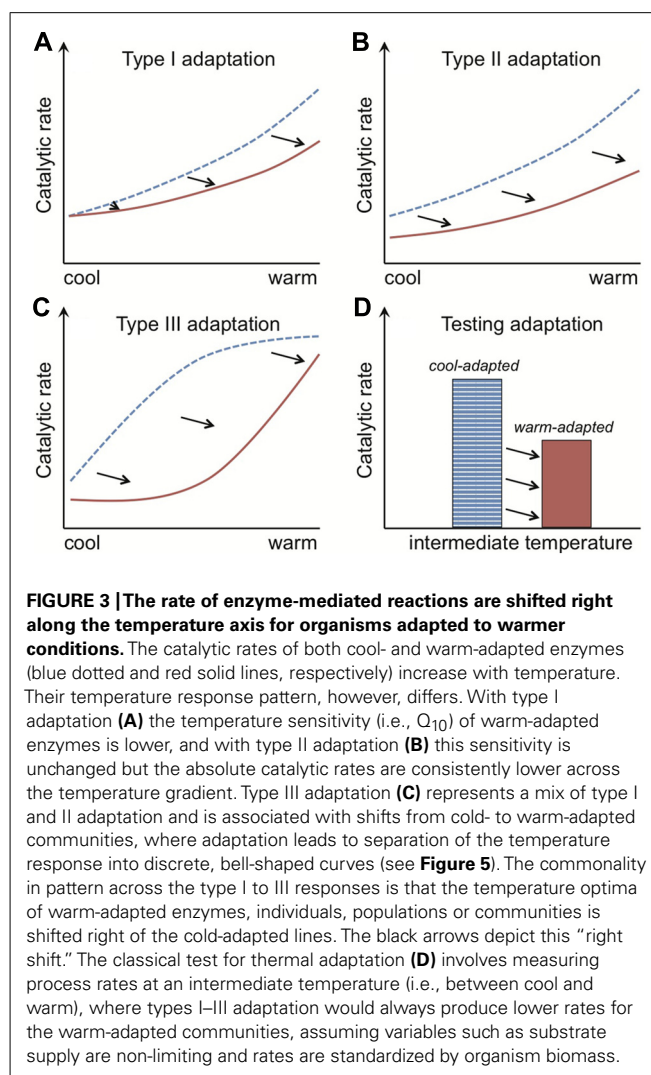
of cold-adapted organisms (Hall et al., 2008; Tjoelker et al., 2008; Bradford et al., 2010).

The “right shift” in temperature optima sets up the classical test for thermal adaptation of respiration (**Figure 3D**). This classical test relies on the fact that cold-adapted organisms should have higher R_{mass} rates, at intermediate temperatures, than warm-adapted organisms because the temperature optimum for the latter has been shifted right (**Figure 3**). The test needs to be performed under conditions where other factors do not limit respiration. For example, for the soil microbial community one should ensure that soil moisture and substrate availability are non-limiting (Bradford et al., 2008a, 2010). Both moisture and substrate limitation restrict respiration responses to temperature (Gu et al., 2004; Bengtson and Bengtsson, 2007; Almagro et al., 2009; Davidson et al., 2012; Suseela et al., 2012; Tucker et al., 2013), and substrate limitation at least in part explains diminishing soil respiration rates under sustained experimental warming (Hartley et al., 2007; Bradford et al., 2008a; Tucker et al., 2013). Tests for thermal adaptation must account also for differences in microbial biomass because higher biomass usually means higher respiration (Waldrop et al., 2000; Allison et al., 2010), which explains why tests for thermal adaptation of respiration must measure R_{mass} .

WHY IS THERMAL ADAPTATION IN SOIL DECOMPOSER COMMUNITIES STILL DEBATED?

When expressed as R_{mass} , both field and laboratory warming experiments have shown thermal adaptation of soil respiration (Bradford et al., 2008a, 2010; but see Bradford et al., 2009; Hartley et al., 2009). Yet purported empirical tests of thermal adaptation of soil respiration rarely control for differences in microbial biomass and/or substrate availability (e.g., Hartley et al., 2007, 2008; Vicca et al., 2009). In modeling studies, both Knorr et al. (2005) and Kirschbaum (2004) concluded that adaptation in soil microbes was not required to explain apparent thermal acclimation in soil respiration because substrate depletion (see Indirect Effects of Temperature on Microbial Activity) generated the respiration response. They could not, however, falsify the hypothesis that thermal adaptation might also explain apparent thermal acclimation because they did not model thermal adaptation as a competing mechanism. This rule in mathematical modeling is often quoted as “pattern does not beget process,” and cautions against accepting as proof of mechanism a model that recreates the observed pattern (Warren et al., 2011b). Indeed, when Allison et al. (2010) modeled both adaptation and substrate depletion, they found both were plausible mechanisms explaining apparent thermal acclimation in soil respiration. The Knorr et al. (2005) and Kirschbaum (2004) studies, however, have >700 citations between them, suggesting they were influential in proliferating the idea that heterotrophic soil microbes might not adapt to warmer temperatures, even though we expect adaptation in other organisms that drive terrestrial carbon cycling (Reich, 2010).

Incorporation of knowledge from other disciplines and direct tests of adaptation seem to underlie recent advances in evaluating how adaptive microbial responses under warming affect SOM dynamics. These advances go beyond respiratory responses and assess other physiological parameters, such as growth efficiencies and extracellular enzyme activities (German et al., 2012; Manzoni



et al., 2012b; Wallenstein et al., 2012; Frey et al., 2013; Tucker et al., 2013). These investigations are finding evidence for adaptation and, as a consequence, generate different expectations for how warming will influence SOM stocks (e.g., accelerated loss vs. protection of stocks; Allison et al., 2010; German et al., 2012; Frey et al., 2013; Tucker et al., 2013). Nevertheless, the extent to which indirect vs. direct temperature effects drive SOM dynamics under warming is largely untested (see Rousk et al., 2012), although the effect types co-occur in field experiments (Bradford et al., 2008a). To provide sufficient space to review and discuss direct temperature effects, I only briefly cover indirect effects. This brevity should not be misinterpreted: indirect effects undoubtedly have a major influence on SOM responses to warming and a synthesis of these effects seems warranted.

INDIRECT EFFECTS OF TEMPERATURE ON MICROBIAL ACTIVITY

Apparent thermal acclimation of soil respiration can arise through multiple processes because biological CO_2 effluxes represent the cumulative activity of microbes, plants and animals (Boone et al.,

1998; Ostle et al., 2009). Conventional SOM models assume that indirect effects provide the sole explanation for longer-term respiration and SOM responses to sustained warming (Kirschbaum, 2004; Eliasson et al., 2005; Knorr et al., 2005) and make no predictions as to how microbial community composition and biomass are affected. For example, substrate depletion is the classic indirect mechanism by which soil respiration is “down regulated” under prolonged warming (**Figure 1**). The mechanism has both observational and experimental support. For example, labile carbon availability to the microbial biomass is lower in experimentally warmed soils (Hartley et al., 2007; Bradford et al., 2008a; Curiel Yuste et al., 2010). Further, seasonal patterns in soil respiration responses to temperature are strongly dependent on substrate availability, with temperature having minimal effects on respiration rates at times of the year when substrate is depleted, and strong effects when substrate supply is abundant (Gu et al., 2004; Bengtson and Bengtsson, 2007). This all makes perfect sense: if an ectothermic heterotroph cannot get much to eat, and has depleted any internal stores, then its R_{mass} rate will decrease under otherwise constant environmental conditions.

Apparent thermal acclimation in the respiration responses of conventional SOM models also appears consistent with substrate depletion. When warming is imposed in conventional SOM models, the turnover rate of the SOM pools increases. The implicit biological assumption is that temperature constraints on microbial activity are relaxed under warming. As the modeled SOM pools turnover, a constant fraction of the carbon is lost as CO_2 . Hence faster turnover is associated with greater losses of CO_2 per unit time (**Figure 4**). This dynamic causes the initial stimulation of soil respiration under warming (**Figure 1**). Gradually, however, the SOM pool of interest decreases in size (i.e., substrate depletion) and, as it does so, there is a proportional decline in respiration from this pool. At the new steady state (i.e., conditions under which SOM pool sizes are constant), soil respiration rates equal carbon input rates to the soil (**Figure 4**). Conventional SOM modeling studies have kept carbon input rates equal under ambient and warmed conditions (Kirschbaum, 2004; Knorr et al., 2005). Hence, under this assumption respiration rates at steady state from ambient and warmer conditions are identical (**Figures 1, 4**), although faster cycling SOM pools are smaller in warmed soils (**Figure 4**). These effects of warming appear consistent with our understanding of limiting factors on microbes in mineral soils: SOM decomposers typically exist in an environment where substrate is limiting (Schimel and Weintraub, 2003).

Although depletion of SOM-substrates has received most attention as the mechanism underlying indirect effects of warming on microbial activity, temperature also influences numerous other processes that influence substrate availability to microbes and so would be expected to modify microbial activity in warming soils. For example, substrate supply rates are a critical control on SOM stocks and turnover, and the temperature response of respiration (Cheng et al., 1996; Fontaine and Barot, 2005; Bradford et al., 2008b; Gershenson et al., 2009; Kuzyakov, 2010; Dorrepaal et al., 2013). Substrate supply is affected by plant carbon input rates, which have been shown to both decrease and increase under warming (Uelman et al., 2000; Ise et al., 2010; Yin et al., 2013). Similarly divergent responses have been shown for fine roots, which are

important not only for rhizodeposition but as carbon substrates themselves (Pregitzer et al., 2000; Rinnan et al., 2008; Melillo et al., 2011; Sistla et al., 2013). Substrate supply within the soil will also be altered if warming affects soil moisture because water availability affects the rate at which enzymes, substrates and/or products of degradation diffuse between microbes and their immediate environment (Xu and Saiers, 2010; Davidson et al., 2012; Manzoni et al., 2012a). For example, if warming dries a soil then diffusion rates may decrease, reducing substrate availability to microbes. Temperature also controls the rate at which substrates sorb and desorb from organo-mineral surfaces (Conant et al., 2011), and hence become available to microbes. Further, temperature may decrease overall SOM substrate quality because labile substrates are depleted faster than more recalcitrant substrates, decreasing both the availability and quality of SOM-substrates (Davidson and Janssens, 2006).

Warming effects on substrate availability – through the mechanisms outlined above – in addition to other warming-induced effects on soil variables such as nitrogen availability (Rustad et al., 2001; Melillo et al., 2011), seem likely to lead to changes in microbial decomposer communities that in turn influence soil respiration rates under warming. For example, substrate limitation might shift enzyme expression toward higher affinity enzymes (Steinweg et al., 2008), where the trade-off is a reduction in maximum catalytic rates. Such a shift in enzyme expression would favor a slower growing microbial biomass, and lower respiration rates, recreating expected reductions in microbial biomass and respiration under sustained warming. Overall, then, a broad array of indirect mechanisms under warming likely affect microbial physiology and community composition, and these indirect effects likely co-occur with the direct effects of warming that are discussed next.

DIRECT EFFECTS OF TEMPERATURE ON MICROBIAL ACTIVITY

Temperature is a fundamental determinant of the distribution and abundance of organisms across time and space (Angilletta, 2009). Organisms occupy different thermal niches because of their physiological tolerances and because temperature modulates the strength of both positive and negative biotic interactions (e.g., Warren et al., 2011a; A’Bear et al., 2012). These individual responses and biotic interactions translate to differences in fitness across genotypes and species. As a result populations subdivide into thermal ecotypes and communities differ in composition as species sort based on environmental temperature (Porankiewicz et al., 1998; Hall et al., 2008, 2010; Wallenstein and Hall, 2012; Garcia-Pichel et al., 2013). Not surprisingly then, experimental and observational studies demonstrate that temperature drives microevolution and speciation (Leroi et al., 1994; Turner et al., 1996; Cooper et al., 2001; Angilletta, 2009). Warming should then directly affect microbial community physiology, biomass and composition (Zogg et al., 1997; Bardgett et al., 1999; Frey et al., 2008, 2013; Bradford et al., 2010; German et al., 2012; Rousk et al., 2012). Investigations of adaptive responses to warming of soil decomposer communities, however, have primarily focused on community-level respiration and growth (Rannekleiv and Bååth, 2001; Bradford

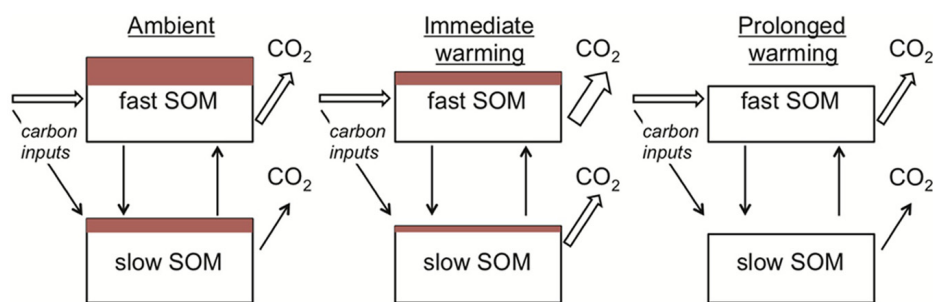


FIGURE 4 | The mechanistic basis for apparent thermal acclimation of soil respiration rates as represented in conventional soil organic matter (SOM) models. Under ambient conditions (left) a proportion of the SOM in both fast and slow pools is vulnerable to loss if temperatures increase (depicted as red-filled rectangles). On warming (middle) respiration rates increase because the rate of SOM turnover is positively related to temperature and a fixed proportion of this turnover is lost as CO_2 . The

red-filled rectangles decrease because carbon losses in respiration are greater than plant-carbon inputs to the soil. With prolonged warming (right) all of the vulnerable SOM is lost as CO_2 , depicted as loss of the red-filled rectangles and reductions in the SOM pool sizes. Respiration rates under ambient and prolonged warming conditions are the same because carbon input rates equal loss rates under steady-state conditions, creating “apparent” thermal acclimation (see Definitions for definition).

et al., 2008a, 2010; Bárcenas-Moreno et al., 2009; Rinnan et al., 2009; Rousk and Bååth, 2011; Rousk et al., 2012). I review these two processes first, before discussing how warming might affect biotic interactions, such as microbivory, that could mitigate or exacerbate microbial respiration and growth responses to temperature change.

A focus on the aggregate, or community-level, response of microbial respiration and/or growth means that adaptation might manifest through multiple mechanisms, ranging from shifts in individual physiology to changes in species composition. That multiple mechanisms are operating obscures our ability to ascribe specific causation as to why we observe thermal adaptation. For example, R_{mass} rates of warm-adapted individuals are expected to be lower than those of cool-adapted individuals at intermediate temperatures, but this result could arise through a change in enzyme expression and/or changes in cell membrane structure. These individual responses might translate to the community R_{mass} response, but equally there could be turnover in community composition toward warm-adapted genotypes or species (Bradford et al., 2008a, 2010; Hartley et al., 2008; Wallenstein and Hall, 2012). In their work on biochemical adaptation, Hochachka and Somero (2002) cautioned that the physiological mechanism explaining adaptation in the rate of a process could be obscured when working at the level of an organ within an individual animal. They advised working at the intracellular level to explain causation. Such work is no doubt required for soil decomposer organisms but we are far from such a reality. Can any of us even state categorically what the most important microbial taxa are for decomposing SOM? We just do not know which study species to choose.

If we do observe a change in microbial community composition under warming, relating such shifts to changes in soil functioning, let alone the pattern of thermal adaptation, is still a major challenge for soil microbial ecologists (Allison and Martiny, 2008; Bradford and Fierer, 2012; Wallenstein and Hall, 2012). Isolation and pure-culture offer an approach to look at physiological responses to warming that might be expected to reflect general responses and hence universal constraints on organisms (Lennon

and Jones, 2011). However, linking these single species back to the aggregate responses of multi-species communities is challenging. The best approach seems to be to recognize that multiple processes might underlie thermal adaptation responses. We must then investigate each mechanism, to determine which contribute most to ecosystem-level carbon cycling responses to climate change. In the subsections below I therefore explore mechanisms that span from the individual- to community-level, and do not pretend to know which matter most for explaining warming effects on carbon cycling at the ecosystem-level.

RESPIRATION

Trade-offs in enzyme structure and function

Enzyme-mediated reactions are generally temperature sensitive. An increase in temperature accelerates reaction rates in the short-term, when all other variables are non-limiting (e.g., enzyme and substrate availabilities). At high temperatures proteins denature and so enzyme function and hence reaction rates drop precipitously. Long-before reaching denaturing temperatures, however, the rate of increase in an enzyme-mediated reaction decreases. Fundamental trade-offs in enzyme structure and function underlie this deceleration. Essentially, enzymes need to be folded into certain three-dimensional shapes (i.e., conformations) to bind a substrate and other shapes to release the product. The rate at which they change shape theoretically controls the speed of the reaction the enzyme catalyzes. At lower temperatures, these shape changes are faster for more flexible enzyme structures. At warmer temperatures, however, a flexible enzyme spends less time in shapes that bind substrate, which decreases the affinity of the enzyme for substrate and hence reduces the relative rise in reaction rate with increasing temperature (Figure 5). Temperature then selects (in the Darwinian sense) for more flexible enzymes when it is cooler and for less flexible enzymes when it is warmer.

A less flexible enzyme can maintain binding conformations for a greater proportion of time at warmer temperatures, and so “out-compete” a cool-adapted enzyme for substrate. As such, in any one organism, population or community, we expect a different set of isoenzymes to be expressed at different temperatures (Figure 5).

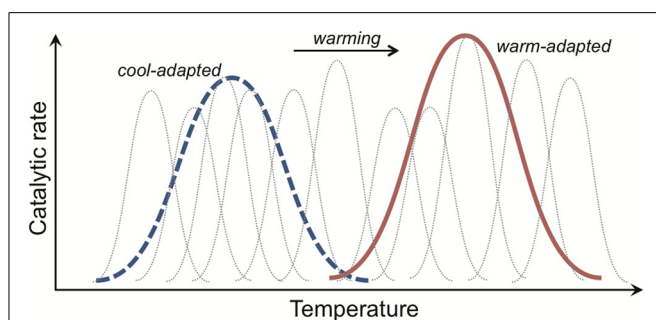


FIGURE 5 | Catalytic rate responses to temperature, caused by the evolutionary trade-off between enzyme structure and function, giving rise to different distributions of isoenzymes in cool- and warm-adapted organisms. An isoenzyme (gray dotted curves) is an enzyme with the same function but a different structure. In any one organism, population or community we expect a different set of isoenzymes to be expressed at different temperatures. This is because enzyme structure usually only promotes efficient substrate-binding and product-release across a narrow temperature window. As such, the aggregate activity of a cool- or warm-adapted organism, population or community (depicted as thick blue dotted and red solid lines for cool- and warm-adapted) results from the activities of a family of isoenzymes expressed across the environmental temperature range.

An isoenzyme (aka isozyme) is an enzyme with the same function but a different structure. In environments where temperature varies markedly in the short-term, such as the day-night cycle during the growing season in temperate forest, a broad suite of isoenzymes might be expressed but the relative contribution to catalysis of any one isoenzyme will change with the daily temperature cycle (Figure 5). Across broad latitudinal gradients, however, catabolic responses for communities from lower latitudes appear shifted right along the temperature axis, consistent with expected trade-offs (Balser and Wixon, 2009; German et al., 2012).

Will trade-offs in respiratory enzymes affect ecosystem processes?

Most of the biochemical adaptation work on trade-offs in flexibility under temperature has been conducted with enzymes involved in the metabolic pathways that comprise cellular respiration. The CO_2 of aerobic respiration is generated as an intracellular product. Ecosystem respiration fluxes therefore result from reactions catalyzed by intracellular enzymes. It would be a mistake, however, to infer that physiological adjustments in R_{mass} rates in warm vs. cold-adapted microbial communities (Figure 3) necessarily underlie apparent thermal acclimation of soil respiration. For example, Schimel and Schaeffer (2012) posited that microbial communities only control ecosystem processes when two conditions hold: (a) organisms differ in their functional traits and (b) the biological process is the rate-limiting step in the reaction sequence.

Condition (a) should hold for the traits of cold- and warm-adapted communities, given the fundamental evolutionary trade-offs between enzyme structure and function across temperature. That is, R_{mass} rates will differ if measured under standard conditions (Figure 3) because of physiological adjustments that arise through individual or community responses. There is some evidence for these shifts in functional traits for soil microbial communities and for laboratory-grown heterotrophic microbes

(Balser and Wixon, 2009; Bradford et al., 2010; German et al., 2012; Crowther and Bradford, 2013). If we look more broadly, we find evidence for the trade-off in mycorrhizal fungi (Heinemeyer et al., 2006; Malcolm et al., 2008) and for heterotrophic microbes and their communities across a range of other systems, where there are distinct latitudinal and seasonal patterns in R_{mass} as a consequence of environmental temperature (Porankiewicz et al., 1998; Lange and Green, 2005; Clarke, 2006; Hall and Cotner, 2007; Tjoelker et al., 2008). Given the theoretical and empirical evidence, the physiological function of heterotrophic soil microbial communities must adapt to warming.

For functional trait differences to influence soil respiration responses to warming requires Schimel and Schaeffer's (2012) condition (b) to hold. That is, that microbial activity is the rate-limiting step in SOM decomposition. Yet they argue this is not the case for mineral soils. Instead, they suggest that physical protection of SOM (e.g., sorption) regulates the breakdown rate of SOM. We should therefore expect temperature-induced changes in intracellular microbial physiology not to scale to soil and ecosystem respiration rates. Schimel and Schaeffer (2012) argue that formation of SOM may, however, be conditional on microbial community composition because organisms differ in their biochemical make-up (e.g., the amount of lipids) and compounds differ in the extent to which they are physically protected from decay. If true, SOM decomposition rates might be independent of physiological adjustments but SOM stock sizes will be dependent on the physiology of the overall microbial community. Specifically, stock sizes are a product of both losses (i.e., decomposition) and inputs (i.e., formation). Understanding soil respiration responses to warming is then likely a poor indicator of SOM stock responses (Conant et al., 2011; Hamdi et al., 2013), meaning that SOM stocks and turnover must be measured directly to understand climate-carbon cycle feedbacks.

Warming does influence the biochemical composition of microorganisms and hence could affect SOM formation rates. For example, as with enzymes, there are trade-offs between the structure and function of lipids in cell membranes (Hazel and Williams, 1990; Hazel, 1995). These trade-offs influence cell membrane permeability and translate to lower R_{mass} values for warm- vs. cold-adapted organisms when testing for thermal adaptation (Hochachka and Somero, 2002). There is no direct evidence of warming-induced changes in cell membrane structure for soil decomposers, but the trade-offs have been shown for aquatic microbial heterotrophs (Hall et al., 2010). The potential for shifts in the chemical composition of soil microbes to affect SOM formation rates, however, remains to be tested. Given that stable SOM in well-drained mineral soils appears to be largely composed of microbial-derived products (Lundberg et al., 2001; Grandy and Neff, 2008), this possibility seems a research priority.

The idea that microbial activity in mineral soils does not regulate SOM decomposition rates is controversial (Kemmitt et al., 2008; Kuzyakov et al., 2009; Paterson, 2009; Schimel and Schaeffer, 2012; Thiessen et al., 2013). It does seem certain, however, that microbial community composition affects the breakdown and mineralization rates of leaf litter (Strickland et al., 2009; Wallenstein et al., 2010, 2012; Keiser et al., 2011; Schimel and Schaeffer, 2012). In some systems litter breakdown (foliar and woody) can

account for a substantial fraction of ecosystem respiration (Wu et al., 2005; Weedon et al., 2009), and so in these systems we might expect physiological shifts in microbial communities under warming to translate to the ecosystem-level. At the very least, for mineral soils, such shifts will influence nutrient cycling because they regulate litter decomposition rates, and so might indirectly affect ecosystem-level carbon fluxes through influences on plant growth. In organic soils the SOM is not protected by organo-mineral interactions, and so this presumably also makes its breakdown sensitive to microbial physiology. As high-latitude systems warm, constraints on microbial activity such as frozen water may be relaxed, making huge stocks of SOM in organic permafrost soils vulnerable to mineralization. Physiological responses of microbes to warming will then influence climate-carbon cycle feedbacks if microbial activity is a rate-limiting step in the breakdown of organic soil carbon stocks.

Extracellular enzymes

If microbial activity does regulate how temperature affects the breakdown of SOM stores, the accepted wisdom is that extracellular (not intracellular) biological processes provide the rate-limiting step (Allison et al., 2011; Wallenstein and Hall, 2012). Specifically, soil microbes catalyze the breakdown of SOM using extracellular enzymes, where the dissolved, low molecular weight products can be assimilated. The enzymes involved in assimilation, intracellular metabolism, and extracellular degradation should all be under the same evolutionary pressure to generate the trade-off between structure and function. Enzymes involved in assimilation of dissolved compounds from the soil environment have not been investigated for this trade-off, but the aggregate activity of classes (e.g., cellulases) of extracellular enzymes expressed by soil decomposer communities do respond to seasonal, latitudinal and experimental warming in a manner consistent with thermal adaptation (Fenner et al., 2005; Wallenstein et al., 2009; Brzostek and Finzi, 2011, 2012; Brzostek et al., 2012; German et al., 2012; Stone et al., 2012). Thermal adaptation in extracellular enzymes could affect warming responses of ecosystem and soil respiration if they provide a rate-limiting step for the acquisition of substrate by the soil microbial community, which in turn controls how much substrate microbes have available for respiration. The classical test for thermal adaptation of R_{mass} rates (Figure 3), however, would not detect thermal adaptation of extracellular enzymes because substrate is supplied in a form not requiring decay prior to assimilation. The test then only examines warming-induced shifts in cellular physiology, such as membrane structure and isoenzyme expression of assimilatory and intracellular enzymes.

GROWTH

Microbial growth is likely much more important than respiration for determining climate-carbon cycle feedbacks. Colonization rates and hence the breakdown of new resources are a function of growth rates, extracellular enzyme production is tied to biomass production, and so are SOM formation rates (Waldrop et al., 2000; Rousk and Bååth, 2011; Schmidt et al., 2011; Bradford et al., 2013; Cotrufo et al., 2013; Thiessen et al., 2013). Microbial growth efficiency (MGE; aka carbon use efficiency) was the physiological parameter in the microbial SOM model of Allison et al. (2010)

to which SOM stocks were most sensitive. Growth efficiency is broadly defined as the proportion of assimilated substrate allocated to growth vs. other fates such as respiration (Brant et al., 2006; Thiet et al., 2006; Frey et al., 2013). Under model scenarios where efficiencies declined in a constant linear fashion with increasing temperature, Allison et al. (2010) demonstrated that associated decreases in microbial biomass and hence extracellular enzyme activities meant that SOM stocks were protected from loss. Understanding how MGEs respond to temperature in the shorter- and longer-term is a research priority if we are to project reliably climate-carbon cycle feedbacks.

Microbial growth efficiency

That MGEs decline as environmental temperature increases is controversial. For heterotrophic microbial communities in aquatic systems, debate has raged as to whether substrate quality alone vs. temperature explains differences in growth efficiencies (del Giorgio and Cole, 1998; Rivkin and Legendre, 2001; Apple et al., 2006; Apple and del Giorgio, 2007; López-Urrutia and Morán, 2007). The idea that substrate quality matters is not controversial. More chemically recalcitrant substrates require greater energy investment to breakdown, reducing net energy gain and hence leaving less energy available for growth (Fierer et al., 2005; Davidson and Janssens, 2006; Craine et al., 2010). The mechanism by which increasing temperature reduces efficiencies is often thought to depend on maintenance energy costs being higher as temperature rises (Manzoni et al., 2012b). Greater maintenance costs then reduce the proportion of energy acquired that is available to growth. The two maintenance activities requiring most energy are likely protein synthesis and the maintenance of ionic gradients across membranes (Clarke and Fraser, 2004). The metabolic costs of maintaining these two processes, for an individual or community, immediately increase with warming because proteins (including enzymes) are less stable and membranes more permeable. These physiological consequences heighten ATP demand, driving respiration, and hence for a fixed substrate intake rate reduce the energy remaining for growth. In the intermediate-term, evolutionary trade-offs (see Respiration) suggest that isoenzymes and membranes will shift toward structures that are more warm-adapted. These shifts should explain thermal adaptation of MGEs. The empirical evidence in soil decomposer communities for shifts in efficiency with sustained warming is, however, limited to a single study and was observed for only one of four tested substrates (Frey et al., 2013).

Original observations that MGEs in soils declined with temperature were confounded by the complexity of substrates on which the microbial biomass was growing (Devêvre and Horwath, 2000; van Ginkel et al., 2000; Pietikäinen et al., 2005). Increasing temperatures permitted the microbes to use more chemically recalcitrant substrates, which have lower efficiencies. Frey et al.'s (2013) observation that MGEs declined with increasing temperature only for substrates requiring extracellular enzyme decay, helped resolve apparently conflicting results that MGEs were temperature insensitive (for glucose, Dijkstra et al., 2011) vs. sensitive (for cellobiose which requires degradation prior to assimilation, Steinweg et al., 2008). When MGEs of whole communities are temperature sensitive, they decline linearly or curvi-linearly with

increasing temperature (Devèvre and Horwath, 2000; van Ginkel et al., 2000; Steinweg et al., 2008; Frey et al., 2013). In contrast, efficiencies are distinctly unimodal for isolates of free-living microbial heterotrophs, that produce extracellular enzymes for substrate decay, from both soils and other environments (Russell, 2007; Crowther and Bradford, 2013). Specifically, there appears to be an optimum MGE that matches the ambient temperature regime from the organism's environment, which declines at cooler and warmer temperatures than this optimum. Growth efficiencies for individuals and communities should, like R_{mass} , then conform to a suite of unimodal response curves (Figure 5). Why are individual growth efficiencies unimodal and those for soil communities unresponsive or only negatively affected by warming?

There is no clear answer as to why MGEs of soil communities are temperature insensitive (e.g., for glucose) or, when sensitive (e.g., for phenol), respond only negatively to temperature. It seems that our understanding of how MGEs respond to warming is woefully inadequate. In short, the theoretical basis is a physiological quagmire, arising from the complexity and associated unknowns of metabolism across all forms of life. For example, why do MGEs of whole communities decline with increasing temperature when using substrates that require extracellular decay (Steinweg et al., 2008; Frey et al., 2013)? There is weak support for the idea that declines occur because maintenance energy costs increase with temperature, leaving less for growth, albeit this explanation is commonly invoked in soil and ecosystem ecology (Clarke and Fraser, 2004; Clarke, 2006; Russell, 2007; Allison et al., 2010; Manzoni et al., 2012b). If maintenance costs do increase at the expense of growth, then catabolic and anabolic energy demands must be uncoupled, with more energy diverted to the former. These energy demands certainly do become uncoupled, with efficiencies declining at temperatures both above and below the optimum for growth in individuals (Angilletta, 2009; Crowther and Bradford, 2013). Yet higher maintenance energies only account for a small proportion of the elevated catabolic demand and there are even arguments that higher maintenance costs do not uncouple anabolic and catabolic processes.

One explanation for why maintenance- and growth-energy demands should remain coupled under warming for soil communities relies on the fact that differences in maintenance costs across species tend to co-vary with life histories. For example, resting metabolic rate, which we might think of as largely reflecting maintenance costs, increases with the temperature at which organisms live in cross-species syntheses (Clarke and Fraser, 2004; Clarke, 2006). Yet life histories also shift toward more active and more rapidly growing organisms as ambient temperature increases, and hence food intake rates are greater. So, maintenance costs increase because more active strategies are associated with higher intracellular enzyme and membrane (at least for eukaryotes) densities (Adadi et al., 2012). Yet these increases in maintenance costs plausibly increase proportionally with growth energy demands which are met by higher food intake, meaning that MGEs are invariant (Figure 6). For individual organisms, faster growth rates are often even associated with an increase in efficiencies because maintenance costs may be a relatively constant demand whether you are growing or not (Pirt, 1965; Ng, 1969). An increase in substrate availability and/or temperature then should increase

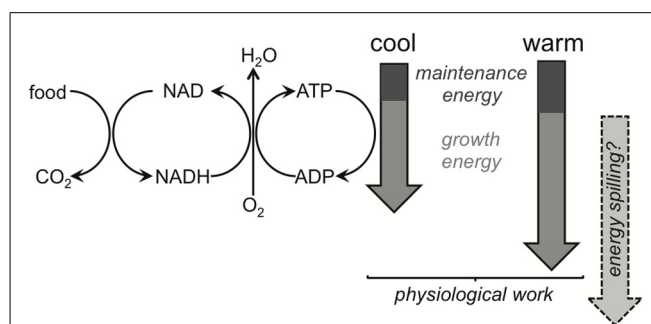


FIGURE 6 | Simplified schematic of metabolism, highlighting ATP-supply being driven by the demand of physiological work and/or energy spilling. Organisms catabolize substrates to provide energy demanded by maintenance and growth. Catabolism and anabolism are then coupled under this metabolic scheme. Decreases in microbial growth efficiency (MGE) are expected under warming, and the commonly cited mechanism involves maintenance energy demands responding more strongly to warming than growth energy demands. The theoretical basis for this expectation remains to be demonstrated and MGEs in field soils can be invariant to temperature. Maintenance demands more likely increase proportionally with growth demands (shown by the cool and warm block arrows), and if temperature accelerates growth then maintenance costs might even become proportionally smaller, increasing MGEs. Energy spilling is an alternative explanation for the uncoupling of catabolism and anabolism under warming. Its direct temperature response is uncertain (depicted by the question mark) but we know it does respond strongly to substrate limitation.

MGEs, explaining the rise in efficiency of the unimodal temperature response observed for individuals (Crowther and Bradford, 2013). Notably, cross-species syntheses of resting metabolic rate (a proxy for maintenance energy) seem restricted to fish, which show the rise with environmental temperature, or with terrestrial insects, which conversely show a weak negative response of resting metabolic rate with temperature (Clarke and Fraser, 2004; Clarke, 2006). The evidential basis is weak, then, for varying maintenance costs to explain the decline in MGE with warming.

Energy spilling (waste metabolism)

So what physiological response can explain varying MGEs with temperature? The most likely explanation is “energy spilling” (reviewed in detail by Russell and Cook, 1995; Russell, 2007). This phenomenon encompasses a range of physiological pathways across different organisms, from bacteria to humans, and is also referred to as waste metabolism, spilling, uncoupling, overflow metabolism and waste respiration (Echtay, 2007; Russell, 2007). Use of the words “waste” and “overflow,” however, may be misleading because energy spilling may be beneficial. The benefits posited include: (a) resource interception, where even if a microbe can not grow it can prevent a competitor from doing so; (b) maintenance of a growth-ready (or metabolically alert) strategy, where energy acquisition can proceed in the absence of growth, and so be immediately available when conditions are favorable; (c) protection from toxins or charge differentials that arise from excessive metabolic activity, where energy spilling is essentially a safety valve; and (d) heat generation, energy spilling by bacteria can raise the temperature of biofilms above ambient and so presumably improve growth conditions (Russell, 2007; Tabata et al.,

2013). Perhaps the best indicator that energy spilling is beneficial is the rapid death in certain environments of those microbes that do not energy spill (Russell, 2007).

In bacteria, energy spilling seems particularly high when the energy source (i.e., organic carbon) is in excess and nitrogen is strongly limiting (Russell and Cook, 1995). That is, where there is plenty of energy but no growth because of nutrient limitation. Conversely under carbon limitation, MGEs are ~10-times higher because catabolism and anabolism are again coupled (Schimel and Weintraub, 2003; Hackmann et al., 2013). These observations in pure culture make Frey et al.'s (2013) results even more intriguing: why were MGEs not higher in long-term warmed soils given the increased nitrogen availability (Rustad et al., 2001; Melillo et al., 2011)? We can also ask why MGEs on glucose were insensitive to temperature, when higher growth rates should have tipped energy demands proportionally toward anabolism? These questions highlight the difficulties of inferring how processes in culture translate to a complex environment such as soil, where a broad suite of growth strategies is represented. The majority, if not all, microbes in the soil use glucose. This ubiquitous use then likely aggregates a broad array of growth strategies, whereas more recalcitrant compounds are used by a small proportion of more specialized species (Hanson et al., 2008; Goldfarb et al., 2011). If traits such as storage of glycogen, which are associated with invariance in MGEs across environment, are differentially distributed across microbial groups then this could explain different MGEs for different substrates (Ng, 1969; Russell, 2007). We expect suites of traits to be related and so if glycogen storage is negatively related to extracellular enzyme production (Russell, 2007), then this and not the cost of extracellular enzyme production, which is relatively low (Allison et al., 2011), could explain declining MGEs with temperature on more recalcitrant substrates. Other explanations, such as differences in growth efficiencies between bacteria and fungi, or *r*- vs. *K*-strategies, now seem largely dismissed (Thiet et al., 2006; Strickland and Rousk, 2010).

Given uncertainties about the physiological mechanisms that determine MGEs, it is unlikely that we will be able to explain in the near term how they might adapt to warming. If energy spilling is beneficial, then under some environments (e.g., nitrogen limitation) reductions in MGE might even be adaptive! What seems likely is that substrate quality and availability, nutrient supply and microbial traits all contribute to observed MGEs. Direct warming effects on MGE are uncertain because conventional views of maintenance vs. growth energy demands fall short of explaining changing efficiencies with temperature. This means we may need to be satisfied with black-boxing the efficiency response of the soil microbial community to warming for current SOM modeling studies. The uncertainty also demands that we redress the paucity of observations we have for how warming affects MGEs of soil communities on specific substrates (Steinweg et al., 2008; Frey et al., 2013), where caveats such as changing substrate quality are controlled for. Culture-based studies can target specific mechanisms and should use isolates that are representative of soil decomposers because variation in microbial traits markedly influences how efficiencies respond to environment (see Russell, 2007).

Growth rates

Whereas MGE responses to temperature are far from clear, thermal adaptation in the growth of the soil microbial community resembles patterns expected from evolutionary trade-offs in the structure and function of both enzymes and membranes. That is, growth rates show the same unimodal temperature response as R_{mass} (Figure 5). These unimodal responses are shifted to the right under experimental warming (Bárcenas-Moreno et al., 2009; Rousk et al., 2012) and across spatial gradients in ambient temperature (Rinnan et al., 2009). These community-level responses match those for isolates of heterotrophic soil microbes (Crowther and Bradford, 2013), for aquatic microbial communities (Hall and Cotner, 2007) and for plants, vertebrates and invertebrates (Angilletta, 2009). These consistent, unimodal patterns suggest that trade-offs at the cellular-level translate to population and community performance (Angilletta, 2009).

Right-shifts in the unimodal growth response of soil microbial biomass to experimental warming are thought caused by species sorting (Bárcenas-Moreno et al., 2009), following the same explanation as for respiration (Bradford et al., 2010; Zhou et al., 2012). This sorting mechanism then explains the time taken (weeks to months) for these effects to manifest. The exception seems to be for very high temperatures; for example, Ranneklev and Bååth (2001) demonstrated that mimicking self-heating of peat by incubation at 55°C caused dramatic right-shifts in thermal optima for growth, resulting from the rapid growth of thermophilic bacteria. However, mesophilic and psychrophilic microorganisms take longer to grow (Ranneklev and Bååth, 2001), perhaps because they have to compete for resources with the more thermophilic organisms that can tolerate, at least for some time, cooler conditions. In contrast, at high temperatures, more thermophilic organisms may be able to grow unrestricted by competition because the original community is poorly adapted to the new temperature conditions. Whatever the mechanism, it seems likely that low growth rates do not permit species turnover within the time course of many cooling experiments (but see Curiel Yuste et al., 2010), explaining why shifts in optimum growth temperatures for communities are not observed under short-term cooling. What remains a mystery is why warming-induced phenotypic shifts in the individual physiologies of active soil microbes do not often translate to community-level processes at the same time-scale. For example, thermal adaptation in the growth and respiration of individual, mesophilic heterotrophic soil microbes occurs in just a few days (Crowther and Bradford, 2013) but shifts in community optima take weeks. Perhaps such responses are obscured from detection because of the host of other processes, such as desorption, that co-occur with warming (Subke and Bahn, 2010; Nie et al., 2013).

The consequences for SOM stocks of thermal adaptation in the growth rates of soil microbial communities have received little attention but may be minimal. Rousk et al. (2012) demonstrated that increases in the potential growth rates of soil bacteria were overwhelmed by reductions in growth rates caused by substrate depletion under experimental warming, meaning growth rates in control and warmed soils were essentially equivalent. Although not yet evaluated, I would argue that thermal adaptation of microbial R_{mass} , growth rates and extracellular enzyme activity should accelerate the rate at which substrate depletion is achieved in warmed

soils. That is, the theoretical “right shift” in these physiological parameters should lead to a microbial biomass that grows and degrades SOM more rapidly than non-adapted communities. How warming then influences substrate availability – through plant inputs, sorption/desorption and perceived chemical recalcitrance – therefore seems a key regulatory gate of SOM dynamics.

A key issue that I have not yet touched on, with regards measuring respiration and growth responses to temperature, is that we still have no direct methods for measuring soil microbial biomass and turnover (Bradford et al., 2009). We have many methods, including chloroform-fumigation extraction, substrate-induced respiration, total PLFA and semi-quantitative PCR, but all provide only correlated estimates of standing biomass (Wardle and Ghani, 1995). Estimates of turnover are even more uncertain. We should think about the uncertainty this generates in our observational and experimental data, and probably carry this forward into SOM and ecosystem models, to provide reliable error estimates for projected respiration and SOM stock responses to warming.

Biotic interactions

Heterotrophic soil microbes are part of a community that includes other microbes, such as arbuscular mycorrhizae and chemoautotrophs, as well as viruses, animals (e.g., Protozoa, nematodes, Collembola) and plants. These groups of organisms are faced with the same suite of physiological trade-offs in response to warming that heterotrophic microbes are (Van Dooremalen et al., 2013). Physiological responses of plants, animals and other microbes might influence soil microbial decomposer responses to warming but they are outside the purview of this review. Yet it is worth emphasizing that (a) temperature modulates the strengths of biotic interactions, and (b) interactions strongly determine the respiration, growth and community composition of soil microbes. For example, short-term increases in the overall growth of the soil microbial biomass under warming might be mitigated by concomitant increases in the growth of their predators, which in turn can promote microbial turnover and limit biomass. This microbial loop (*sensu* Clarholm, 1994) could explain increased availability of ammonium under experimental soil warming, but alternatively higher animal feeding can limit the growth and hence decomposer activity of heterotrophic microbes, as well as induce microbivore-defense, which represents a different energy cost (A’Bear et al., 2012; Crowther et al., 2012). Higher nitrogen availabilities could decrease fine root growth and exudation, limiting substrate available to soil microbes and shifting the soil community toward a more *K*-selected community (but see Zhou et al., 2012). Virtually no warming studies put microbial biomass responses in the full context of these biotic interactions, and yet we expect them to be major drivers of microbial activity.

A PLACE FOR THERMAL ADAPTATION IN COUPLED CLIMATE-CARBON CYCLE MODELS

Implicit assumptions in conventional SOM models are that biological processes, such as respiration, conform to the principles of invariance, probability and simplicity (Bradford and Fierer, 2012). Such principles derive from classical physics and assume that past conditions do not influence future responses (invariance), that all organisms respond identically (probability), and that only a

few, measurable variables influence outcomes (simplicity). Even if microorganisms are included as an SOM pool in the conventional models, they exert no control on respiration rates (Allison and Martiny, 2008). That is, if you removed the microbial pool, respiration would continue unabated because microbial activity is implicitly represented and donor-controlled. Specifically, respiration under this paradigm is represented as a first order reaction, where CO₂ evolution from an SOM pool is a function of the pool size, and a decay rate constant that responds positively to temperature and moisture (Todd-Brown et al., 2012). Biological systems do not follow this paradigm because, in contrast to the principles of classical physics, organisms adapt to, differ in their tolerances of, and interact dependent on, environmental temperature. Such adaptive responses of organisms can, for example, scale to the level of ecosystem carbon exchange (Niu et al., 2012). Yet even when global convergence in the temperature sensitivity of ecosystem respiration was observed, Mahecha et al. (2010) cautioned that prescriptions of a constant Q₁₀ value across systems was not justified. Instead they suggested that projections from coupled climate-carbon cycle models would be improved with a deeper understanding of the factors and processes affecting SOM mineralization.

Incorporating thermal adaptation and microbes into coupled climate-carbon cycle models is not, however, a straightforward exercise and the many challenges are reviewed elsewhere (e.g., Allison and Martiny, 2008; Ostle et al., 2009; Todd-Brown et al., 2012; Smith and Dukes, 2013; Todd-Brown et al., 2013). I wish to emphasize here only what I consider to be the major theoretical question related to incorporation of soil microbial processes and their responses to temperature. The question is: how best to represent soil microbes in models? I simplify this discussion by referring to SOM models and describe in the paragraph below the reason for focusing on these models, and in the subsequent paragraph elaborate on the question itself.

The land components of ESMs represent SOM dynamics relatively simply, but more complex representations are emerging. For example DAYCENT is the daily time-step version of CENTURY, one of the most widely used conventional SOM models, and is incorporated in version 4.5 of the land ecosystem model of the Community ESM (see Bonan et al., 2013). By considering SOM models we can then evaluate SOM responses that scale to local and global warming effects. At global scales SOM stocks are important in terms of a carbon store, whose loss might provide a positive feedback to climate change (Denman et al., 2007). At local scales, SOM stocks are inherently tied to ecosystem health because, for example, of the role SOM plays in preventing soil erosion, retaining moisture and nutrients, and providing soil structure and habitat (Lal, 2004).

The major theoretical question about representing soil microbes in models breaks down into two, broad choices: (a) as a supply driven pool, as in the conventional models; or (b) as a demand-driven pool, as in the new family of microbial SOM models? The primary difference between the two approaches is that the demand-driven approach creates a feedback between SOM turnover and microbial response (Allison and Martiny, 2008). In the conventional, supply driven approach microbes can be eliminated from the model and SOM continues to turnover. In

the microbial SOM model approach, loss of the microbes brings SOM turnover to a halt because turnover is explicitly dependent on microbial activity. Representing the same microbial response to warming in the two different model structures can then have divergent consequences for SOM stocks. For example, a decline in MGEs with warming reduced SOM decomposition in the microbial SOM model of Allison et al. (2010), leading to no net change in SOM stocks. The same decline in efficiency, in contrast, led to greater losses of SOM for a conventional model (Frey et al., 2013). This decline occurred because in conventional models SOM decomposition rates are determined by temperature and formation rates by the assumed MGE. Hence warming translated to accelerated SOM decomposition, along with reduced formation rates because of declining growth efficiencies. In both model structures SOM formation rates are then a function of microbial growth, but the structures diverge because microbial activity explicitly regulates decomposition rates in the microbial SOM models but implicitly regulates it through temperature in the conventional models.

The conventional and microbial SOM model structures both assume that SOM turnover rates are dependent on microbial activity (Parton et al., 1988; Schimel, 2001; Lawrence et al., 2009; Allison et al., 2010). A third family of SOM models is required for hypothesis testing where only physico-chemical processes regulate SOM decomposition and formation rates (Kemmitt et al., 2008). My expectation is that such a family of models will be equivalent to neutral models: largely unrepresentative of what actually occurs (Clark, 2009; Warren et al., 2011b) but excellent at advancing our understanding of those processes that do regulate SOM turnover. I expect us to find that both biological and physico-chemical processes play important roles in SOM dynamics under warming, as argued by Conant et al. (2011) and as represented in conventional SOM models such as DAYCENT and RothC (Bonan et al., 2013).

CONCLUSIONS

Thermal adaptation of organism respiration and growth rates should occur through fundamental evolutionary trade-offs in cellular physiology, such as between the structure and function of both enzymes and membranes. Individuals can adjust their physiology in response to sustained warming by producing warm-adapted isoenzymes and membrane structures, but changes in the physiology of the soil microbial biomass as a whole likely arise through shifts from colder- to warmer-adapted species (or at least genotypes). These physiological responses to warming are consistent with the idea that indirect warming effects, such as substrate depletion, at least partly explain apparent thermal acclimation of soil and ecosystem respiration to prolonged warming. Indeed, I hope that I have demonstrated in this review that thermal adaptation must occur in soil decomposer communities. As such, questions related to the consequences of thermal adaptation for carbon cycling must move from asking whether adaptation occurs, to asking what role adaptation plays in shaping ecosystem carbon stocks and flows in a warming world.

The idea that growth efficiencies of the soil microbial biomass decline with increasing temperature should be viewed as controversial. There is little empirical evidence that temperature directly elicits this response in soil communities and the physiological

basis for the decline is not resolved. We should explore whether maintenance demands vs. energy spilling is the primary mechanism that uncouples anabolism and catabolism. Energy spilling seems more plausible but how it will adapt to warming is unclear because rather than “waste metabolism,” it likely has many fitness benefits for microbes. Uncoupling in favor of catabolism vs. anabolism causes declines in MGEs, which then prevent warming-induced SOM losses in microbial models and exacerbate them in conventional SOM models. Despite these divergent responses, both model structures assume that microbes regulate SOM turnover, an idea that has recently been questioned. Microbial ecologists thus face two challenges to the explicit incorporation of microbes in ESMs. We need to show conclusively that microbial activity does regulate SOM dynamics, and that adjustments in microbial physiology under warming can be represented in a manner commensurate with observed responses of soil respiration, microbial biomass and SOM stocks.

ACKNOWLEDGMENTS

A grant (DEB-1021098) from the U.S. National Science Foundation supported this work and comments from the Editor, and four reviewers, improved the text.

REFERENCES

- A'Bear, A. D., Boddy, L., and Jones, T. H. (2012). Impacts of elevated temperature on the growth and functioning of decomposer fungi are influenced by grazing collembola. *Glob. Change Biol.* 18, 1823–1832. doi: 10.1111/j.1365-2486.2012.02637.x
- Adadi, R., Volkmer, B., Milo, R., Heinemann, M., and Shlomi, T. (2012). Prediction of microbial growth rate versus biomass yield by a metabolic network with kinetic parameters. *PLoS Comput. Biol.* 8:e1002575. doi: 10.1371/journal.pcbi.1002575 Epub 2012 Jul 5.
- Ågren, G. I. (2010). Microbial mitigation. *Nat. Geosci.* 3, 303–304. doi: 10.1038/ngeo857
- Ågren, G. I., and Bosatta, E. (1996). *Theoretical Ecosystem Ecology: Understanding Element Cycles*. Cambridge: Cambridge University Press.
- Ågren, G. I., and Bosatta, E. (2002). Reconciling differences in predictions of temperature response of soil organic matter. *Soil Biol. Biochem.* 34, 129–132. doi: 10.1016/S0038-0717(01)00156-0
- Allison, S. D., and Martiny, J. B. H. (2008). Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci. U.S.A.* 105, 115212–11519. doi: 10.1073/pnas.0801925105
- Allison, S. D., Wallenstein, M. D., and Bradford, M. A. (2010). Soil-carbon response to warming dependent on microbial physiology. *Nat. Geosci.* 3, 336–340. doi: 10.1038/ngeo846
- Allison, S. D., Weintraub, M. N., Gartner, T. B., and Waldrop, M. P. (2011). “Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function,” in *Soil Enzymology*, eds G. Shukla and A. Varma. (Heidelberg: Springer-Verlag), 229–243.
- Almagro, M., Lopez, J., Querejeta, J. I., and Martinez-Mena, M. (2009). Temperature dependence of soil CO₂ efflux is strongly modulated by seasonal patterns of moisture availability in a Mediterranean ecosystem. *Soil Biol. Biochem.* 41, 594–605. doi: 10.1016/j.soilbio.2008.12.021
- Angilletta, M. J. Jr. (2009). *Thermal Adaptation. A theoretical and Empirical Synthesis*. Oxford: Oxford University Press. doi: 10.1093/acprof:oso/9780198570875.001.1
- Apple, J. K., and del Giorgio, P. A. (2007). Organic substrate quality as the link between bacterioplankton carbon demand and growth efficiency in a temperate salt-marsh estuary. *ISME J.* 1, 729–742. doi: 10.1038/ismej.2007.86
- Apple, J. K., Del Giorgio, P. A., and Kemp, W. M. (2006). Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary. *Aquat. Microbial. Ecol.* 43, 243–254. doi: 10.3354/ame043243
- Atkin, O. K., and Tjoelker, M. G. (2003). Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends Plant Sci.* 8, 343–351. doi: 10.1016/S1360-1385(03)00136-5

- Balser, T. C., and Wixon, D. L. (2009). Investigating biological control over soil carbon temperature sensitivity. *Glob. Change Biol.* 15, 2935–2949. doi: 10.1111/j.1365-2486.2009.01946.x
- Bárcenas-Moreno, G., Gómez-Brandón, M., Rousk, J., and Bååth, E. (2009). Adaptation of soil microbial communities to temperature: comparison of fungi and bacteria in a laboratory experiment. *Glob. Change Biol.* 15, 2950–2957. doi: 10.1111/j.1365-2486.2009.01882.x
- Bardgett, R. D., Kandeler, E., Tschirko, D., Hobbs, P. J., Bezemer, T. M., Jones, T. H., et al. (1999). Below-ground microbial community development in a high temperature world. *Oikos* 85, 193–203. doi: 10.2307/3546486
- Bengtson, P., and Bengtsson, G. (2007). Rapid turnover of DOC in temperate forests accounts for increased CO₂ production at elevated temperatures. *Ecol. Lett.* 10, 783–790. doi: 10.1111/j.1461-0248.2007.01072.x
- Bonan, G. B., Hartman, M. D., Parton, W. J., and Wieder, W. R. (2013). Evaluating litter decomposition in earth system models with long-term litterbag experiments: an example using the Community Land Model version 4 (CLM4). *Glob. Change Biol.* 19, 957–974. doi: 10.1111/gcb.12031
- Boone, R. D., Nadelhoffer, K. J., Canary, J. D., and Kaye, J. P. (1998). Roots exert a strong influence on the temperature sensitivity of soil respiration. *Nature* 396, 570–572. doi: 10.1038/25119
- Bradford, M. A., Davies, C. A., Frey, S. D., Maddox, T. R., Melillo, J. M., Mohan, J. M., et al. (2008a). Thermal adaptation of soil microbial respiration to elevated temperature. *Ecol. Lett.* 11, 1316–1327. doi: 10.1111/j.1461-0248.2008.01251.x
- Bradford, M. A., and Fierer, N. (2012). “The biogeography of microbial communities and ecosystem processes: implications for soil and ecosystem models,” in *Soil Ecology and Ecosystem Services*, eds D. H. Wall, R. D. Bardgett, V. Behan-Pelletier, J. E. Herrick, H. Jones, K. Ritz, J. et al. (UK: Oxford University Press), 189–200.
- Bradford, M. A., Fierer, N., and Reynolds, J. F. (2008b). Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils *Funct. Ecol.* 22, 964–974. doi: 10.1111/j.1365-2435.2008.01404.x
- Bradford, M. A., Keiser, A. D., Davies, C. A., Mersmann, C. A., and Strickland, M. S. (2013). Empirical evidence that soil carbon formation from plant inputs is positively related to microbial growth. *Biogeochemistry* 113, 271–281. doi: 10.1007/s10533-012-9822-0
- Bradford, M. A., Wallenstein, M. D., Allison, S. D., Treseder, K. K., Frey, S. D., Watts, B. W., et al. (2009). Decreased mass specific respiration under experimental warming is robust to the microbial biomass method employed. *Ecol. Lett.* 12, E15–E18. doi: 10.1111/j.1461-0248.2009.01332.x
- Bradford, M. A., Watts, B. W., and Davies, C. A. (2010). Thermal adaptation of heterotrophic soil respiration in laboratory microcosms. *Glob. Change Biol.* 16, 1576–1588. doi: 10.1111/j.1365-2486.2009.02040.x
- Brant, J. B., Sulzman, E. W., and Myrold, D. D. (2006). Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. *Soil Biol. Biochem.* 38, 2219–2232. doi: 10.1016/j.soilbio.2006.01.022
- Brzostek, E. R., Blair, J. M., Dukes, J. S., Frey, S. D., Hobbie, S. E., Melillo, J. M., et al. (2012). The effect of experimental warming and precipitation change on proteolytic enzyme activity: positive feedbacks to nitrogen availability are not universal. *Glob. Change Biol.* 18, 2617–2625. doi: 10.1111/j.1365-2486.2012.02685.x
- Brzostek, E. R., and Finzi, A. C. (2011). Substrate supply, fine roots, and temperature control proteolytic enzyme activity in temperate forest soils. *Ecology* 92, 892–902. doi: 10.1890/10-1803.1
- Brzostek, E. R., and Finzi, A. C. (2012). Seasonal variation in the temperature sensitivity of proteolytic enzyme activity in temperate forest soils. *J. Geophys. Res. Biogeosci.* 117. doi: 10.1029/2011JG001688
- Bullock, T. H. (1955). Compensation for temperature in the metabolism and activity of poikilotherms. *Biol. Rev. Camb. Philos. Soc.* 30, 311–342. doi: 10.1111/j.1469-185X.1955.tb01211.x
- Cheng, W. X., Zhang, Q. L., Coleman, D. C., Carroll, C. R., and Hoffman, C. A. (1996). Is available carbon limiting microbial respiration in the rhizosphere? *Soil Biol. Biochem.* 28, 1283–1288. doi: 10.1016/S0038-0717(96)00138-1
- Clarholm, M. (1994). “Microbial loop in soil,” in *Beyond the Biomass: Compositional and Functional Analysis of Soil Microbial Communities*, eds K. Ritz, J. Dighton, and K. E. Giller (London: Wiley-Sayce), 221–230.
- Clarke, A. (2006). Temperature and the metabolic theory of ecology. *Funct. Ecol.* 20, 405–412. doi: 10.1111/j.1365-2435.2006.01109.x
- Clarke, A., and Fraser, K. P. P. (2004). Why does metabolism scale with temperature? *Funct. Ecol.* 18, 243–251. doi: 10.1111/j.0269-8463.2004.00841.x
- Clark, J. S. (2009). Beyond neutral science. *Trends Ecol. Evol.* 24, 8–15. doi: 10.1016/j.tree.2008.09.004
- Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., et al. (2013). Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science* 339, 1615–1618. doi: 10.1126/science.1231923
- Conant, R. T., Ryan, M. G., Ågren, G. I., Birge, H. E., Davidson, E. A., Eliasson, P. E., et al. (2011). Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. *Glob. Change Biol.* 17, 3392–3404. doi: 10.1111/j.1365-2486.2011.02496.x
- Cooper, V. S., Bennett, A. F., and Lenski, R. E. (2001). Evolution of thermal dependence of growth rate of *Escherichia coli* populations during 20,000 generations in a constant environment. *Evolution* 55, 889–896. doi: 10.1554/0014-3820(2001)055[0889:EOTDOG]2.0.CO;2
- Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., and Paul, E. (2013). The microbial efficiency-matrix stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Glob. Change Biol.* 19, 988–995. doi: 10.1111/gcb.12113
- Craine, J. M., Fierer, N., and Mclauchlan, K. K. (2010). Widespread coupling between the rate and temperature sensitivity of organic matter decay. *Nat. Geosci.* 3, 854–857. doi: 10.1038/ngeo1009
- Crowther, T. W., and Bradford, M. A. (2013). Thermal acclimation in widespread heterotrophic soil microbes. *Ecol. Lett.* 16, 469–477. doi: 10.1111/ele.12069
- Crowther, T. W., Littleboy, A., Jones, T. H., and Boddy, L. (2012). Interactive effects of warming and invertebrate grazing on the outcomes of competitive fungal interactions. *FEMS Microbiol. Ecol.* 81, 419–426. doi: 10.1111/j.1574-6941.2012.01364.x
- Curiel Yuste, J., Ma, S., and Baldocchi, D. D. (2010). Plant-soil interactions and acclimation to temperature of microbial-mediated soil respiration may affect predictions of soil CO₂ efflux. *Biogeochemistry* 98, 127–138. doi: 10.1007/s10533-009-9381-1
- Davidson, E. A., and Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440, 165–173. doi: 10.1038/nature04514
- Davidson, E. A., Samanta, S., Caramori, S. S., and Savage, K. (2012). The Dual Arrhenius and Michaelis-Menten kinetics model for decomposition of soil organic matter at hourly to seasonal time scales. *Glob. Change Biol.* 18, 371–384. doi: 10.1111/j.1365-2486.2011.02546.x
- del Giorgio, P. A., and Cole, J. J. (1998). Bacterial growth efficiency in natural aquatic ecosystems. *Annu. Rev. Ecol. Syst.* 29, 503–541. doi: 10.1146/annurev.ecolsys.29.1.503
- Denman, K. L., Brasseur, G., Chidthaisong, A., Ciais, P., Cox, P. M., Dickinson, R. E., et al. (2007). “Couplings between changes in the climate system and biogeochemistry,” in *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, eds S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, et al. (Cambridge: Cambridge University Press), 499–587.
- Devèvre, O. C., and Horwath, W. R. (2000). Decomposition of rice straw and microbial carbon use efficiency under different soil temperatures and moistures. *Soil Biol. Biochem.* 32, 1773–1785. doi: 10.1016/S0038-0717(00)00096-1
- Dijkstra, P., Dalder, J. J., Selman, P. C., Hart, S. C., Koch, G. W., Schwartz, E., et al. (2011). Modeling soil metabolic processes using isotopologue pairs of position-specific C-13-labeled glucose and pyruvate. *Soil Biol. Biochem.* 43, 1848–1857. doi: 10.1016/j.soilbio.2011.05.001
- Dorrepaal, E., Toet, S., Van Logtestijn, R. S. P., Swart, E., Van De Weg, M. J., Callaghan, T. V., et al. (2013). Carbon respiration from subsurface peat accelerated by climate warming in the subarctic. *Nature* 460, 616–619. doi: 10.1038/nature08216
- Echtay, K. S. (2007). Mitochondrial uncoupling proteins – What is their physiological role? *Free Radic. Biol. Med.* 43, 1351–1371. doi: 10.1016/j.freeradbiomed.2007.08.011
- Eliasson, P. E., Mcmurtrie, R. E., Pepper, D. A., Strömberg, M., Linder, S., and Ågren, G. I. (2005). The response of heterotrophic CO₂ flux to soil warming. *Glob. Change Biol.* 11, 167–181. doi: 10.1111/j.1365-2486.2004.00878.x
- Falkowski, P., Scholes, R. J., Boyle, E., Canadell, J., Canfield, D., Elser, J., et al. (2000). The global carbon cycle: a test of our knowledge of earth as a system. *Science* 290, 291–296. doi: 10.1126/science.290.5490.291
- Fenner, N., Freeman, C., and Reynolds, B. (2005). Observations of a seasonally shifting thermal optimum in peatland carbon-cycling processes; implications for

- the global carbon cycle and soil enzyme methodologies. *Soil Biol. Biochem.* 37, 1814–1821. doi: 10.1016/j.soilbio.2005.02.032
- Fierer, N., Craine, J. M., McLaughlin, K., and Schimel, J. P. (2005). Litter quality and the temperature sensitivity of decomposition. *Ecology* 86, 320–326. doi: 10.1890/04-1254
- Fontaine, S., and Barot, S. (2005). Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. *Ecol. Lett.* 8, 1075–1087. doi: 10.1111/j.1461-0248.2005.00813.x
- Frey, S. D., Drijber, R., Smith, H., and Melillo, J. (2008). Microbial biomass, functional capacity, and community structure after 12 years of soil warming. *Soil Biol. Biochem.* 40, 2904–2907. doi: 10.1016/j.soilbio.2008.07.020
- Frey, S. D., Lee, J., Melillo, J. M., and Six, J. (2013). The temperature response of soil microbial efficiency and its feedback to climate. *Nat. Clim. Change* 3, 395–398. doi: 10.1038/nclimate1796
- García-Pichel, F., Loza, V., Marusenko, Y., Mateo, P., and Potrafka, R. M. (2013). Temperature drives the continental-scale distribution of key microbes in topsoil communities. *Science* 340, 1574–1577. doi: 10.1126/science.1236404
- German, D. P., Marcelo, K. R. B., Stone, M. M., and Allison, S. D. (2012). The Michaelis-Menten kinetics of soil extracellular enzymes in response to temperature: a cross-latitudinal study. *Glob. Change Biol.* 18, 1468–1479. doi: 10.1111/j.1365-2486.2011.02615.x
- Gershenson, A., Bader, N. E., and Cheng, W. (2009). Effects of substrate availability on the temperature sensitivity of soil organic matter decomposition. *Glob. Change Biol.* 15, 176–183. doi: 10.1111/j.1365-2486.2008.01827.x
- Goldfarb, K. C., Karaoz, U., Hanson, C. A., Santee, C. A., Bradford, M. A., Treseder, K. K., et al. (2011). Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. *Front. Microbiol.* 2:94. doi: 10.3389/fmicb.2011.00094
- Grandy, A. S., and Neff, J. C. (2008). Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. *Sci. Total Environ.* 404, 297–307. doi: 10.1016/j.scitotenv.2007.11.013
- Gu, L. H., Post, W. M., and King, A. W. (2004). Fast labile carbon turnover obscures sensitivity of heterotrophic respiration from soil to temperature: a model analysis. *Global Biogeochem. Cycles* 18, GB1022. doi: 10.1029/2003GB002119
- Hackmann, T. J., Diese, L., and Firkins, J. L. (2013). Quantifying the responses of mixed rumen microbes to excess carbohydrate. *Appl. Environ. Microbiol.* 79, 3786–3795. doi: 10.1128/AEM.00482-413. Epub 2013 Apr 12.
- Hall, E. K., and Cotner, J. B. (2007). Interactive effect of temperature and resources on carbon cycling by freshwater bacterioplankton communities. *Aquat. Microb. Ecol.* 49, 35–45. doi: 10.3354/ame01124
- Hall, E. K., Nehauser, C., and Cotner, J. B. (2008). Toward a mechanistic understanding of how natural bacterial communities respond to changes in temperature in aquatic ecosystems. *ISME J.* 2, 471–481. doi: 10.1038/ismej.2008.9
- Hall, E. K., Singer, G. A., Kainz, M. J., and Lennon, J. T. (2010). Evidence for a temperature acclimation mechanism in bacteria: an empirical test of a membrane-mediated trade-off. *Funct. Ecol.* 24, 898–908. doi: 10.1111/j.1365-2435.2010.01707.x
- Hamdi, S., Moyano, F., Sall, S., Bernoux, M., and Chevallier, T. (2013). Synthesis analysis of the temperature sensitivity of soil respiration from laboratory studies in relation to incubation methods and soil conditions. *Soil Biol. Biochem.* 58, 115–126. doi: 10.1016/j.soilbio.2012.11.012
- Hanson, C. A., Allison, S. D., Bradford, M. A., Wallenstein, M. D., and Treseder, K. K. (2008). Fungal taxa target different carbon sources in forest soil. *Ecosystems* 11, 1157–1167. doi: 10.1007/s10021-008-9186-4
- Hartley, I. P., Heinemeyer, A., and Ineson, P. (2007). Effects of three years of soil warming and shading on the rate of soil respiration: substrate availability and not thermal acclimation mediates observed response. *Glob. Change Biol.* 13, 1761–1770. doi: 10.1111/j.1365-2486.2007.01373.x
- Hartley, I. P., Hopkins, D. W., Garnett, M. H., Sommerkorn, M., and Wookey, P. A. (2008). Soil microbial respiration in arctic soil does not acclimate to temperature. *Ecol. Lett.* 11, 1092–1100. doi: 10.1111/j.1461-0248.2008.01223.x
- Hartley, I. P., Hopkins, D. W., Garnett, M. H., Sommerkorn, M., and Wookey, P. A. (2009). No evidence for compensatory thermal adaptation of soil microbial respiration in the study of Bradford et al. (2008). *Ecol. Lett.* 12, E12–E14. doi: 10.1111/j.1461-0248.2009.01300.x
- Hazel, J. R. (1995). Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? *Annu. Rev. Physiol.* 57, 19–42. doi: 10.1146/annurev.ph.57.030195.000315
- Hazel, J. R., and Williams, E. E. (1990). The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Prog. Lipid Res.* 29, 167–227. doi: 10.1016/0163-7827(90)90002-3
- Heinemeyer, A., Ineson, P., Ostle, N., and Fitter, A. H. (2006). Respiration of the external mycelium in the arbuscular mycorrhizal symbiosis shows strong dependence on recent photosynthates and acclimation to temperature. *New Phytol.* 171, 159–170. doi: 10.1111/j.1469-8137.2006.01730.x
- Hochachka, P. W., and Somero, G. N. (2002). *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. New York: Oxford University Press, Inc.
- Ise, T., Litton, C. M., Giardina, C. P., and Ito, A. (2010). Comparison of modeling approaches for carbon partitioning: impact on estimates of global net primary production and equilibrium biomass of woody vegetation from MODIS GPP. *J. Geophys. Res.* 115, G040205. doi: 10.1029/2010JG001326
- Jobbágy, E. G., and Jackson, R. B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol. Appl.* 10, 423–436. doi: 10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2
- Keiser, A. D., Strickland, M. S., Fierer, N., and Bradford, M. A. (2011). The effect of resource history on the functioning of soil microbial communities is maintained across time. *Biogeosciences* 8, 1477–1486. doi: 10.5194/bg-8-1477-2011
- Kemmitt, S. J., Lanyon, C. V., Waite, I. S., Wen, Q., Addiscott, T. M., Bird, N. R. A., et al. (2008). Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass – a new perspective. *Soil Biol. Biochem.* 40, 61–73. doi: 10.1016/j.soilbio.2007.06.021
- Kirschbaum, M. U. F. (2004). Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? *Glob. Change Biol.* 10, 1870–1877. doi: 10.1111/j.1365-2486.2004.00852.x
- Kirschbaum, M. U. F. (2006). The temperature dependence of organic-matter decomposition – still a topic of debate. *Soil Biol. Biochem.* 38, 2510–2518. doi: 10.1016/j.soilbio.2006.01.030
- Knorr, W., Prentice, I. C., House, J. I., and Holland, E. A. (2005). Long-term sensitivity of soil carbon turnover to warming. *Nature* 433, 298–301. doi: 10.1038/nature03226
- Kuzyakov, Y. (2010). Priming effects: interactions between living and dead organic matter. *Soil Biol. Biochem.* 42, 1363–1371. doi: 10.1016/j.soilbio.2010.04.003
- Kuzyakov, Y., Blagodatskaya, E., and Blagodatsky, S. (2009). Comments on the paper by Kemmitt et al. (2008) ‘Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass – a new perspective’ [Soil Biol. Biochem. 40, 61–73]: the biology of the regulatory gate. *Soil Biol. Biochem.* 41, 435–439. doi: 10.1016/j.soilbio.2008.07.023
- Lal, R. (2004). Soil carbon sequestration impacts on global climate change and food security. *Science* 304, 1623–1627. doi: 10.1126/science.1097396
- Lange, O. L., and Green, T. G. A. (2005). Lichens show that fungi can acclimate their respiration to seasonal changes in temperature. *Oecologia* 142, 11–19. doi: 10.1007/s00442-004-1697-x
- Lawrence, C. L., Neff, J. C., and Schimel, J. S. (2009). Does adding microbial mechanisms of decomposition improve soil organic matter models? A comparison of four models using data from a pulsed rewetting experiment. *Soil Biol. Biochem.* 41, 1923–1934. doi: 10.1016/j.soilbio.2009.06.016
- Lennon, J. T., and Jones, S. E. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nat. Rev. Microbiol.* 9, 119–130. doi: 10.1038/nrmicro2504
- Leroi, A. M., Bennett, A. F., and Lenski, R. E. (1994). Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption. *Proc. of the Natl. Acad. of Sci. U.S.A.* 91, 1917–1921. doi: 10.1073/pnas.91.5.1917
- López-Urrutia, A., and Morán, X. A. (2007). Resource limitation of bacterial production distorts the temperature dependence of oceanic carbon cycling. *Ecology* 88, 817–822. doi: 10.1890/06-1641
- Lundberg, P., Ekblad, A., and Nilsson, M. (2001). ¹³C NMR spectroscopy studies of forest soil microbial activity: glucose uptake and fatty acid biosynthesis. *Soil Biol. Biochem.* 33, 621–632. doi: 10.1016/S0038-0717(00)00206-6
- Luo, Y. (2007). Terrestrial carbon-cycle feedback to climate warming. *Annu. Rev. Ecol. Evol. Syst.* 38, 683–712. doi: 10.1146/annurev.ecolsys.38.091206.095808
- Luo, Y., Wan, S., Hui, D., and Wallace, L. L. (2001). Acclimatization of soil respiration to warming in a tall grass prairie. *Nature* 413, 622–625. doi: 10.1038/35098065

- Mahecha, M., Reichstein, M., Carvalhais, N., Lasslop, G., Lange, H., Seneviratne, S. I., et al. (2010). Global convergence in the temperature sensitivity of respiration at ecosystem level. *Science* 329, 838–840. doi: 10.1126/science.1189587
- Malcolm, G. M., López-Gutiérrez, J. C., Koide, R. T., and Eissenstat, D. M. (2008). Acclimation to temperature and temperature sensitivity of metabolism by ectomycorrhizal fungi. *Glob. Change Biol.* 14, 1169–1180. doi: 10.1111/j.1365-2486.2008.01555.x
- Manzoni, S., Schimel, J. P., and Porporato, A. (2012a). Responses of soil microbial communities to water stress: results from a meta-analysis. *Ecology* 93, 930–938. doi: 10.1890/11-0026.1
- Manzoni, S., Taylor, P., Richter, A., Porporato, A., and Ågren, G. I. (2012b). Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytol.* 196, 79–91. doi: 10.1111/j.1469-8137.2012.04225.x
- Melillo, J. M., Butler, S., Johnson, J., Mohan, J., Steudler, P., Lux, H., et al. (2011). Soil warming, carbon-nitrogen interactions, and forest carbon budgets. *Proc. Natl. Acad. Sci. U.S.A.* 108, 9508–9512. doi: 10.1073/pnas.1018189108
- Melillo, J. M., Steudler, P. A., Aber, J. D., Newkirk, K., Lux, H., Bowles, F. P., et al. (2002). Soil warming and carbon-cycle feedbacks to the climate system. *Science* 298, 2173–2176. doi: 10.1126/science.1074153
- Miltner, A., Bombach, P., Schmidt-Brücken, B., and Kästner, M. (2012). SOM genesis: microbial biomass as a significant source. *Biogeochemistry* 111, 41–55. doi: 10.1007/s10533-011-9658-z
- National_Research_Council. (2009). *A New Biology for the 21st Century*. Washington, D.C.: National Academies Press.
- Ng, H. (1969). Effect of decreasing growth temperature on cell yield of *Escherichia coli*. *J. Bacteriol.* 98, 232–237.
- Nie, M., Pendall, E., Bell, C., Gasch, C. K., Raut, S., Tamang, S., et al. (2013). Positive climate feedbacks of soil microbial communities in a semi-arid grassland. *Ecol. Lett.* 16, 234–241. doi: 10.1111/ele.12034
- Niu, S., Luo, Y., Fei, S., Yuan, W., Schimel, D., Law, B. E., et al. (2012). Thermal optimality of net ecosystem exchange of carbon dioxide and underlying mechanisms. *New Phytol.* 194, 775–783. doi: 10.1111/j.1469-8137.2012.04095.x
- Oechel, W. C., Vourilits, G. L., Hastings, S. J., Zulueta, R. C., Hinzman, L., and Kane, D. (2000). Acclimation of ecosystem CO₂ exchange in the Alaskan Arctic in response to decadal climate warming. *Nature* 406, 978–981. doi: 10.1038/35023137
- Ostle, N. J., Smith, P., Fisher, R., Woodward, F. I., Fisher, J. B., Smith, J. U., et al. (2009). Integrating plant–soil interactions into global carbon cycle models. *J. Ecol.* 97, 851–863. doi: 10.1111/j.1365-2745.2009.01547.x
- Parton, W. J., Stewart, J. W. B., and Cole, C. V. (1988). Dynamics of C, N, P and S in grassland soils – a model. *Biogeochemistry* 5, 109–131. doi: 10.1007/BF02180320
- Paterson, E. (2009). Comments on the regulatory gate hypothesis and implications for C-cycling in soil. *Soil Biol. Biochem.* 41, 1352–1354. doi: 10.1016/j.soilbio.2009.02.012
- Pietikäinen, J. M., Pettersson, M., and Bååth, E. (2005). Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. *FEMS Microbiol. Ecol.* 52, 49–58. doi: 10.1016/j.femsec.2004.10.002
- Pirt, S. J. (1965). Maintenance energy of bacteria in growing cultures. *Proc. R. Soc. B Biol. Sci.* 163, 224–231. doi: 10.1098/rspb.1965.0069
- Porankiewicz, J., Schelin, J., and Clarke, A. K. (1998). The ATP-dependent Clp protease is essential for acclimation to UV-B and low temperature in the cyanobacterium *Synechococcus*. *Mol. Microbiol.* 29, 275–283. doi: 10.1046/j.1365-2958.1998.00928.x
- Pregitzer, K. S., King, J. A., Burton, A. J., and Brown, S. E. (2000). Responses of tree fine roots to temperature. *New Phytol.* 147, 105–115. doi: 10.1046/j.1469-8137.2000.00689.x
- Rannekleiv, S. B., and Bååth, E. (2001). Temperature-driven adaptation of the bacterial community in peat measured by using thymidine and leucine incorporation. *Appl. Environ. Microbiol.* 67, 1116–1122. doi: 10.1128/AEM.67.3.1116-1122.2001
- Reich, P. B. (2010). The carbon dioxide exchange. *Science* 329, 774–775. doi: 10.1126/science.1194353
- Rinnan, R., Michelsen, A., and Jonasson, S. (2008). Effects of litter addition and warming on soil carbon, nutrient pools and microbial communities in a subarctic heath ecosystem. *Appl. Soil Ecol.* 39, 271–281. doi: 10.1016/j.apsoil.2007.12.014
- Rinnan, R., Rousk, J., Yergeau, E., Kowalchuk, G. A., and Bååth, E. (2009). Temperature adaptation of soil bacterial communities along an Antarctic climate gradient: predicting responses to climate warming. *Glob. Change Biol.* 15, 2615–2625. doi: 10.1111/j.1365-2486.2009.01959.x
- Rivkin, R. B., and Legendre, L. (2001). Biogenic carbon cycling in the upper ocean: effects of microbial respiration. *Science* 291, 2398–2400. doi: 10.1126/science.291.5512.2398
- Rousk, J., and Bååth, E. (2011). Growth of saprotrophic fungi and bacteria in soil. *FEMS Microbiol. Ecol.* 78, 17–30. doi: 10.1111/j.1574-6941.2011.01106.x
- Rousk, J., Frey, S. D., and Bååth, E. (2012). Temperature adaptation of bacterial communities in experimentally warmed forest soils. *Glob. Change Biol.* 18, 3252–3258. doi: 10.1111/j.1365-2486.2012.02764.x
- Russell, J. B. (2007). The energy spilling reactions of bacteria and other organisms. *J. Mol. Microbiol. Biotechnol.* 13, 1–11. doi: 10.1159/000103591
- Russell, J. B., and Cook, G. M. (1995). Energetics of bacterial growth: balance of anabolic and catabolic reactions. *Microbiol. Rev.* 59, 48–62.
- Rustad, L., Campbell, J. L., Marion, G. M., Norby, R. J., Mitchell, M. J., Hartley, A. E., et al. (2001). A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* 126, 543–562. doi: 10.1007/s004420000544
- Schimel, J. P. (2001). “Biogeochemical models: implicit vs. explicit microbiology,” in *Global Biogeochemical Cycles in the Climate System*, eds E. D. Schulze, S. P. Harrison, M. Heimann, E. A. Holland, J. J. Lloyd, I. C. Prentice et al. (San Diego: Academic Press), 177–183. doi: 10.1016/B978-012631260-7/50015-7
- Schimel, J. P., and Schaeffer, S. M. (2012). Microbial control over carbon cycling in soil. *Front. Microbiol.* 3:348. doi: 10.3389/fmicb.2012.00348
- Schimel, J. P., and Weintraub, M. N. (2003). The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biol. Biochem.* 35, 549–563. doi: 10.1016/S0038-0717(03)00015-4
- Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., et al. (2011). Persistence of soil organic matter as an ecosystem property. *Nature* 478, 49–56. doi: 10.1038/nature10386
- Sistla, S. A., Moore, J. C., Simpson, R. T., Gough, L., Shaver, G. R., and Schimel, J. P. (2013). Long-term warming restructures Arctic tundra without changing net soil carbon storage. *Nature* 497, 615–618. doi: 10.1038/nature12129
- Smith, N. G., and Dukes, J. S. (2013). Plant respiration and photosynthesis in global-scale models: incorporating acclimation to temperature and CO₂. *Glob. Change Biol.* 19, 45–63. doi: 10.1111/j.1365-2486.2012.02797.x
- Steffen, W., Noble, I., Canadell, J., Apps, M., Schulze, E. D., Jarvis, P. G., et al. (1998). The terrestrial carbon cycle: implications for the Kyoto Protocol. *Science* 280, 1393–1394. doi: 10.1126/science.280.5368.1393
- Steinweg, J. M., Plante, A. F., Conant, R. T., Paul, E. A., and Tanaka, D. L. (2008). Patterns of substrate utilization during long-term incubations at different temperatures. *Soil Biol. Biochem.* 40, 2722–2728. doi: 10.1016/j.soilbio.2008.07.002
- Stone, M. M., Weiss, M. S., Goodale, C. L., Adams, M. B., Fernandez, I. J., German, D. P., et al. (2012). Temperature sensitivity of soil enzyme kinetics under N-fertilization in two temperate forests. *Glob. Change Biol.* 18, 1173–1184. doi: 10.1111/j.1365-2486.2011.02545.x
- Strickland, M. S., Lauber, C., Fierer, N., and Bradford, M. A. (2009). Testing the functional significance of microbial community composition. *Ecology* 90, 441–451. doi: 10.1890/08-0296.1
- Strickland, M. S., and Rousk, J. (2010). Considering fungal:bacterial dominance in soils – methods, controls, and ecosystem implications. *Soil Biol. Biochem.* 42, 1385–1395. doi: 10.1016/j.soilbio.2010.05.007
- Subke, J.-A., and Bahn, M. (2010). On the ‘temperature sensitivity’ of soil respiration: can we use the immeasurable to predict the unknown? *Soil Biol. Biochem.* 42, 1653–1656. doi: 10.1016/j.soilbio.2010.05.026
- Suseela, V., Conant, R. T., Wallenstein, M. D., and Dukes, J. S. (2012). Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment. *Glob. Change Biol.* 18, 336–348. doi: 10.1111/j.1365-2486.2011.02516.x
- Tabata, K., Hida, F., Kiriya, T., Ishizaki, N., Kamachi, T., and Okura, I. (2013). Measurement of soil bacterial colony temperatures and isolation of a high heat-producing bacterium. *BMC Microbiol.* 13:56. doi: 10.1186/1471-2180-13-56
- Thiessen, S., Gleixner, G., Wutzler, T., and Reichstein, M. (2013). Both priming and temperature sensitivity of soil organic matter decomposition depend on microbial biomass – an incubation study. *Soil Biol. Biochem.* 57, 739–748. doi: 10.1016/j.soilbio.2012.10.029
- Thiet, R. K., Frey, S. D., and Six, J. (2006). Do growth yield efficiencies differ between soil microbial communities differing in fungal:bacterial ratios?

- Reality check and methodological issues. *Soil Biol. Biochem.* 38, 837–844. doi: 10.1016/j.soilbio.2005.07.010
- Tjoelker, M. G., Oleksyn, J., Reich, P. B., and Zytzkowiak, R. (2008). Coupling of respiration, nitrogen, and sugars underlies convergent temperature acclimation in *Pinus banksiana* across wide-ranging sites and populations. *Glob. Change Biol.* 14, 782–797. doi: 10.1111/j.1365-2486.2008.01548.x
- Todd-Brown, K. E. O., Hopkins, F. M., Kivlin, S. N., Talbot, J. M., and Allison, S. D. (2012). A framework for representing microbial decomposition in coupled climate models. *Biogeochemistry* 109, 19–33. doi: 10.1007/s10533-011-9635-6
- Todd-Brown, K. E. O., Randerson, J. T., Post, W. M., Hoffman, F. M., Tarnocai, C., Schuur, E. A. G., et al. (2013). Causes of variation in soil carbon simulations from CMIP5 Earth system models and comparison with observations. *Biogeosciences* 10, 1717–1736. doi: 10.5194/bg-10-1717-2013
- Treseder, K. K., Balser, T. C., Bradford, M. A., Brodie, E. L., Dubinsky, E. A., Eviner, V. T., et al. (2012). Integrating microbial ecology into ecosystem models: challenges and priorities. *Biogeochemistry* 109, 7–18. doi: 10.1007/s10533-011-9636-5
- Tucker, C. L., Bell, J., Pendall, E., and Ogle, K. (2013). Does declining carbon-use efficiency explain thermal acclimation of soil respiration with warming? *Glob. Change Biol.* 19, 252–263. doi: 10.1111/gcb.12036
- Turner, P. E., Souza, V., and Lenski, R. E. (1996). Tests of ecological mechanisms promoting the stable coexistence of two bacterial genotypes. *Ecology* 77, 2119–2129. doi: 10.2307/2265706
- Uselman, S. M., Qualls, R. G., and Thomas, R. B. (2000). Effects of increased atmospheric CO₂, temperature, and soil N availability on root exudation of dissolved organic carbon by a N-fixing tree (*Robinia pseudoacacia* L.). *Plant Soil* 222, 191–202. doi: 10.1023/A:1004705416108
- Van Dooremalen, C., Berg, M. P., and Ellers, J. (2013). Acclimation responses to temperature vary with vertical stratification: implications for vulnerability of soil-dwelling species to extreme temperature events. *Glob. Change Biol.* 19, 975–984. doi: 10.1111/gcb.12081
- van Ginkel, J. H., Gorissen, A., and Polci, D. (2000). Elevated atmospheric carbon dioxide concentration: effects of increased carbon input in a *Lolium perenne* soil on microorganisms and decomposition. *Soil Biol. Biochem.* 32, 449–456. doi: 10.1016/S0038-0717(99)00097-8
- Vicca, S., Fizez, L., Kockelbergh, F., Van Pelt, D., Segers, J. J. R., Meire, P., et al. (2009). No signs of thermal acclimation of heterotrophic respiration from peat soils exposed to different water levels. *Soil Biol. Biochem.* 41, 2014–2016. doi: 10.1016/j.soilbio.2009.07.007
- Waldrop, M. P., Balser, T. C., and Firestone, M. K. (2000). Linking microbial community composition to function in a tropical soil. *Soil Biol. Biochem.* 32, 1837–1846. doi: 10.1016/S0038-0717(00)00157-7
- Wallenstein, M. D., Haddix, M. L., Lee, D. D., Conant, R. T., and Paul, E. A. (2012). A litter-slurry technique elucidates the key role of enzyme production and microbial dynamics in temperature sensitivity of organic matter decomposition. *Soil Biol. Biochem.* 47, 18–26. doi: 10.1016/j.soilbio.2011.12.009
- Wallenstein, M. D., and Hall, E. K. (2012). A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. *Biogeochemistry* 109, 35–47. doi: 10.1007/s10533-011-9641-8
- Wallenstein, M. D., Hess, A. M., Lewis, M. R., Steltzer, H., and Ayres, E. (2010). Decomposition of aspen leaf litter results in unique metabolomes when decomposed under different tree species. *Soil Biol. Biochem.* 42, 484–490. doi: 10.1016/j.soilbio.2009.12.001
- Wallenstein, M. D., McMahon, S. K., and Schimel, J. P. (2009). Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. *Glob. Change Biol.* 15, 1631–1639. doi: 10.1111/j.1365-2486.2008.01819.x
- Wardle, D. A., and Ghani, A. (1995). Why is the strength of relationships between pairs of methods for estimating soil microbial biomass often so variable? *Soil Biol. Biochem.* 27, 821–828. doi: 10.1016/0038-0717(94)00229-T
- Warren, R. J. II, Bahn, V., and Bradford, M. A. (2011a). Temperature cues phenological synchrony in ant-mediated seed dispersal. *Glob. Change Biol.* 17, 2444–2454. doi: 10.1111/j.1365-2486.2010.02386.x
- Warren, R. J. II, Skelly, D. K., Schmitz, O. J., and Bradford, M. A. (2011b). Universal ecological patterns in college basketball communities. *PLoS ONE* 6:e17342. doi: 10.1371/journal.pone.0017342
- Weedon, J. T., Cornwell, W. K., Cornelissen, J. H. C., Zanne, A. E., Wirth, C., and Coomes, D. A. (2009). Global meta-analysis of wood decomposition rates: a role for trait variation among tree species? *Ecol. Lett.* 12, 45–56. doi: 10.1111/j.1461-0248.2008.01259.x
- Wu, J.-B., Guan, D.-X., Han, S.-J., and Jin, C.-J. (2005). Ecological functions of coarse woody debris in forest ecosystem. *J. For. Res.* 16, 247–252. doi: 10.1007/BF02856826
- Xu, N., and Saiers, J. (2010). Temperature and hydrologic controls on dissolved organic matter mobilization and transport within a forest topsoil. *Environ. Sci. Technol.* 44, 5423–5429. doi: 10.1021/es1002296
- Yin, H., Li, Y., Xiao, J., Xu, Z., Cheng, X., and Liu, Q. (2013). Enhanced root exudation stimulates soil nitrogen transformations in a subalpine coniferous forest under experimental warming. *Glob. Change Biol.* 19, 2158–2167. doi: 10.1111/gcb.12161
- Yvon-Durocher, G., Jones, J. I., Trimmer, M., Woodward, G., and Montoya, J. M. (2010). Warming alters the metabolic balance of ecosystems. *Philos. Trans. R. Soc. B Biol. Sci.* 365, 2117–2126. doi: 10.1098/rstb.2010.0038
- Zhou, J., Xue, K., Xie, J., Deng, Y., Wu, L., Cheng, X., et al. (2012). Microbial mediation of carbon-cycle feedbacks to climate warming. *Nat. Clim. Change* 2, 106–110. doi: 10.1038/nclimate1331
- Zogg, G. P., Zak, D. R., Ringelberg, D. B., Macdonald, N. W., Pregitzer, K. S., and White, D. C. (1997). Compositional and functional shifts in microbial communities due to soil warming. *Soil Sci. Soc. Am. J.* 61, 475–481. doi: 10.2136/sssaj1997.03615995006100020015x

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 14 May 2013; accepted: 21 October 2013; published online: 12 November 2013.

Citation: Bradford MA (2013) Thermal adaptation of decomposer communities in warming soils. *Front. Microbiol.* 4:333. doi: 10.3389/fmicb.2013.00333

This article was submitted to *Terrestrial Microbiology*, a section of the journal *Frontiers in Microbiology*.

Copyright © 2013 Bradford. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Controls on soil microbial community stability under climate change

Franciska T. de Vries^{1*} and Ashley Shade²

¹ Faculty of Life Sciences, The University of Manchester, Manchester, UK

² Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT, USA

Edited by:

Johannes Rousk, Lund University, Sweden

Reviewed by:

Romain L. Barnard, Institut National de la Recherche Agronomique, France

Jennifer Talbot, Stanford University, USA

*Correspondence:

Franciska T. De Vries, Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester, M13 9PT, UK
e-mail: franciska.devries@manchester.ac.uk

Soil microbial communities are intricately linked to ecosystem functioning because they play important roles in carbon and nitrogen cycling. Still, we know little about how soil microbial communities will be affected by disturbances expected with climate change. This is a significant gap in understanding, as the stability of microbial communities, defined as a community's ability to resist and recover from disturbances, likely has consequences for ecosystem function. Here, we propose a framework for predicting a community's response to climate change, based on specific functional traits present in the community, the relative dominance of r- and K-strategists, and the soil environment. We hypothesize that the relative abundance of r- and K-strategists will inform about a community's resistance and resilience to climate change associated disturbances. We also propose that other factors specific to soils, such as moisture content and the presence of plants, may enhance a community's resilience. For example, recent evidence suggests microbial grazers, resource availability, and plant roots each impact on microbial community stability. We explore these hypotheses by offering three vignettes of published data that we re-analyzed. Our results show that community measures of the relative abundance of r- and K-strategists, as well as environmental properties like resource availability and the abundance and diversity of higher trophic levels, can contribute to explaining the response of microbial community composition to climate change-related disturbances. However, further investigation and experimental validation is necessary to directly test these hypotheses across a wide range of soil ecosystems.

Keywords: disturbance, drought, fungi, bacteria, PLFA, pyrosequencing, resistance, resilience

INTRODUCTION

Soil microbial communities are intricately linked to ecosystem functioning because they play important roles in carbon (C) and nitrogen (N) cycling, and feed back to plant communities as mutualists and pathogens (Van der Heijden et al., 2008). Although much research has been done to study the impacts of a range of disturbances on soil microbial communities and their functioning (Griffiths and Philippot, 2013), many uncertainties remain about the controls on soil microbial community stability (**Box 1**), and the consequences of disturbance-induced changes in microbial communities for their capacity to withstand further disturbances. This may be in part because most studies measured the stability of bulk microbial properties, such as biomass and respiration, rather than of community structure (the number of different taxa and their relative abundances; **Box 1**). However, changes in the abundances or relative contributions of community members may have implications for the stability of a microbial community, and these kinds of membership changes may not be apparent when measuring bulk microbial properties. In addition, soils are unique and highly heterogeneous environments, and controls on microbial community stability in soil might differ from other systems. We argue that knowledge

on what controls soil microbial community stability is pivotal for predicting the impacts of climate change on soil microbial communities and the processes that they drive.

Here, drawing from findings from both terrestrial and aquatic systems, we formulate hypotheses on the controls of resistance and resilience of microbial communities in soil, focusing on disturbances associated with climate change (**Box 1**). Climate change is expected to result in increased frequency of drought and heavy rainfall, increases in temperature, and increased litter inputs and plant root exudates through elevated concentrations of atmospheric CO₂, which all have significant impacts on soil microbial community structure and functioning (Bardgett et al., 2013). Here, we focus on pulse disturbances associated with climate change, such as drought, increased rainfall, and increased litter inputs, because the clear start and end point of these disturbances allows for assessing both resistance and resilience of microbial community composition (**Box 1**). We use three case studies in which we re-analyze published data on the impact of these disturbances on microbial communities to further develop our proposed hypotheses. Finally, we synthesize our findings, and recommend ways of testing our hypotheses about controls of soil microbial community stability.

Box 1 | Glossary

Microbial community composition: the assortment of microbial taxa that comprises a community (Hunter, 1990).

Microbial community structure: the membership and (relative) abundances of microbial taxa in a community (Anderson et al., 2011).

Trait: phenotypic characteristic or attribute of an individual microbe that is affected by genotype and the environment (Campbell and Reece, 2006).

Functional trait: trait with a direct functional role that defines a microbe in terms of its ecological role, i.e., its interaction with other microbes and its environment (Lavorel and Garnier, 2002; Wallenstein and Hall, 2012).

Disturbance: causal event that alters a community directly or indirectly, typically through its effect on the community's environment (Rykiel, 1985; Glasby and Underwood, 1996).

Pulse disturbance: relatively discrete (with a clear start and end point), short-term events with a clear start and end point (Lake, 2000).

Press disturbance: long term event or continuous change (Lake, 2000).

Climate change: statistically significant variation in the mean state of the climate or its variability, caused either by natural internal processes or external forcing, or by persistent anthropogenic-induced changes in the composition of the atmosphere or land use (IPCC, 2007). Here, we focus on disturbances associated with climate change that are relevant to soil communities and processes, namely elevated atmospheric CO₂ and its indirect effects (increased soil C inputs through roots, root exudates, and increased litter fall), extreme weather events (drought and heavy rainfall), and warming.

Global change: changes in the global environment that may alter the capacity of the Earth to sustain life (Schlesinger, 2006), including both land-use and climate change. Here, we focus on global change disturbances such as land use change and N deposition rather than on climate change disturbances.

Stability: the tendency of a community to return to a mean condition after a disturbance (Pimm, 1984); includes the components of resistance and resilience (see also Worm and Duffy, 2003; Shade et al., 2012a).

Resistance: the ability of a community property or process to remain unchanged in the face of a specific disturbance (Pimm, 1984; Allison and Martiny, 2008).

Resilience: the ability of a community property or process to recover after a specific disturbance, often reported as a rate of return (Allison and Martiny, 2008).

Adaptation: the process through which a microbe increases its fitness in a particular environment (Wallenstein and Hall, 2012), i.e., optimization of traits that increase fitness.

Evolutionary adaptation: changes in the relative abundance of gene frequencies in a gene pool to optimize traits that increase fitness as a result of changes in environmental conditions (Campbell and Reece, 2006; Orsini et al., 2013).

for relationships between microbial communities and stability (resistance or resilience under disturbance) comes from aquatic microcosm studies (e.g., Wertz et al., 2007; Wittebolle et al., 2009; Eisenhauer et al., 2012). The majority of these studies have focused on the stability of processes or bulk microbial properties (e.g., biomass or functioning) under disturbance, rather than the stability of community structure itself. Disturbance influences microbial community structure if species differ in their trade-off between growth rate and disturbance tolerance (Engelmoer and Rozen, 2009). Therefore, specific functional traits (**Box 1**) may be more informative of community stability in disturbed ecosystems than community composition and structure (Lennon et al., 2012; Wallenstein and Hall, 2012; Mouillot et al., 2013). For example, the ability to resist dehydration via synthesis of the sugar trehalose to maintain cell membrane integrity (e.g., McIntyre et al., 2007; Zhang and Van, 2012) may be an important soil microbial trait to consider for drought resistance, whereas the ability to use specific C or N forms that are released when a drought ends might inform about resilience (Borken and Matzner, 2009) (**Table 1**). In contrast, more general stress-response pathways, such as the sporulation pathway of *Bacillus subtilis* (e.g., Higgins and Dworkin, 2012) may be universally useful for maintaining stability in the face of a variety of disturbances.

Dispersal mechanisms and connectivity are important for the resilience of microbial communities because the success of regional dispersal affects the maintenance of local diversity (e.g., Matthiessen et al., 2010; Lindstrom and Langenheder, 2012). Connectedness of metapopulations has been shown to be an important factor in the response of aquatic communities to disturbance (e.g., Altermatt et al., 2011; Carrara et al., 2012), but such evidence is lacking for soils. Dispersal mechanisms are likely to play an even more important role for the recovery of microbial communities in soil because of its heterogeneous nature (Ritz et al., 2004), and low moisture content can hamper dispersal of soil microbes by spatially isolating metacommunities (Treves et al., 2003). However, soil microbes can also disperse via aboveground mechanisms. For example, fungi that rely on active dispersal through airborne spores (e.g., Roper et al., 2010) may have greater resilience than bacteria that lack more active dispersal mechanisms (Kasel et al., 2008; but see Barcenas-Moreno et al., 2011). On the other hand, bacteria, archaea, and phytoplankton cells are thought to passively disperse easily because of their large populations and small body sizes (e.g., Baas-Becking, 1934; Finlay and Clarke, 1999).

From the above, we infer that specific microbial traits are pivotal for determining microbial community response to disturbance, and that the ability of a microbial community to resist or recover from a specific disturbance may be informed by the dominance, or community-weighted mean, of a specific functional trait (e.g., Wallenstein and Hall, 2012) (**Table 1**). Recent advances in sequence-based metagenomics allow for identification of functional genes in a microbial community (Thomas et al., 2012). However, although the presence and expression of specific functional genes in soil microbial communities has been shown to respond to global change and climate change disturbances (e.g., Baldrian et al., 2012; Yergeau et al., 2012; Yarwood et al., 2013), the relative abundance of functional genes has never been used

MICROBIAL COMMUNITY STRUCTURE, SPECIFIC TRAITS PRESENT IN A COMMUNITY, AND THE R-K SPECTRUM

Much work has been done on the relationship between the diversity and structure of microbial communities and their response to disturbance, often with contrasting results. Most evidence

Table 1 | Examples of microbial traits and the genes involved that might play a role in the resistance and resilience of microbial communities to climate change.

Trait	Genes involved	Process	Climate change driver	References
Desiccation and heat resistance	otsBA, otsA neuO	Trehalose synthesis Capsule O- acetylation	Drought, warming	Canovas et al., 2001; McIntyre et al., 2007; Miller and Ingram, 2008; Mordhorst et al., 2009; Zhang and Van, 2012
Sporulation	>500	Multiple	Wide range of disturbances	Higgins and Dworkin, 2012
Use of specific N forms	amoA cnorB nosZ narG nirK, nirS nifH	Ammonia oxidation Nitric oxide reduction Nitrous oxide reduction Nitrate reduction Nitrite reduction Nitrogen fixation	Increased nitrogen availability through warming and rewetting after drought, changes in dominant N forms through warming, changes in soil moisture, and changes in soil C availability through elevated CO ₂	Lamb et al., 2011; Long et al., 2012; Yergeau et al., 2012; Yarwood et al., 2013
Use of specific C forms	chiA mcrA pmoA gtlA cbhl lcc βglu	Chitin degradation Methanogenesis Methane oxidation Citrate synthesis Cellulose degradation Lignin and phenol oxidation Glucose oxidation	Changes in soil C availability through rewetting after drought, and elevated CO ₂	Theuerl and Buscot, 2010; Theuerl et al., 2010; Edwards et al., 2011; Baldrian et al., 2012; Castro et al., 2012; Nannipieri et al., 2012

to infer a community's ability to withstand and recover from disturbances. This approach still has many caveats; newly discovered gene sequences often lack homology to known genes in current databases and remain unknown until biochemical characterization and annotation of their functional abilities, and microorganisms may carry the genetic capacity to exhibit a certain functional trait, but, ultimately, not express the gene or produce an active gene product in nature. Thus, to capitalize on sequence-based metagenomic tools for the understanding of functional traits, the traits of interest and their genes and regulatory pathways must be well-characterized.

In addition to specific traits, microorganisms can be characterized according to their life-history strategy: *r*-strategists (termed ruderals in plant ecology, and copiotrophs in microbial ecology) have high growth rates and low resource use efficiency, and *K*-strategists (termed competitors in plant ecology, and oligotrophs in microbial ecology) have low growth rates and high resource use efficiency (Klappenbach et al., 2000; Fierer et al., 2007). This assumed fundamental trade-off between growth rate and resource use efficiency (Hall et al., 2009) may underlie the capacity of microbial communities to respond to disturbance (Schimel et al., 2007; Wallenstein and Hall, 2012), as community structure will change if the taxa present differ in this trade-off (Engelmoer and Rozen, 2009). There is evidence from both plant and soil communities that *K*-strategists are more resistant, but less resilient, to climate change-related disturbances than *r*-strategists (Grime, 2001; Haddad et al., 2008; Bapiri et al., 2010; De Vries et al., 2012a; Lennon et al., 2012), and a trade-off between resistance and resilience is widely documented (Pimm, 1984; Hedlund et al., 2004; De Vries et al., 2012a). Different soils with different

microbial communities have been compared in their response to disturbances (mostly in terms of bulk biomass and function), and changes in the abundances or relative contributions of community members have been linked to the overarching stability of the microbial community structure itself (Griffiths and Philippot, 2013). As some taxa may be more sensitive to certain disturbances than other taxa, it is possible that their differential responses impact not only the abundances of insensitive community members (for instance, through changes in the strengths of microbial interactions, such as the release of an insensitive taxon from competition due to the decrease in abundance of a taxon sensitive to disturbance), but also the overarching resistance and resilience of the community. Here, we propose that community-level measures that have a theoretical relationship with a specific functional trait, or with the *r*-*K*-strategist spectrum, might predict the response of soil microbial community structure to pulse disturbances associated with climate change.

HYPOTHESIS 1: THE RESISTANCE OF MICROBIAL COMMUNITY STRUCTURE TO DISTURBANCE INCREASES WITH INCREASING RELATIVE ABUNDANCE OF K STRATEGISTS (OR OLIGOTROPHS), BUT THE RESILIENCE DECREASES.

Gram-positive bacteria often are slower growing than Gram-negative bacteria (Prescott et al., 1996), and therefore the ratio between Gram-positives and Gram-negatives of a soil microbial community might be indicative of the prevalence of *K*-strategists in that community. In addition, the ability of many Gram-positive bacteria to sporulate allows them to withstand a variety of disturbances, including drought (Drenovsky et al., 2010; Higgins and Dworkin, 2012). Therefore, we propose that the resistance

of microbial community structure will increase with increasing Gram-positive/Gram-negative ratio, or increasing relative abundance of Gram-positive bacteria.

Similarly, microbial communities that have a high proportion of fungi compared to bacteria are associated with nutrient [N and phosphorus (P)] poor conditions that require high resource use efficiency, and fungi typically are considered to be slower growing than bacteria (Six et al., 2006). Therefore, we argue that the fungal/bacterial ratio of a soil microbial community may also be indicative of the prevalence of K-strategists in that community, and, following this, the resistance of microbial community structure will increase with increasing fungi-to-bacteria (F/B) ratio, or increasing relative abundance of fungi, whereas the resilience will decrease. The carbon-to-nitrogen (C/N) ratio of microbial communities may be also be linked to intrinsic growth rate; fungi are slower-growing and have wider C/N ratios than bacteria (Van Veen and Paul, 1979; Bloem et al., 1997; but see Cleveland and Liptzin, 2007), thus, microbial communities that are dominated by fungi rather than bacteria will have a wider C/N ratio.

Finally, the resilience of microbial community structure will increase with increasing abundance of bacteria that can be classified as copiotrophs, such as many members of the β -proteobacteria and Bacteroidetes, and decreasing abundance of oligotrophs, such as many members of the Acidobacteria (Fierer et al., 2007). Notably, many oligotrophic microorganisms may be r-strategists, while many copiotrophic microorganisms may also be K-strategists, and so there is likely overlap between the two types of classification. Although we propose here that the above community attributes can be used to predict the resistance and resilience of microbial community composition, we acknowledge that within the categories and distinctions we propose, there will of course be exceptions that do not respond as we suggest.

At first, it may seem circular that quickly-growing organisms will be less resistant but more resilient to disturbances, and that communities with frequent disturbance regimes may be dominated by microorganisms exhibiting these strategies because of selection. However, we believe that our hypothesis is not merely self-affirming because microorganisms may respond to disturbances not only by growing and dying, but also, for example, by temporarily changing their physiological state or metabolism (e.g., entering dormancy), maintaining stochastic gene expression, exhibiting phenotypic plasticity, or being rescued by dispersal from nearby meta-communities (e.g., Shade et al., 2012a). Therefore, given the array of complex responses that microorganisms may have when challenged with a disturbance, growth is not the only mechanism that could maintain community stability.

HIGHER TROPHIC LEVELS

Although there is some evidence from aquatic and terrestrial studies that the presence of higher trophic levels can enhance the recovery of microbial biomass and activity (Maraun et al., 1998; Downing and Leibold, 2010), almost no attention has been given to the role of higher trophic levels of the soil food web in controlling resilience of microbial community structure. Microbial grazers have the potential to affect resilience of microbial community structure via two mechanisms. First, they can aid the dispersal of microbes by carrying them in their guts or

on their surfaces. For example, bacterial-feeding nematodes disperse bacteria by carrying them both their surfaces and in their guts (Ingham, 1999), fungal spores are dispersed by the movement of fungal grazers such as collembolans (Renker et al., 2005), and bacterioplankton may “hitchhike” on zooplankton carapaces to overcome otherwise impenetrable gradients in water columns (Grossart et al., 2010). In addition, microbial grazers affect microbial communities by preferentially feeding on specific taxa or functional groups, thereby either reducing their abundance or stimulating their turnover and activity (Chen and Ferris, 2000; Cole et al., 2004; Fu et al., 2005; Postma-Blaauw et al., 2005). As an example, heterotrophic nanoflagellates, prominent bacteriovores in aquatic systems, often preferentially graze on medium-sized bacterioplankton, leaving the small and large-bodied organisms behind (Miki and Jacquet, 2008).

HYPOTHESIS 2: THE RESILIENCE OF MICROBIAL COMMUNITY STRUCTURE INCREASES WITH GREATER DIVERSITY OF ORGANISMS OF HIGHER TROPHIC LEVELS

Different microbial grazers have different feeding preferences, and different soil faunal species often have different movement patterns. Thus, we hypothesize that a greater diversity or species richness of higher trophic levels in the soil food web enhances resilience of soil microbial communities after disturbance, because they stimulate the growth and dispersal of a wider range of soil microbes than faunal communities of lower diversity.

RESOURCE AVAILABILITY

As suggested by Wallenstein and Hall (2012) resource availability might constrain the rate of soil microbial community adaptation and recovery; in low resource environments, shifts in microbial community structure will be slow, whereas in high resource environments, communities will respond rapidly. Indeed, resource availability has been linked to resilience of microbial and faunal biomass several times (Orwin et al., 2006; De Vries et al., 2012b). It was observed (but not quantified in regards to community composition) that the resilience of both microbial and faunal communities seemed to be increased by the presence of plants (De Vries et al., 2012b) presumably because plants offer substantial belowground carbon inputs for microbial communities. Resource availability has the potential to both enhance and retard microbial community resilience, depending on the remaining microbial traits after a disturbance: low resource availability may give slow-growing (oligotrophic) microbes a competitive advantage, whereas high resource availability may favor fast-growing (copiotrophic) microbes. Therefore, we propose that a greater resource availability, diversity, and heterogeneity would increase community resilience after a disturbance, and indeed, several studies report a positive effect of plant species diversity (with presumably a diversity of belowground root exudates and litter inputs) on the stability of microbial biomass and microbial processes (Milcu et al., 2010; Royer-Tardif et al., 2010). Moreover, root exudates form a tight evolutionary link between plants and microbial communities (Badri and Vivanco, 2009), and recent evidence showed that different chemical compositions of *Arabidopsis* root exudates select for different microbial communities (Badri et al., 2013), thereby potentially affecting the

response of those communities to climate change. Because plants respond to climate change by modifying their C balance (Atkin and Tjoelker, 2003; Chaves et al., 2003), temporal changes in root exudation especially have great potential to affect microbial community responses to climate change.

HYPOTHESIS 3: THE RESILIENCE OF MICROBIAL COMMUNITY STRUCTURE INCREASES WITH GREATER RESOURCE AVAILABILITY. BECAUSE OF THE BELOWGROUND C INPUTS BY PLANT, THE PRESENCE OF A PLANT WILL INCREASE THE RESILIENCE OF THE MICROBIAL COMMUNITY

Increased concentrations of labile carbon, nitrogen, and phosphorus as a result of greater resource availability might allow microbial taxa to maximize their intrinsic growth rate and thus increases the resilience of microbial community composition. We also hypothesize that the presence of a plant enhances the resilience of microbial community structure through its below-ground carbon inputs.

MOISTURE AVAILABILITY

Moisture availability plays a crucial role for microbial activity and survival, because microbes are in close contact with water and have semi-permeable cell walls. In addition and as briefly mentioned earlier, low soil moisture content limits the dispersal of microorganisms (Carson et al., 2010; Kravchenko et al., 2013). However, moisture is also limiting for the movement of microbial grazers such as nematodes (Young et al., 1998), which, as hypothesized above, might promote growth and dispersal of microbes and increase microbial community resilience.

HYPOTHESIS 4: MOISTURE AVAILABILITY INCREASES RESILIENCE OF MICROBIAL COMMUNITY STRUCTURE

We hypothesize that relatively higher moisture availability increases the recovery of microbial community structure after drought, and also after other types of disturbance, such as changes in N and C availability (as a result of increased atmospheric CO₂ concentrations) or heat waves.

METHODS

We analyzed three case studies to test the hypotheses about soil microbial community resistance and resilience outlined above, focusing on drought, rainfall, and increased litter inputs. In all three case studies, we calculated Bray-Curtis similarities between disturbed and control microbial communities as a measure of both resistance and resilience of microbial community structure. For resistance, this was the similarity between the disturbed treatment and the control at the end of the disturbance; for resilience, it was the similarity between the disturbed treatment and the control after ending the disturbance. In both cases, a similarity of 1 would mean maximum resistance (no effect of disturbance) or resilience (complete recovery). We used axis scores from ordination plots as metrics of microbial community structure, as well as F/B ratio and Gram-positive/Gram-negative ratio. We fitted single-variable linear and non-linear models [including a quadratic term of the significant explanatory variable(s)] (lm function in R) to explain resistance and resilience from metrics of microbial community structure, as well as from higher

trophic level richness and numbers, soil C and N availability, and soil moisture content. If the quadratic term was significant, we performed an ANOVA to test whether the non-linear model significantly improved model fit. Finally, we fitted the best explaining additive model for microbial community resistance and resilience using parameters that had shown to be significant in the single-variable models. All analyses were performed in R [version 2.15.2, (2012)].

CASE STUDY 1: RESPONSES OF GRASSLAND AND WHEAT FIELD MICROBIAL COMMUNITIES TO MULTIPLE DROUGHT EVENTS

The data from case study 1 were originally published in two papers: De Vries et al. (2012a) and De Vries et al. (2012b). The experiment investigated the responses of the entire soil food web and of C and N cycling in grassland and wheat soil to drought. The experiment included two phases: a field-based drought and a glasshouse-based drought. During the glasshouse-based experiment, the response of biomass of functional groups and processes in both control and drought treatments was monitored directly 1, 3, 10, and 77 days after ending the drought. This, in combination with 32 experimental units (land use × field drought × glasshouse drought × 4 replicates) per sampling, and an extra set of pots in which a wheat plant was grown to assess the impact of plant presence on the recovery of the soil food web, resulted in a total of 192 observations. Microbial communities were analyzed using analysis of phospholipid-derived fatty acid profiles (PLFA). In addition, soil concentrations of available C, N, and moisture were measured, as well as leaching and gaseous losses of C and N. For more details on methods and experimental set up see De Vries et al. (2012a,b).

The original publications focused on the impact of drought on biomass and activity of soil food webs, with only a minor role for changes in community composition. The biomass and activity of fungal-based soil food webs of grasslands were found to be more resistant to drought, whereas biomass and activity bacterial-based soil food webs were more resilient. In addition, the presence of a plant increased the resilience of microbial biomass, and resilience of microbial biomass was positively related to C availability. Here, we re-analyzed microbial community data to test our four hypotheses about resistance and resilience of microbial community structure. We calculated F/B ratio (the ratio between the fungal PLFA 18:2ω6 and the bacterial PLFAs i-15:0, a-15:0, 15:0, i-16:0, 16:1ω7, 17:0, a-17:0, cyclo-17:0, 18:1ω7, and cyclo-19:0), Gram-positive/Gram-negative ratio (the ratio between Gram-positive PLFAs i-15:0 and i-17:0 and Gram-negative PLFAs a-C15:0, 16:1ω7, cyclo-17:0, and cyclo-19:0) and PCA scores of relative abundances of PLFAs [widely used in ecology for analyzing PLFA profiles, e.g., in De Vries et al. (2012c)].

We found that both the resistance and the resilience of microbial communities were explained by community structure. In line with hypothesis 1, resistance decreased with greater PC1 scores, along which Gram-negative abundance increased (Table A4), and increased with greater Gram positive/Gram negative ratio (quadratic relationship, Table 2). However, resistance decreased with greater F/B ratio, which is in contrast with hypothesis 1, and with earlier findings that resistance of biomass and activity

to drought increased with greater relative abundance of fungi (Bapiri et al., 2010; De Vries et al., 2012a). A possible explanation for this is that there is only one PLFA that represents fungi, whereas there are ten PFAs for bacteria. Thus, changes in microbial community structure therefore are dominated by changes in the bacterial members, and the ratio between fungal and bacterial PLFA might not be the most informative for those changes. In addition, the bacterial community in a fungal-dominated microbial community might undergo more dramatic shifts in composition because of intense competition with fungi.

In contrast to hypothesis 1, resilience decreased with greater PC1 scores, whereas it increased with greater C/N ratio of microbial biomass and greater F/B ratio (included in best model, **Table 3**) and Gram positive/Gram negative ratio (**Table 3**). The positive relationship between resilience and F/B ratio as well as Gram-positive/Gram-negative ratio might reflect the fact that the initial changes in communities dominated by fungi and Gram-positives were smaller and thus these remained more similar to their undisturbed counterparts throughout. This is further supported by the lack of evidence for a trade-off between resistance and resilience. In comparison, the resilience index proposed by Orwin et al. (2010) calculates the resilience relative to the initial change in a parameter, and thus a low resistance is more likely followed by a high resilience. It goes beyond the scope of this paper to compare the use of different resilience indices, but it is noteworthy that different methods of calculating these indices can give different results.

Our results partly support hypotheses 2, 3, and 4. As hypothesized, resilience of microbial community structure increased with greater microarthropod richness. However, it decreased with greater protozoa numbers (**Table 3**). When only the last sampling (77 days after ending the drought) was analyzed, the positive relationship of resilience with greater microarthropod richness was also significant (adjusted R-squared = 0.30, $P = 0.017$), but resilience increased with protozoa numbers (adjusted R-squared = 0.22, $P = 0.037$). Notably, the presence of a plant strongly increased overall microbial community resilience, although within land use and field drought treatments this effect was not, or only marginally, significant (**Figure 1**). Within the plant treatment, resilience increased with increasing soil dissolved organic C availability (adjusted R-squared = 0.22, $P = 0.038$). These results support our hypothesis that plant belowground C inputs increase microbial community resilience. However, the lack of explanatory power of overall resource availability for community resilience might indicate that other mechanisms are more important, such as the greater abundance of higher trophic levels in plant treatments (De Vries et al., 2012b), or plant impacts on soil structure and aeration, which were not measured here.

CASE STUDY 2: RESPONSE OF MICROBIAL COMMUNITIES FROM INTENSIVELY MANAGED AND EXTENSIVELY MANAGED GRASSLAND TO DROUGHT

In the study published by Gordon et al. (2008) the impact of a glasshouse-based drought was assessed on microbial

Table 2 | Case study 1: regression models explaining microbial community resistance to the glasshouse-based drought.

Model	Intercept	P	Independent variables included in model	Parameter value	P	Adj. R ²
Single, linear	0.93	<0.0001	PC1 scores	−0.008	<0.0001	0.79
Single, linear	1.01	<0.0001	F/B ratio	−1.48	0.0005	0.56
Single, non-linear	0.75	<0.0001	Gram+/gram− ratio	+3.7*10 ^{−3}	<0.0001	0.88
			(Gram+/gram− ratio) ²	−1.8*10 ^{−5}	<0.0001	
Multiple, non-linear	0.83	<0.0001	PC1	−5.0*10 ^{−3}	0.034	0.91
			Gram+/gram− ratio	+2.3*10 ^{−4}	0.006	
			(Gram+/gram− ratio) ²	−1.3*10 ^{−5}	0.002	

Table 3 | Case study 1: regression models explaining variation in microbial community resilience after the glasshouse-based drought.

Model	Intercept	P	Independent variables included in model	Parameter value	P	Adj. R ²
Single, linear	0.93	<0.0001	Microarthropod richness	+0.004	0.001	0.12
Single, linear	0.96	<0.0001	Protozoa numbers	−7.0*10 ^{−8}	<0.0001	0.33
Single, linear	0.95	<0.0001	PC1	−0.005	<0.0001	0.50
Single, linear	0.93	<0.0001	Microbial biomass C/N	+0.006	0.009	0.07
Single, non-linear	0.91	<0.0001	Gram+/gram− ratio	+4.1*10 ^{−4}	0.006	0.16
			(Gram+/gram− ratio) ²	−6.2*10 ^{−6}	0.05	
Multiple, linear	0.91	<0.0001	Protozoa numbers	−5.1*10 ^{−9}	<0.0001	0.63
			PC1	−4.1*10 ^{−3}	<0.0001	
			Gram+/gram− ratio	+3.2*10 ^{−4}	0.001	
			F/B ratio	0.34	0.002	

communities from extensively managed, unfertilized, species rich grassland, and from intensively managed, fertilized, and heavily grazed grassland, alongside measurements of C and N leaching. The response of microbial biomass C and N, and C and N leaching, was measured 1, 3, 9, 16, 30, and 50 days after rewetting, while microbial community structure (as PLFAs) was measured only at day 30. With two land uses, a drought vs. a control, and four replicates, this resulted in 16 observations for microbial community structure.

In the original publication, the authors found that biomass N of the (fungal-dominated) microbial community of extensively managed grassland was less affected by drought than that of the bacterial-dominated microbial community of intensively managed grassland. Moreover, this was paralleled by smaller leaching losses of C and N from the grassland soil. Changes in microbial community composition were not analyzed quantitatively. Here, we re-analyzed microbial community data to test our hypotheses that microbial community resilience can be explained by

microbial community structure. As in case study 1, we used PCA scores as microbial community metrics, alongside F/B ratio and Gram-positive/Gram-negative ratio.

The results from this case study support hypothesis 1. We found that resilience was negatively related to the F/B ratio and the Gram-positive/Gram-negative ratio. In addition, resilience increased with greater PC1 scores (Table 4), along which most Gram-negative PLFAs increased and fungal PLFA decreased (Table A5). This dataset did not allow for testing the other hypotheses.

CASE STUDY 3: TROPICAL FOREST SOIL MICROBIAL COMMUNITIES RESPONSES TO LITTER ADDITION, LITTER REMOVAL, AND RAINFALL EXCLUSION IN A FIELD EXPERIMENT

Nemergut et al. (2010) published a study assessing the impact of organic matter content through on soil microbial communities in Costa Rican tropical forest soils. The design included three experimental treatments (litter exclusion, litter addition, and throughfall exclusion) and one control, each observed over time in triplicate plots. The control plots were sampled at the beginning of the experiment, in April 2007, and then subsequently in June and October 2008. The experimental plots were sampled in June and October 2008, resulting in 27 total observations. Pyrosequencing of the 16S rRNA gene was used to measure of bacterial and archaeal community structure, and a suite of soil environmental parameters were also assessed, including: soil water content, microbial biomass, CO₂ efflux, dissolved oxygen, and ammonium and nitrate concentrations. The sequencing data, contextual data, and metadata were deposited in MG-RAST and made publicly available. The Nemergut et al. (2010) dataset was selected as a case study because parameters of interest to global change disturbance were measured (microbial community structure, soil resources, and soil moisture), and because it provided a sequence-based assessment of composition to complement the PLFA-based assessments of Case Studies 1 and 2.

In the original work, the authors reported that certain phyla of bacteria and archaea were more prevalent in some of the experimental treatments than others, and, more specifically, that oligotrophic taxa (e.g., Acidobacteria) were more prevalent in plots that were compromised in organic matter availability. To query the dataset specifically about community resistance and resilience, we first calculated resistance as the Bray-Curtis similarity (averaged across replicates) between the initial time point

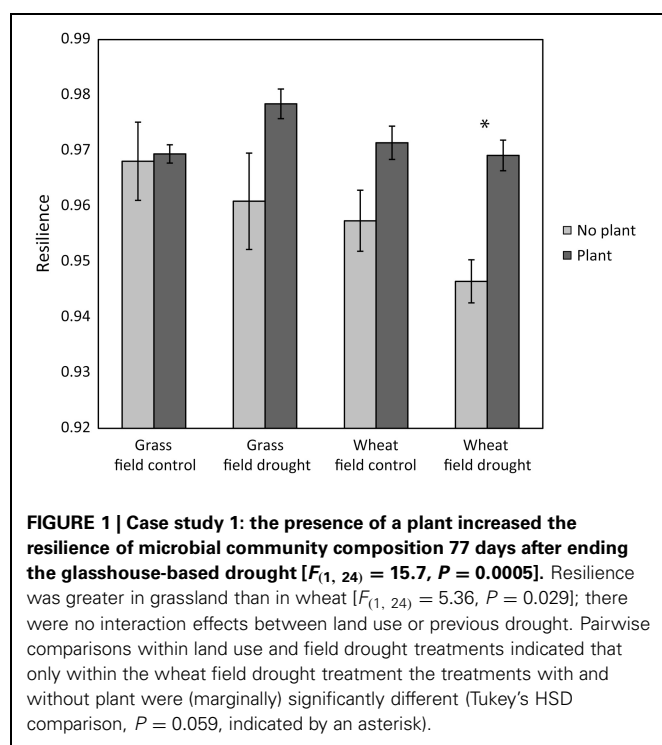


Table 4 | Case study 2: regression models explaining variation in microbial community resilience at day 30 after ending the glasshouse-based drought.

Model	Intercept	P	Independent variables included in model	Parameter value	P	Adj. R ²
Single, linear	0.95	<0.0001	F/B ratio	−3.76	0.0094	0.65
Single, linear	0.94	<0.0001	PC1 scores	+0.003	0.013	0.62
Single, non-linear	1.04	<0.0001	Gram+/gram− ratio	−0.137	0.021	0.77
			(Gram+/gram− ratio) ²	+0.0359	0.028	
Single, linear	0.96	<0.001	Microbial biomass	−1.7*10 ^{−5}	0.024	0.53

(pre-disturbance) control and the post-manipulation time point for each experimental treatment (April control vs. June treatment). Then, we calculated resilience as the Bray-Curtis similarity between the final time point and the April pre-disturbance control (April control vs. October treatment). We used unconstrained correspondence analysis to determine axis scores as a metric of microbial community structure.

We found that microbial community structure (axis 1 CA scores) explained variability in resistance across treatments (non-linear model: resistance was explained by main and quadratic term of axis 1 scores, adjusted R squared = 0.89, $p < 0.0001$ and $p = 0.004$, respectively)—resistance increased with axis 1 scores. The axis 1 gradient corresponded to transition from communities with a high representation of Proteobacteria-affiliated taxa (many of which can be classified as copiotrophs) to communities with a high representation of Acidobacteria-affiliated taxa (many of which can be classified as oligotrophs; **Table A6** online Suppl. Data). Thus, this result supports hypothesis 1 that resistance increases with increasing abundance of oligotrophs. Axis 2 CA scores and microbial biomass did not provide explanatory value for resistance. Of all the available environmental measurements, only nitrate concentrations and moisture content explained variability in resilience (Pearson's correlation between moisture and nitrate -0.123 , $P = 0.538$); resilience increased with nitrate availability, but decreased with moisture content (**Table 5**). This suggests that nitrate availability and moisture are important for resilience of microbial communities in tropical soils, and supports hypothesis 3, but not hypothesis 4, which pose that resilience increases with nutrient and water availability, respectively. The dataset did not allow for testing the remaining hypotheses.

Notably, there were only small changes in community composition within treatments over time, which prompted the authors to combine the time points for their original analysis. This is, in some ways, expected because spatial variability often exceeds temporal variability in soil communities (Bardgett et al., 1997; Ettema and Wardle, 2002). However, the resistance and resilience determined by these small changes were well-explained by community structure, nitrate, and water content.

SUPPORT FOR THE HYPOTHESES – A FRAMEWORK FOR PREDICTING MICROBIAL COMMUNITY RESISTANCE AND RESILIENCE TO CLIMATE CHANGE

In all three case studies, the resistance and the resilience of microbial communities could be explained by community

properties associated with the r-K spectrum. We found that the measures that significantly explained resistance and resilience and were indicative for shifts from r-strategists to K-strategists were strongly interrelated (**Tables A1–A3**), confirming that these measures inform about broad shifts in community structure linked to changes in the abundance of r- and K-strategists. Moreover, the presence and abundance of higher trophic levels, resource availability, and moisture content were strong predictors for microbial community resilience. Although the structure of the data we analyzed does not allow for drawing conclusions on the relative importance of those controls, and the relationships we found are not necessarily causal, these results are a first observation and exploration of a framework for predicting the response of soil microbial communities to climate change based on the ratio between r- and K-strategists, and the environment (**Figure 2**, top panel). We propose that, although the underlying specific functional genes present in a microbial community determine its response to climate change, simple measures that characterize microbial communities along the r-K spectrum can inform its ability to resist and recover from climate change related disturbances. Our framework also takes into account the effect of the environment, and interrelationships between environment and r-K dominance of microbial communities, in the three-dimensional response plane.

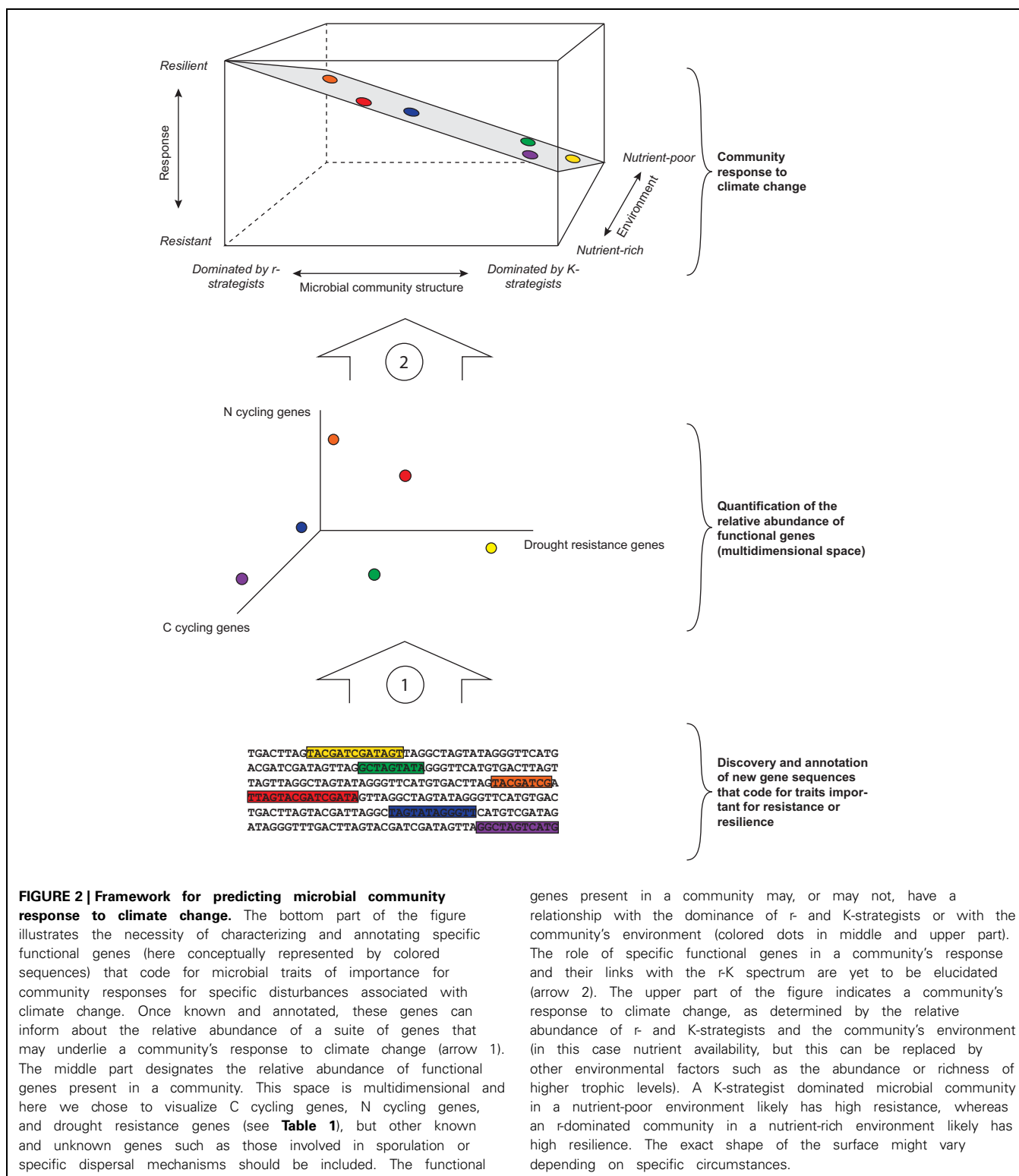
Furthermore, we propose that the abundance of specific functional genes such as those involved in desiccation resistance will predict a community's response to drought, but genes involved in C and N cycling might link to the r-K spectrum and thus be useful for predicting microbial community response to climate change (**Table 1**; **Figure 2**). For example, the abundance of amoA genes is likely to be greater in N-poor environments in which the dominant N form is ammonia than in nutrient rich environments in which the dominant form is nitrate (Schimel and Bennett, 2004), and might thus be associated with microbial communities dominated by oligotrophs. Ultimately, our framework allows for plotting specific functional traits onto this plane for predicting microbial community stability under a range of specific disturbances.

FUTURE DIRECTIONS: THE ROLES OF MULTIPLE DISTURBANCES AND ADAPTATION FOR SOIL MICROBIAL COMMUNITY STABILITY

By selecting for specific traits among community members, a disturbance may affect a community's ability to respond

Table 5 | Case study 3: regression models explaining variation in microbial community resilience after litter addition, litter removal, and rainfall exclusion.

Model	Intercept	P	Independent variables included in model	Parameter value	P	Adj. R ²
Single, linear	0.22	<0.0001	Nitrate	+0.006	0.026	0.34
Single, non-linear	0.44	<0.0001	Moisture	−0.004	0.005	0.52
Multiple, linear	0.40	<0.0001	Nitrate	+0.004	0.023	0.71
			Moisture	−0.035	0.005	



to a subsequent disturbance or to a series of compounded disturbances. For example, it has been shown that the order of different types of disturbances influences the outcome of community structure, suggesting that selection for a specific trait

affects the ability to respond to a subsequent disturbance of a different type (Fukami, 2001). Thus, we may expect that when a microbial community is exposed to two subsequent disturbances of the same type, its composition will be more resistant

to the second disturbance because of selection for the tolerant trait by the first disturbance. There is some support for this hypothesis from soils. Precipitation regime affected the response of soil bacterial community composition to subsequent drought and rewetting events (Evans and Wallenstein, 2012), and extremophiles are often tolerant to a wide range of disturbances (Mangold et al., 2013). In contrast, microbial communities exposed to severe drought appeared to be more resistant to a subsequent heat wave, suggesting that the microbial traits responsible for drought tolerance are related to those of heat-tolerance (Berard et al., 2012). However, very little is known about the interrelatedness between specific functional traits in soil microbes, which makes it difficult to predict responses to multiple disturbances. In contrast, the r-K spectrum might inform about a microbial community's ability to withstand different types of disturbance: r-strategists thrive in nutrient (N and P) rich, disturbed environments compared to K-strategists, but are less resistant to climate change than K-strategists (Hedlund et al., 2004; De Vries et al., 2012a).

Adaptation also may be an important strategy for individual microbial taxa to cope with a changing climate (Box 1). A microbe's ability to adapt to disturbance is linked to its generation time or turnover rate, and therefore r-strategists may show quicker adaptation than K-strategists. Moreover, warming can increase growth rates, but also horizontal gene transfer between bacterial taxa (Pritchard, 2011). In addition, for example, it has been shown that *E. coli* can acquire stress resistance to a range of disturbances after pretreatment with a different disturbance after only 500 generation times (Dragosits et al., 2013). This so called cross-stress protection has been shown for a range of species across kingdoms. Similar to microbial community resilience, rates of adaptation and evolution are likely influenced by environmental factors such as the abundance and richness of higher trophic levels, moisture availability, and resource availability. Although not within the scope of this paper, these findings suggest that evolutionary changes might be of equal importance to shifts in community structure for determining the response of microbial communities to climate change (Orsini et al., 2013).

CONCLUSION

Our aim in this paper was to hypothesize controls on microbial community resistance and resilience to climate change, and to explore our hypotheses by carefully re-analyzing three vignettes of published data. Our results show that both microbial community properties associated with the r-K spectrum and environmental factors such as the abundance and richness of higher trophic levels, plant presence, and resource availability can explain the response of microbial community structure to climate change-related disturbances. A clear limitation to our study is the relatively narrow focus on three vignettes of case studies, and further investigation and experimental validation is necessary to directly test these hypotheses across a wide range of soil ecosystems. Although querying publicly available data can be used to formulate hypotheses on the potential controls of

microbial community resistance and resilience, disentangling the interwoven controls on microbial community resistance and resilience requires mechanistic experiments designed to test specific questions about the hypothesized controls (Jansson and Prosser, 2013).

As a final consideration, it is possible that routine successional trajectories of microbial communities (for example, seasonal trajectories in temperate soils) may be altered permanently as a result of a disturbance. However, the nature of these alterations will depend on the traits present in the community and on the type of disturbance. In temperate aquatic systems, it has been suggested that annual seasonal succession in bacterial community composition may serve as a baseline from which a community's response to a pulse disturbances can be measured, while gradual shifts in this succession may be used as an indicator of long-term adaptations to press disturbances such as global climate changes (Shade et al., 2012a,b). Similarly, soil community successional trajectories may be quantified and monitored to detect gradual shifts in composition over the long term, such as in response to the press disturbance of increased temperature, and how these shifts affect short-term responses to pulse disturbances, such as drought. However, typical rates of community turnover in soil systems are not well documented, especially at the same site on inter-annual scales, and in the absence of any disturbance (Shade et al., 2013). Knowledge of these baseline seasonal dynamics for soils is crucial for providing context for community responses to pulse disturbances, like drought and flooding. Therefore, collecting time series of soil communities and quantifying baseline fluctuations should be prioritized toward the goal of further understanding microbial community stability given ongoing and compounded global climate change disturbances. Combined with long-term experiments that directly manipulate anticipated global change disturbances [e.g., free-air carbon dioxide enrichment experiments (Ainsworth and Long, 2005)], we think that these time series will provide essential insights into the important microbial traits and environmental conditions that may alter or maintain ecosystem services in the face of global changes.

ACKNOWLEDGMENTS

We thank the Joint EU-US Workshop "Microbial Community Dynamics: Cooperation and Competition" of the European-United States Task Force on Biotechnology Research for encouraging our collaboration. AS is a Gordon and Betty Moore Foundation Fellow of the Life Sciences Research Foundation. We thank Diana Nemergut for permission to re-analyze the dataset for case study 3 from MG-RAST, and we thank Helen Gordon for providing us with the dataset for case study 2. We also thank two anonymous referees and the editor for their constructive comments on the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/Terrestrial_Microbiology/10.3389/fmicb.2013.00265/abstract

REFERENCES

- Ainsworth, E. A., and Long, S. P. (2005). What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytol.* 165, 351–371. doi: 10.1111/j.1469-8137.2004.01224.x
- Allison, S. D., and Martiny, J. B. H. (2008). Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci. U.S.A.* 105, 11512–11519. doi: 10.1073/pnas.0801925105
- Altermatt, F., Bieger, A., Carrara, F., Rinaldo, A., and Holyoak, M. (2011). Effects of connectivity and recurrent local disturbances on community structure and population density in experimental metacommunities. *PLoS ONE* 6:e19525. doi: 10.1371/journal.pone.0019525
- Anderson, M. J., Crist, T. O., Chase, J. M., Vellend, M., Inouye, B. D., Freestone, A. L., et al. (2011). Navigating the multiple meanings of beta diversity: a roadmap for the practicing ecologist. *Ecol. Lett.* 14, 19–28. doi: 10.1111/j.1461-0248.2010.01552.x
- Atkin, O. K., and Tjoelker, M. G. (2003). Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends Plant Sci.* 8, 343–351. doi: 10.1016/S1360-1385(03)00136-5
- Baas-Becking, L. G. M. (1934). *Geobiologie of Inleiding tot de Milieukunde*. The Hague: Van Stockum and Zon.
- Badri, D. V., Chaparro, J. M., Zhang, R. F., Shen, Q. R., and Vivanco, J. M. (2013). Application of natural blends of phytochemicals derived from the root exudates of arabidopsis to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. *J. Biol. Chem.* 288, 4502–4512. doi: 10.1074/jbc.M112.433300
- Badri, D. V., and Vivanco, J. M. (2009). Regulation and function of root exudates. *Plant Cell Environ.* 32, 666–681. doi: 10.1111/j.1365-3040.2009.01926.x
- Baldrian, P., Kolarik, M., Stursova, M., Kopecky, J., Valaskova, V., Vetrovsky, T., et al. (2012). Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME J.* 6, 248–258. doi: 10.1038/ismej.2011.95
- Bapiri, A., Bååth, E., and Rousk, J. (2010). Drying-rewetting cycles affect fungal and bacterial growth differently in an arable soil. *Microb. Ecol.* 60, 419–428. doi: 10.1007/s00248-010-9723-5
- Barcenas-Moreno, G., Garcia-Orenes, F., Mataix-Solera, J., Mataix-Beneyto, J., and Baath, E. (2011). Soil microbial recolonisation after a fire in a Mediterranean forest. *Biol. Fertil. Soils* 47, 261–272. doi: 10.1007/s00374-010-0532-2
- Bardgett, R. D., Leemans, D. K., Cook, R., and Hobbs, P. J. (1997). Seasonality of the soil biota of grazed and ungrazed hill grasslands. *Soil Biol. Biochem.* 29, 1285–1294. doi: 10.1016/S0038-0717(97)00019-9
- Bardgett, R. D., Manning, P., Morriën, E., and De Vries, F. T. (2013). Hierarchical responses of plant–soil interactions to climate change: consequences for the global carbon cycle. *J. Ecol.* 101, 334–343. doi: 10.1111/1365-2745.12043
- Berard, A., Ben Sassi, M., Renault, P., and Gros, R. (2012). Severe drought-induced community tolerance to heat wave. An experimental study on soil microbial processes. *J. Soils Sediments* 12, 513–518. doi: 10.1007/s11368-012-0469-1
- Bloem, J., De Ruiter, P. C., and Bouwman, L. A. (1997). “Soil food webs and nutrient cycling in agroecosystems,” in *Modern Soil Microbiology*, eds J. D. Van Elsas, J. T. Trevors, and E. M. H. Wellington (New York, NY: Marcel Dekker, Inc.), 245–278.
- Borken, W., and Matzner, E. (2009). Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Glob. Change Biol.* 15, 808–824. doi: 10.1111/j.1365-2486.2008.01681.x
- Campbell, N. A., and Reece, J. B. (2006). *Biology*. San Francisco, CA: Pearson Education, Inc.
- Canovas, D., Fletcher, S. A., Hayashi, M., and Csonka, L. N. (2001). Role of trehalose in growth at high temperature of *Salmonella enterica* serovar typhimurium. *J. Bacteriol.* 183, 3365–3371. doi: 10.1128/JB.183.11.3365-3371.2001
- Carrara, F., Altermatt, F., Rodriguez-Iturbe, I., and Rinaldo, A. (2012). Dendritic connectivity controls biodiversity patterns in experimental metacommunities. *Proc. Natl. Acad. Sci. U.S.A.* 109, 5761–5766. doi: 10.1073/pnas.1119651109
- Carson, J. K., Gonzalez-Quinones, V., Murphy, D. V., Hinz, C., Shaw, J. A., and Gleeson, D. B. (2010). Low pore connectivity increases bacterial diversity in soil. *Appl. Environ. Microbiol.* 76, 3936–3942. doi: 10.1128/AEM.03085-09
- Castro, H. F., Classen, A. T., Austin, E. E., Crawford, K. M., and Schadt, C. W. (2012). Development and validation of a citrate synthase directed quantitative PCR marker for soil bacterial communities. *Appl. Soil Ecol.* 61, 69–75. doi: 10.1016/j.apsoil.2012.05.007
- Chaves, M. M., Maroco, J. P., and Pereira, J. S. (2003). Understanding plant responses to drought - from genes to the whole plant. *Funct. Plant Biol.* 30, 239–264. doi: 10.1071/FP02076
- Chen, J., and Ferris, H. (2000). Growth and nitrogen mineralization of selected fungi and fungal-feeding nematodes on sand amended with organic matter. *Plant Soil* 218, 91–101. doi: 10.1023/A:1014914827776
- Cleveland, C. C., and Liptzin, D. (2007). C: N: P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry* 85, 235–252. doi: 10.1007/s10533-007-9132-0
- Cole, L., Staddon, P. L., Sleep, D., and Bardgett, R. D. (2004). Soil animals influence microbial abundance, but not plant-microbial competition for soil organic nitrogen. *Funct. Ecol.* 18, 631–640. doi: 10.1111/j.0269-8463.2004.00894.x
- De Vries, F. T., Liiri, M., Björnlund, L., Bowker, M., Christensen, S., Setälä, H., et al. (2012a). Land use alters the resistance and resilience of soil food webs to drought. *Nat. Clim. Change* 2, 276–280. doi: 10.1038/nclimate1368
- De Vries, F. T., Liiri, M., Björnlund, L., Setälä, H., Christensen, S., and Bardgett, R. D. (2012b). Legacy effects of drought on plant growth and the soil food web. *Oecologia* 170, 821–833. doi: 10.1007/s00442-012-2331-y
- De Vries, F. T., Manning, P., Tallwin, J. R. B., Mortimer, S. R., Pilgrim, E. S., Harrison, K. A., et al. (2012c). Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. *Ecol. Lett.* 15, 1230–1239. doi: 10.1111/j.1461-0248.2012.01844.x
- Downing, A. L., and Leibold, M. A. (2010). Species richness facilitates ecosystem resilience in aquatic food webs. *Freshw. Biol.* 55, 2123–2137. doi: 10.1111/j.1365-2427.2010.02472.x
- Dragosits, M., Mozhayskiy, V., Quinones-Soto, S., Park, J., and Tagkopoulou, I. (2013). Evolutionary potential, cross-stress behavior and the genetic basis of acquired stress resistance in *Escherichia coli*. *Mol. Syst. Biol.* 9, 1–13. doi: 10.1038/msb.2012.76
- Drenovsky, R. E., Steenwerth, K. L., Jackson, L. E., and Scow, K. M. (2010). Land use and climatic factors structure regional patterns in soil microbial communities. *Glob. Ecol. Biogeogr.* 19, 27–39. doi: 10.1111/j.1466-8238.2009.00486.x
- Edwards, I. P., Zak, D. R., Kellner, H., Eisenlord, S. D., and Pregitzer, K. S. (2011). Simulated atmospheric N deposition alters fungal community composition and suppresses ligninolytic gene expression in a Northern Hardwood Forest. *PLoS ONE* 6:e20421. doi: 10.1371/journal.pone.0020421
- Eisenhauer, N., Scheu, S., and Jousset, A. (2012). Bacterial diversity stabilizes community productivity. *PLoS ONE* 7:e34517. doi: 10.1371/journal.pone.0034517
- Engelmoer, D. J. P., and Rozen, D. E. (2009). Fitness trade-offs modify community composition under contrasting disturbance regimes in *Pseudomonas fluorescens* microcosms. *Evolution* 63, 3031–3037. doi: 10.1111/j.1558-5646.2009.00758.x
- Ettema, C. H., and Wardle, D. A. (2002). Spatial soil ecology. *Trends Ecol. Evol.* 17, 177–183. doi: 10.1016/S0169-5347(02)02496-5
- Evans, S. E., and Wallenstein, M. D. (2012). Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? *Biogeochemistry* 109, 101–116. doi: 10.1007/s10533-011-9638-3
- Fierer, N., Bradford, M. A., and Jackson, R. B. (2007). Toward an ecological classification of soil bacteria. *Ecology* 88, 1354–1364. doi: 10.1890/05-1839
- Finlay, B. J., and Clarke, K. J. (1999). Ubiquitous dispersal of microbial species. *Nature* 400, 828–828. doi: 10.1038/23616
- Fu, S. L., Ferris, H., Brown, D., and Plant, R. (2005). Does the positive feedback effect of nematodes on the biomass and activity of their bacteria prey vary with nematode species and population size? *Soil Biol. Biochem.* 37, 1979–1987. doi: 10.1016/j.soilbio.2005.01.018
- Fukami, T. (2001). Sequence effects of disturbance on community structure. *Oikos* 92, 215–224. doi: 10.1034/j.1600-0706.2001.920203.x
- Glasby, T. M., and Underwood, A. J. (1996). Sampling to differentiate between pulse and press perturbations. *Environ. Monit. Assess.* 42, 241–252. doi: 10.1007/BF00414371

- Gordon, H., Haygarth, P. M., and Bardgett, R. D. (2008). Drying and rewetting effects on soil microbial community composition and nutrient leaching. *Soil Biol. Biochem.* 40, 302–311. doi: 10.1016/j.soilbio.2007.08.008
- Griffiths, B. S., and Philippot, L. (2013). Insights into the resistance and resilience of the soil microbial community. *FEMS Microbiol. Rev.* 37, 112–129. doi: 10.1111/j.1574-6976.2012.00343.x
- Grime, J. P. (2001). *Plant Strategies, Vegetation Processes, and Ecosystem Properties*. Chichester: John Wiley and Sons Ltd.
- Grossart, H.-P., Dziallas, C., Leunert, F., and Tang, K. W. (2010). Bacteria dispersal by hitchhiking on zooplankton. *Proc. Natl. Acad. Sci. U.S.A.* 107, 11959–11964. doi: 10.1073/pnas.1000668107
- Haddad, N. M., Holyoak, M., Mata, T. M., Davies, K. F., Melbourne, B. A., and Preston, K. (2008). Species' traits predict the effects of disturbance and productivity on diversity. *Ecol. Lett.* 11, 348–356. doi: 10.1111/j.1461-0248.2007.01149.x
- Hall, E. K., Dzialowski, A. R., Stoxen, S. M., and Cotner, J. B. (2009). The effect of temperature on the coupling between phosphorus and growth in lacustrine bacterioplankton communities. *Limnol. Oceanogr.* 54, 880–889. doi: 10.4319/lo.2009.54.3.0880
- Hedlund, K., Griffiths, B., Christensen, S., Scheu, S., Setälä, H., Tscharnkte, T., et al. (2004). Trophic interactions in changing landscapes: responses of soil food webs. *Basic Appl. Ecol.* 5, 495–503. doi: 10.1016/j.baec.2004.09.002
- Higgins, D., and Dworkin, J. (2012). Recent progress in *Bacillus subtilis* sporulation. *FEMS Microbiol. Rev.* 36, 131–148. doi: 10.1111/j.1574-6976.2011.00310.x
- Hunter, M. L. J. (1990). *Wildlife Forests, and Forestry*. Englewood Cliffs, NJ: Regents/Prentice Hall.
- Ingham, E. R. (1999). *Nematodes* [Online]. Available online at: http://soils.usda.gov/sqi/soil_quality/soil_biology/soil_biology_prime_r.html
- IPCC. (2007). "Summary for policymakers," in *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, eds S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller (Cambridge, UK and New York, NY: Cambridge University Press).
- Jansson, J. K., and Prosser, J. I. (2013). Microbiology: the life beneath our feet. *Nature* 494, 40–41. doi: 10.1038/494040a
- Kasel, S., Bennett, L. T., and Tibbitts, J. (2008). Land use influences soil fungal community composition across central Victoria, south-eastern Australia. *Soil Biol. Biochem.* 40, 1724–1732. doi: 10.1016/j.soilbio.2008.02.011
- Klappenbach, J. A., Dunbar, J. M., and Schmidt, T. M. (2000). rRNA operon copy number reflects ecological strategies of bacteria. *Appl. Environ. Microbiol.* 66, 1328–1333. doi: 10.1128/AEM.66.4.1328-1333.2000
- Kravchenko, A., Chun, H. C., Mazer, M., Wang, W., Rose, J. B., Smucker, A., et al. (2013). Relationships between intra-aggregate pore structures and distributions of *Escherichia coli* within soil macroaggregates. *Appl. Soil Ecol.* 63, 134–142. doi: 10.1016/j.apsoil.2012.10.001
- Lake, P. S. (2000). Disturbance, patchiness, and diversity in streams. *J. N. Am. Benthol. Soc.* 19, 573–592. doi: 10.2307/1468118
- Lamb, E. G., Han, S., Lanoil, B. D., Henry, G. H. R., Brummell, M. E., Banerjee, S., et al. (2011). A high Arctic soil ecosystem resists long-term environmental manipulations. *Glob. Change Biol.* 17, 3187–3194. doi: 10.1111/j.1365-2486.2011.02431.x
- Lavorel, S., and Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Funct. Ecol.* 16, 545–556. doi: 10.1046/j.1365-2435.2002.00664.x
- Lennon, J. T., Aanderud, Z. T., Lehmkuhl, B. K., and Schoolmaster, D. R. Jr. (2012). Mapping the niche space of soil microorganisms using taxonomy and traits. *Ecology* 93, 1867–1879. doi: 10.1890/11-1745.1
- Lindstrom, E. S., and Langenheder, S. (2012). Local and regional factors influencing bacterial community assembly. *Environ. Microbiol. Rep.* 4, 1–9. doi: 10.1111/j.1758-2229.2011.00257.x
- Long, X., Chen, C. R., Xu, Z. H., Linder, S., and He, J. Z. (2012). Abundance and community structure of ammonia oxidizing bacteria and archaea in a Sweden boreal forest soil under 19-year fertilization and 12-year warming. *J. Soils Sediments* 12, 1124–1133. doi: 10.1007/s11368-012-0532-y
- Mangold, S., Potrykus, J., Bjorn, E., Lovgren, L., and Dopson, M. (2013). Extreme zinc tolerance in acidophilic microorganisms from the bacterial and archaeal domains. *Extremophiles* 17, 75–85. doi: 10.1007/s00792-012-0495-3
- Maraun, M., Visser, S., and Scheu, S. (1998). Oribatid mites enhance the recovery of the microbial community after a strong disturbance. *Appl. Soil Ecol.* 9, 175–181. doi: 10.1016/S0929-1393(98)00072-9
- Matthiessen, B., Ptacnik, R., and Hillebrand, H. (2010). Diversity and community biomass depend on dispersal and disturbance in microalgal communities. *Hydrobiologia* 653, 65–78. doi: 10.1007/s10750-010-0349-x
- McIntyre, H. J., Davies, H., Hore, T. A., Miller, S. H., Dufour, J.-P., and Ronson, C. W. (2007). Trehalose biosynthesis in *Rhizobium leguminosarum* bv. trifolii and its role in desiccation tolerance. *Appl. Environ. Microbiol.* 73, 3984–3992. doi: 10.1128/AEM.00412-07
- Miki, T., and Jacquet, S. (2008). Complex interactions in the microbial world: underexplored key links between viruses, bacteria and protozoan grazers in aquatic environments. *Aquat. Microb. Ecol.* 51, 195–208. doi: 10.3354/ame01190
- Milcu, A., Thebault, E., Scheu, S., and Eisenhauer, N. (2010). Plant diversity enhances the reliability of belowground processes. *Soil Biol. Biochem.* 42, 2102–2110. doi: 10.1016/j.soilbio.2010.08.005
- Miller, E. N., and Ingram, L. O. (2008). Sucrose and overexpression of trehalose biosynthetic genes (otsBA) increase desiccation tolerance of recombinant *Escherichia coli*. *Biotechnol. Lett.* 30, 503–508. doi: 10.1007/s10529-007-9573-5
- Mordhorst, I. L., Claus, H., Ewers, C., Lappann, M., Schoen, C., Elias, J., et al. (2009). O-acetyltransferase gene neuO is segregated according to phylogenetic background and contributes to environmental desiccation resistance in *Escherichia coli* K1. *Environ. Microbiol.* 11, 3154–3165. doi: 10.1111/j.1462-2920.2009.02019.x
- Mouillot, D., Graham, N. A. J., Villéger, S., Mason, N. W. H., and Bellwood, D. R. (2013). A functional approach reveals community responses to disturbances. *Trends Ecol. Evol.* 28, 167–177. doi: 10.1016/j.tree.2012.10.004
- Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., Masciandaro, G., et al. (2012). Soil enzymology: classical and molecular approaches. *Biol. Fertil. Soils* 48, 743–762. doi: 10.1007/s00374-012-0723-0
- Nemergut, D. R., Cleveland, C. C., Wieder, W. R., Washenberger, C. L., and Townsend, A. R. (2010). Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial community composition in a lowland tropical rain forest. *Soil Biol. Biochem.* 42, 2153–2160. doi: 10.1016/j.soilbio.2010.08.011
- Orsini, L., Schwenk, K., De Meester, L., Colbourne, J. K., Pfender, M. E., and Weider, L. J. (2013). The evolutionary time machine: using dormant propagules to forecast how populations can adapt to changing environments. *Trends Ecol. Evol.* 28, 274–282. doi: 10.1016/j.tree.2013.01.009
- Orwin, K. H., Wardle, D. A., and Greenfield, L. G. (2006). Context-dependent changes in the resistance and resilience of soil microbes to an experimental disturbance for three primary plant chronosequences. *Oikos* 112, 196–208. doi: 10.1111/j.0030-1299.2006.13813.x
- Orwin, K. H., Buckland, S. M., Johnson, D., Turner, B. L., Smart, S., Oakley, S., et al. (2010). Linkages of plant traits to soil properties and the functioning of temperate grassland. *J. Ecol.* 98, 1074–1083. doi: 10.1111/j.1365-2745.2010.01679.x
- Pimm, S. L. (1984). The complexity and stability of ecosystems. *Nature* 307, 321–326. doi: 10.1038/307321a0
- Postma-Blauw, M. B., De Vries, F. T., De Goede, R. G. M., Bloem, J., Faber, J. H., and Brussaard, L. (2005). Within-trophic group interactions of bacterivorous nematode species and their effects on the bacterial community and nitrogen mineralization. *Oecologia* 142, 428–439. doi: 10.1007/s00442-004-1741-x
- Prescott, L. M., Harley, J. P., and Klein, D. A. (1996). *Microbiology*. Boston, MA: WCB McGraw-Hill.
- Pritchard, S. G. (2011). Soil organisms and global climate change. *Plant Pathol.* 60, 82–99. doi: 10.1111/j.1365-3059.2010.02405.x
- R-Core-Team. (2012). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
- Renker, C., Otto, P., Schneider, K., Zimdars, B., Maraun, M., and Buscot, F. (2005). Oribatid mites as potential vectors for soil microfungi: study of mite-associated fungal species. *Microb. Ecol.* 50, 518–528. doi: 10.1007/s00248-005-5017-8

- Ritz, K., McNicol, W., Nunan, N., Grayston, S., Millard, P., Atkinson, D., et al. (2004). Spatial structure in soil chemical and microbiological properties in an upland grassland. *FEMS Microbiol. Ecol.* 49, 191–205. doi: 10.1016/j.femsec.2004.03.005
- Roper, M., Seminara, A., Bandi, M. M., Cobb, A., Dillard, H. R., and Pringle, A. (2010). Dispersal of fungal spores on a cooperatively generated wind. *Proc. Natl. Acad. Sci. U.S.A.* 107, 17474–17479. doi: 10.1073/pnas.1003577107
- Royer-Tardif, S., Bradley, R. L., and Parsons, W. F. J. (2010). Evidence that plant diversity and site productivity confer stability to forest floor microbial biomass. *Soil Biol. Biochem.* 42, 813–821. doi: 10.1016/j.soilbio.2010.01.018
- Rykiel, E. J. (1985). Towards a definition of ecological disturbance. *Aust. J. Ecol.* 10, 361–365. doi: 10.1111/j.1442-9993.1985.tb00897.x
- Schimel, J., Balser, T. C., and Wallenstein, M. (2007). Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88, 1386–1394. doi: 10.1890/06-0219
- Schimel, J. P., and Bennett, J. (2004). Nitrogen mineralization: Challenges of a changing paradigm. *Ecology* 85, 591–602. doi: 10.1890/03-8002
- Schlesinger, W. H. (2006). Global change ecology. *Trends Ecol. Evol.* 21, 348–351. doi: 10.1016/j.tree.2006.03.004
- Shade, A., Gregory Caporaso, J., Handelsman, J., Knight, R., and Fierer, N. (2013). A meta-analysis of changes in bacterial and archaeal communities with time. *ISME J.* 7, 1493–1506. doi: 10.1038/ismej.2013.54
- Shade, A., Peter, H., Allison, S. D., Baho, D. L., Berga, M., Burgmann, H., et al. (2012a). Fundamentals of microbial community resistance and resilience. *Front. Microbiol.* 3:417. doi: 10.3389/fmicb.2012.00417
- Shade, A., Read, J. S., Youngblut, N. D., Fierer, N., Knight, R., Kratz, T. K., et al. (2012b). Lake microbial communities are resilient after a whole-ecosystem disturbance. *ISME J.* 6, 2153–2167. doi: 10.1038/ismej.2012.56
- Six, J., Frey, S. D., Thiet, R. K., and Batten, K. M. (2006). Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Sci. Soc. Am. J.* 70, 555–569. doi: 10.2136/sssaj2004.0347
- Theuerl, S., and Buscot, F. (2010). Laccases: toward disentangling their diversity and functions in relation to soil organic matter cycling. *Biol. Fertil. Soils* 46, 215–225. doi: 10.1007/s00374-010-0440-5
- Theuerl, S., Dorr, N., Guggenberger, G., Langer, U., Kaiser, K., Lamersdorf, N., et al. (2010). Response of recalcitrant soil substances to reduced N deposition in a spruce forest soil: integrating laccase-encoding genes and lignin decomposition. *FEMS Microbiol. Ecol.* 73, 166–177. doi: 10.1111/j.1574-6941.2010.00877.x
- Thomas, T., Gilbert, J., and Meyer, F. (2012). Metagenomics - a guide from sampling to data analysis. *Microb. Inform. Exp.* 2, 3. doi: 10.1186/2042-5783-2-3
- Treves, D. S., Xia, B., Zhou, J., and Tiedje, J. M. (2003). A two-species test of the hypothesis that spatial isolation influences microbial diversity in soil. *Microb. Ecol.* 45, 20–28. doi: 10.1007/s00248-002-1044-x
- Van der Heijden, M. G. A., Bardgett, R. D., and Van Straalen, N. M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* 11, 296–310. doi: 10.1111/j.1461-0248.2007.01139.x
- Van Veen, J. A., and Paul, E. A. (1979). Conversion of biovolume measurements of soil organisms, grown under various moisture tensions, to biomass and their nutrient content. *Appl. Environ. Microbiol.* 37, 686–692.
- Wallenstein, M. D., and Hall, E. K. (2012). A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. *Biogeochemistry* 109, 35–47. doi: 10.1007/s10533-011-9641-8
- Wertz, S., Degrange, V., Prosser, J. I., Poly, F., Commeaux, C., Guillaumaud, N., et al. (2007). Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance. *Environ. Microbiol.* 9, 2211–2219. doi: 10.1111/j.1462-2920.2007.01335.x
- Wittebolle, L., Marzorati, M., Clement, L., Balloi, A., Daffonchio, D., Heylen, K., et al. (2009). Initial community evenness favours functionality under selective stress. *Nature* 458, 623–626. doi: 10.1038/nature07840
- Worm, B., and Duffy, J. E. (2003). Biodiversity, productivity and stability in real food webs. *Trends Ecol. Evol.* 18, 628–632. doi: 10.1016/j.tree.2003.09.003
- Yarwood, S., Brewer, E., Yarwood, R., Lajtha, K., and Myrold, D. (2013). Soil microbe active community composition and capability of responding to litter addition after 12 years of no inputs. *Appl. Environ. Microbiol.* 79, 1385–1392. doi: 10.1128/AEM.03181-12
- Yergeau, E., Bokhorst, S., Kang, S., Zhou, J., Greer, C. W., Aerts, R., et al. (2012). Shifts in soil microorganisms in response to warming are consistent across a range of Antarctic environments. *ISME J.* 6, 692–702. doi: 10.1038/ismej.2011.124
- Young, I. M., Griffiths, B. S., Robertson, W. M., and McNicol, J. W. (1998). Nematode (*Caenorhabditis elegans*) movement in sand as affected by particle size, moisture and the presence of bacteria (*Escherichia coli*). *Eur. J. Soil Sci.* 49, 237–241. doi: 10.1046/j.1365-2389.1998.00151.x
- Zhang, Q., and Van, T. (2012). Correlation of intracellular trehalose concentration with desiccation resistance of soil *Escherichia coli* populations. *Appl. Environ. Microbiol.* 78, 7407–7413. doi: 10.1128/AEM.01904-12

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 04 April 2013; accepted: 17 August 2013; published online: 05 September 2013.

Citation: de Vries FT and Shade A (2013) Controls on soil microbial community stability under climate change. *Front. Microbiol.* 4:265. doi: 10.3389/fmicb.2013.00265

This article was submitted to *Terrestrial Microbiology*, a section of the journal *Frontiers in Microbiology*.

Copyright © 2013 de Vries and Shade. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

APPENDIX

Table A1 | Pearson correlation coefficients between variables explaining microbial community resistance in Case study 1.

	PC1 axis	F/B ratio	Gram+/Gram– ratio
PC1 axis			
F/B ratio	<u>0.75</u>		
Gram+/Gram– ratio	<u>–0.87</u>	<u>1.7 * 10^{–5}</u>	

Underlines values designate significant correlations ($P < 0.05$).

Table A2 | Pearson correlation coefficients between variables explaining microbial community resilience in Case study 1.

	Protozoa	Micro arthropods	PC1	F/B ratio	Gram+/Gram– ratio	Microbial C/N ratio
Protozoa						
Microarthropods	<u>–0.35</u>					
PC1	<u>0.43</u>	<u>–0.35</u>				
F/B ratio	<u>0.26</u>	0.10	<u>0.36</u>			
Gram+/Gram– ratio	–0.08	0.015	<u>–0.43</u>	<u>–0.67</u>		
Microbial C/N ratio	–0.09	0.18	<u>–0.25</u>	–0.21	0.21	

Underlines values designate significant correlations ($p < 0.05$).

Table A3 | Pearson correlation coefficients between variables explaining microbial community resilience in Case study 2.

	F/B ratio	PC1	Gram+/Gram– ratio	Microbial biomass
F/B ratio				
PC1	<u>–0.71</u>			
Gram+/Gram– ratio	0.59	<u>–0.97</u>		
Microbial biomass	0.63	<u>–0.90</u>	<u>0.92</u>	

Underlines values designate significant correlations ($P < 0.05$).

Table A4 | Axis loadings of individual PLFA in Case study 1.

	PC1 score	PC2 score
i.C14.0	–0.27999	–3.67E-05
C14.0	–0.25943	–0.15196
i.C15.0	–0.28069	–0.04368
a.C15.0	–0.28717	–0.0606
C15.0	0.01904	–0.19215
X3.hydroxy.C12.0	–0.03272	–0.17065
methyl.C16.0	–0.00739	0.243093
C16.0	0.007681	0.201414
C16.1w7	0.024379	–0.45926
X10.methyl.C16.0	0.110574	0.294226
i.C17.0	0.168	0.275936
a.C17.0	0.220549	0.157182
i.C17.1w6	0.067054	–0.13123
n.methyl.C17.0	0.250714	–0.13939
C17.0	0.266306	–0.05702
C17.0.cyclo	0.205752	–0.30061
X10.methyl.C17.0	0.194742	–0.16161
C18.0	0.266156	0.049383
C18.1	0.058706	0.156452
trans.C18.1w9	0.241332	–0.06568
cis.C18.1w9	0.222525	–0.07776
X10.methyl.C18.0	0.267682	–0.11721
cis.C18.2w6	0.020817	0.373708
C18.3	–0.0547	0.096218
C19.0.cyclo	0.250798	0.192782
C20.0	0.251506	–0.11747

Axis 1 explained 43% of variation, axis 2 explained 14% of variation. PLFAs marked green, red, and yellow are representative of Gram-positive, Gram-negative, and fungi, respectively.

Table A5 | Axis loadings of individual PLFA in Case study 2.

	PC1 score	PC2 score
Methyl.2.hydroxydecanoate	0.218345	−0.04861
i.C14.0	0.215282	−0.04387
C14.0	−0.11147	−0.16243
i.C15.0	−0.21785	−0.1755
a.C15.0	0.241314	0.032725
C15.0	0.241365	0.052056
X14.methyl.C15.0	−0.24439	−0.03885
X3.hydroxy.C12.0	−0.23551	−0.11515
methyl.C16.0	−0.24441	−0.0385
C16.0	−0.16101	0.30498
C16.1w7	0.222117	0.165607
C16.1 and C17.0merged	0.057127	−0.32505
i.C17.0	0.117704	−0.11584
a.C17.0	0.130821	0.029151
X2.hydroxy.C14.0	−0.14187	−0.12541
i.C17.1w6	0.201859	−0.01235
n.methyl.C17.0	−0.08864	−0.21781
C17.0	0.107442	0.153301
C17.0cyclo	0.2358	0.02104
X10.methylC17.0	−0.23775	−0.04534
X3.OH.C14.0	−0.23064	−0.10203
C18.0	−0.02399	0.352795
C18.1	0.037532	0.107055
trans.C18.1w9	−0.12215	0.366805
cis.C18.1w9	0.229866	0.126425
X10.methyl.C18.0	−0.08328	0.358585
cis.C18.2w6	−0.15507	0.3251
X2.hydroxy.hexadecanoic.methyl.ester	0.114723	−0.02548
C18.3	−0.14311	0.117455
C19.0.cyclo	−0.2067	0.199156
C20.0	−0.14997	−0.05973

Axis 1 explained 53% of variation, axis 2 explained 15% of variation. PLFAs marked green, red, and yellow are representative of Gram-positive, Gram-negative, and fungi, respectively.

Table A6 | CA axis scores for the 20 most abundant bacterial taxa in Case study 3.

OTU_ID	CA1	CA2	Total no. seqs	Consensus lineage
7721	−0.052755783	0.128596257	1232	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Hyphomicrobiaceae; g__Rhodoplanes; s__
7592	−0.277985389	0.384482383	559	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Bradyrhizobiaceae
5179	−0.031267675	0.274724392	477	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Hyphomicrobiaceae; g__Rhodoplanes; s__
4450	−0.368835276	−0.01104972	434	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Syntrophobacterales; f__Syntrophobacteraceae; g__s__
3664	−0.203535915	0.204631553	397	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Hyphomicrobiaceae; g__Rhodoplanes; s__
232	0.317417102	−0.327066878	291	k__Bacteria; p__Acidobacteria; c__Acidobacteria-5; o__s__f__g__s__
6514	0.374990616	0.045919714	275	k__Bacteria; p__Acidobacteria; c__Acidobacteria-2; o__s__f__g__s__
3615	0.148161131	0.186057802	271	k__Bacteria; p__Acidobacteria; c__Acidobacteria-2; o__s__f__g__s__
6194	0.272492541	−0.440098295	243	k__Bacteria; p__Nitrospirae; c__Nitrospira; o__Nitrospirales; f__Nitrospiraceae; g__Nitrospira; s__
1968	0.406077387	−0.153510689	236	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Hyphomicrobiaceae; g__Rhodoplanes; s__
2877	−0.452435542	0.331937119	214	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Flavobacteriaceae; g__Flavobacterium
5980	1.037736522	−0.623269339	211	k__Bacteria; p__Acidobacteria; c__Acidobacteria-2; o__s__f__g__s__
3158	0.511248597	−0.360815747	177	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__s__f__g__s__
741	0.561319057	−0.242946783	166	k__Bacteria; p__Acidobacteria; c__Acidobacteria; o__Acidobacteriales; f__Koribacteraceae; g__s__
9410	0.330214073	0.053950661	162	k__Bacteria; p__Acidobacteria; c__Acidobacteria; o__Acidobacteriales; f__Koribacteraceae; g__Candidatus Koribacter; s__
4283	−0.21498302	0.197813008	158	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Rhodobiaceae; g__s__
2587	−0.012292612	0.229939177	157	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Hyphomicrobiaceae; g__Rhodoplanes; s__
2250	0.126551331	−0.137573106	154	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Syntrophobacterales; f__Syntrophobacteraceae; g__s__
8618	−0.03958676	0.222012965	153	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Sinobacteraceae; g__s__

CA axis 1 explained 5.47% and CA axis 2 explained 5.05% of variation.



Plant soil interactions alter carbon cycling in an upland grassland soil

Bruce C. Thomson^{1*}, Nick J. Ostle², Niall P. McNamara², Simon Oakley², Andrew S. Whiteley³, Mark J. Bailey¹ and Robert I. Griffiths¹

¹ Centre for Ecology and Hydrology, Wallingford, Oxfordshire, UK

² Centre for Ecology and Hydrology, Lancaster, Lancashire, UK

³ School of Earth and Environment, The University of Western Australia, Crawley, WA, Australia

Edited by:

Per Bengtson, Lund University, Sweden

Reviewed by:

Mark A. Bradford, Yale University, USA

Feike A. Dijkstra, The University of Sydney, Australia

*Correspondence:

Bruce C. Thomson, Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, OX10 8BB, UK
e-mail: brth@ceh.ac.uk

Soil carbon (C) storage is dependent upon the complex dynamics of fresh and native organic matter cycling, which are regulated by plant and soil-microbial activities. A fundamental challenge exists to link microbial biodiversity with plant-soil C cycling processes to elucidate the underlying mechanisms regulating soil carbon. To address this, we contrasted vegetated grassland soils with bare soils, which had been plant-free for 3 years, using stable isotope (¹³C) labeled substrate assays and molecular analyses of bacterial communities. Vegetated soils had higher C and N contents, biomass, and substrate-specific respiration rates. Conversely, following substrate addition unlabeled, native soil C cycling was accelerated in bare soil and retarded in vegetated soil; indicative of differential priming effects. Functional differences were reflected in bacterial biodiversity with *Alphaproteobacteria* and *Acidobacteria* dominating vegetated and bare soils, respectively. Significant isotopic enrichment of soil RNA was found after substrate addition and rates varied according to substrate type. However, assimilation was independent of plant presence which, in contrast to large differences in ¹³CO₂ respiration rates, indicated greater substrate C use efficiency in bare, *Acidobacteria*-dominated soils. Stable isotope probing (SIP) revealed most community members had utilized substrates with little evidence for competitive outgrowth of sub-populations. Our findings support theories on how plant-mediated soil resource availability affects the turnover of different pools of soil carbon, and we further identify a potential role of soil microbial biodiversity. Specifically we conclude that emerging theories on the life histories of dominant soil taxa can be invoked to explain changes in soil carbon cycling linked to resource availability, and that there is a strong case for considering microbial biodiversity in future studies investigating the turnover of different pools of soil carbon.

Keywords: upland acidic grassland, bacteria, substrate-specific respiration, priming effects, substrate carbon use efficiency, T-RFLP, RNA stable isotope probing, soil organic carbon

INTRODUCTION

In terrestrial ecosystems the amount of organic C stored in soil is largely dependent upon a dynamic balance between inputs, mostly from plants (Hopkins and Gregorich, 2005), and respired outputs from soil (Kuzyakov, 2002). Plant communities directly affect soil C storage as they provide a range of C resources, mainly in the forms of root exudates and detritus, which are decomposed at different rates by the soil biota (De Deyn et al., 2008; Paterson et al., 2009). Soil microbial communities are known to be incredibly diverse and are essential for decomposition (Schimel and Schaeffer, 2012), yet there is little evidence that below-ground microbial biodiversity affects soil organic matter turnover (Nannipieri et al., 2003). Indeed, it has been suggested that abiotic factors, and not microbial community structure, regulate mineralization rates (Kemmitt et al., 2008). With major advances in understanding global soil microbial biodiversity (Fierer et al., 2009), it is also imperative we improve our understanding of the diversity and activity of soil microbes within a functional context,

particularly to elucidate the role of soil biodiversity in the terrestrial C balance and wider sustainability of soils (Bardgett et al., 2008; Paterson et al., 2009).

When studying soil organic matter dynamics, isotope labeled substrate additions often reveal changes in the decomposition rates of unlabeled native soil organic carbon (SOC); a phenomenon termed *the priming effect* (Bingeman et al., 1953). Priming effects can be either positive or negative and are thought to be important in determining a soil's C storage potential (Kuzyakov et al., 2000). Positive priming describes increased native soil organic matter cycling following fresh substrate inputs, whereas negative priming is the converse decline in native cycling following inputs. The underlying mechanisms affecting the magnitude and direction of priming are not yet fully understood, despite many lab and field based studies examining potential factors, such as: vegetation presence (Brant et al., 2006; Guenet et al., 2010); resource availability (Kuzyakov and Demin, 1998; Fontaine et al., 2004a; Kuzyakov and Bol, 2006); and substrate

quality and quantity (De Nobili et al., 2001; Pascault et al., 2013). There are further complications as to the source of the native carbon being assessed in priming effects studies, be it from turnover of non-living soil organic matter (termed real priming effects), or from the endogenous metabolism of microorganisms (apparent priming effects) (Dalenberg and Jager, 1981; Bell et al., 2003; Blagodatskaya and Kuzyakov, 2008). We do not enter this debate here, and will henceforth use the term SOC to refer to carbon present in both living and non-living components of existing soil organic matter, prior to fresh organic carbon (FOC) addition.

Several recent theories have proposed that a better understanding of the role of soil microbial communities may be required to fully understand priming effects (Fontaine et al., 2003; Fontaine and Barot, 2005; Kuzyakov and Bol, 2006). Fontaine et al. (2003), in particular, detailed hypothetical models based on microbial population dynamics and soil nutrient conditions which placed microbial diversity and activity as a key driver of priming effects. Here, microbial populations with different life histories are considered to respond differently to added carbon inputs, where copiotrophs (*r*-strategists) rapidly utilize the added FOC, whereas oligotroph (*K*-strategist) populations more efficiently channel the energy gained from FOC into degrading SOC. This theory also emphasizes that plants may have an important role in driving soil priming effects as they determine soil fertility, and affect microbial community structure and functional activity by providing FOC inputs of differing resource value (Griffiths et al., 2003; Johnson et al., 2003; Bardgett, 2005).

In Fontaine's theory (Fontaine et al., 2003) it was posited that, after utilizing FOC, particular microbial populations increase in size resulting in a greater turnover of SOC. Therefore, it is supposed that monitoring differences in soil microbial community structure, alongside soil C fluxes, could provide insights into the microbial populations contributing to the direction and magnitude of soil priming effects. Whilst some studies have simply monitored microbial communities in conjunction with soil respiration measures (Falchini et al., 2003; Landi et al., 2006; De Graaff et al., 2010; Guenet et al., 2010), others have traced ^{13}C -labeled substrates into lipid biomarkers (Nottingham et al., 2009). Additionally, stable isotope probing (SIP) of nucleic acids has been undertaken to permit more detailed molecular analyses of the diversity of active microbes (Bernard et al., 2007, 2012; Pascault et al., 2013). These few studies have shown that population shifts in active microbes can be associated with changes in soil C turnover, yet more studies are still needed to synthesize and strengthen current theories on the relationships between microbial biodiversity and the cycling of different pools of SOC.

In a previous study we showed that field-based removal of vegetation from an acidic grassland decreased soil resource availability, soil respiration rates and changed bacterial community structure to favor presumed oligotrophic taxa (Thomson et al., 2010). Using these same treatments, here we seek to investigate in more detail the role of vegetation (presence or absence), resource availability and bacterial biodiversity on the specific cycling of labile FOC and native SOC. Three ^{13}C labeled substrates will be used to examine how the type of substrate, as a proxy for labile FOC resources, affects the observed patterns. Total bacterial community responses will be assessed and we also seek to

explore the use of an RNA-based SIP approach to investigate active communities degrading different C sources, and to further our understanding of soil priming effects.

MATERIALS AND METHODS

FIELD SITE, SOIL SAMPLING, AND PROPERTIES

Soil (10 cm depth) was collected in autumn at an upland, grassland experiment located at the Rigg Foot field site, Sourhope, Scotland, UK (GR NT854 196 at 300 m above sea level). In a previous experiment, soil bacterial community structure and respiration rates were found to vary at the field scale as a result of the topography of the experimental site, which led to water-logging in certain areas (Thomson et al., 2010). Therefore, in this experiment we chose to examine replicates from within a single experimental block. The experimental block comprised of a 10 m² area of control grassland (hereon referred to as vegetated treatment), within which a 4 m² defoliated plot had been established and covered with a permeable black membrane, for 3 years preceding this experiment, to prevent plant growth (hereon referred to as bare treatment). Three replicate monoliths (60 cm × 60 cm) were sampled from the vegetated and bare areas within close proximity of each other to minimize variation in environmental conditions; ensuring that the main difference between treatments was the presence or absence of plants. Samples were transported immediately to the laboratory where they were fresh sieved to 2 mm, roots removed by hand then stored at 4°C until required for analyses and ^{13}C substrate addition experiments.

Experimental microcosms were set-up using 100 ml air-tight containers (Lock & Lock, Armoriga, Petersfield, UK) modified to include a rubber septum (SubaSeal, Sigma-Aldrich, Poole, UK) for headspace gas sampling. Triplicate microcosms (20 g sieved soil) were established to enable destructive sampling at 0 (prior to substrate addition), 24, 72, and 193 h to examine extracted RNA, and total and functional bacterial communities in vegetated and bare treatments (equaling a total of 78 microcosms). These replicates were also used for CO₂ sampling on ten occasions (0, 12, 24, 48, 72, 96, 120, 144, 168, and 193 h).

C and N contents were analyzed with an Elementar Vario EL elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany). Deionized water was added to soil to provide a 1:1 mixture and pH was measured using an HI 8424 pH meter (Hanna Instruments Srl, Italy). Moisture (%) was calculated by drying overnight at 105°C then re-weighing to measure water loss. Microbial biomass measurements were based on analysis of phospholipid fatty acids (PLFAs), using a previously described method (Bardgett et al., 1996). Phospholipids were extracted from 1.5 g soil (fresh weight) and extracts analyzed using an Agilent 6890 Gas Chromatograph (Zebron ZB-5 Capillary GC Column 60 m × 0.32 mm × 0.25 μm). Individual PLFA peaks were identified based on retention times of known bacterial fatty acid standards (Sigma-Aldrich, Dorset, UK). Concentrations of individual fatty acids were calculated using a standard 19:0 peak as a reference. Total soil microbial PLFA concentrations were calculated from all measured PLFAs (15:0, 15:0i, 15:0a, 14:0, 16:0i, 16:0, 16:1, 16:1ω5, 16:1ω7, 17:1ω8, 7Me-17:0, br17:0, 17:0i, 17:0a, br18:0, 18:1ω5, 18:1ω7, 18:0, 19:1, 7,8cy-19:0).

¹³C SUBSTRATE INCUBATION EXPERIMENT

Fully labeled (99 atom%) ¹³C-glucose, ¹³C-glycine, and ¹³C-phenol (Cambridge Isotope Laboratories Inc. Andover, UK) were weighed and dissolved in distilled H₂O. The ¹³C labeled substrate solutions were sterilized through a 0.2 μm filter (Sartorius, Göttingen, Germany) and added at a rate of 0.4 mg ¹³C g⁻¹ dry soil. For the glucose, glycine and phenol incubations 200, 250, and 220 μl of substrate solution was added, respectively to each microcosm. Filter sterilized, distilled H₂O was also used as a control treatment. After the addition of substrate solution or distilled H₂O, soil was stirred once to mix substrates before the initial gas sampling (0 h). Moisture contents were maintained throughout by weighing and rewetting. At each sampling, microcosms were sealed shut and headspace gas samples taken immediately and after 3 h to determine the soil respiratory CO₂ flux. Microcosms were maintained at approximately 15°C (mean summer temperature) in a temperature controlled room.

CO₂ AND ¹³C-CO₂ ANALYSES

CO₂ measurements were made with a Perkin Elmer Autosystem XL Gas Chromatograph (Perkin Elmer, Waltham, MA, USA), fitted with a flame ionization detector, operated at 350°C. CO₂ was isothermally separated with nitrogen as the carrier gas flowing at 30 cm³ min⁻¹ on a 2 m column packed with Poropak Q. The detector response was calibrated using a certified gas standard containing 500 μl l⁻¹ CO₂ in nitrogen (Air Products, Leeds, UK). Between sampling events lids were left open to allow microcosms to vent.

To analyze respired ¹³CO₂, headspace gas sampled from microcosms was injected into a Trace Gas pre-concentrator unit (Micromass, Manchester, UK), and ¹³C content was subsequently quantified using gas chromatography isotope ratio mass spectrometry (GC-IRMS) (Micromass, Manchester, UK). Analysis was performed at the NERC Life Sciences Mass Spectrometer Facility, CEH Lancaster using their standard protocols (uncertainty better than 0.3%). Abundances of ¹³C were expressed as ¹³C atom% i.e.:

$$^{13}\text{C atom\%} = [(R_{\text{sample}}) \div (R_{\text{sample}} + 1)] \times 100$$

where R_{sample} is the ¹³C:¹²C ratio of analyte CO₂.

RESPIRATION CALCULATIONS

The amount of substrate ¹³C respired as ¹³CO₂-C (mg g⁻¹ dry soil h⁻¹) was calculated for each sampling event using the equation:

$$^{13}\text{C from substrate} = [(\text{respCO}_2) \div 100] \times ^{13}\text{C atom\% excess}$$

where respCO_2 = the flux of total CO₂-C respired from soil over 3 h at each time point, and ¹³C atom% excess = the ¹³C atom% difference between two measurements taken 3 h apart at each sampling time point.

Rates of unlabeled SOC turnover following substrate addition were calculated using an equation previously described by Fontaine et al. (2004a,b):

$$\text{unlabeled SOC turnover} = (\text{tot-substrate}^{13}\text{C} - \text{tot}_{\text{soilresp}})$$

where $\text{tot-substrate}^{13}\text{C}$ = total respiration minus ¹³C respired following substrate amendment (mg g⁻¹ dry soil h⁻¹), and $\text{tot}_{\text{soilresp}}$ = total soil respiration from the water control treatment (mg g⁻¹ dry soil h⁻¹).

TOTAL BACTERIAL COMMUNITY STRUCTURE

Nucleic acids were extracted from 0.5 g soil using a previously described method (Griffiths et al., 2000) and were finally re-suspended in molecular-grade H₂O. Terminal restriction fragment length polymorphism (T-RFLP) analysis of soil bacterial communities was performed using diluted extracted nucleic acids with 16S rRNA gene primers 63F (Marchesi et al., 1998) (fluorescently-labeled with D4 blue dye) (Sigma-Proligo, Dorset, UK) and 519R (Lane, 1991) (MWG Biotech, London, UK). Amplicons were then digested with *MspI* restriction enzyme (New England Biolabs Inc., Ipswich, MA, USA) prior to fragment analysis using a Beckman Coulter CEQ 2000XL capillary sequencer (Beckman Coulter Corporation, California, USA). Terminal restriction fragment (T-RF) relative abundances were calculated as the ratio between the fluorescence of individual T-RFs and the total integrated fluorescence of all T-RFs.

STABLE ISOTOPE ANALYSES OF SOIL RNA

Soil RNA was purified from total nucleic acids extractions using a RNA/DNA minikit (Qiagen, Crawley, UK) according to the manufacturer's instructions. To assess the amount of substrate ¹³C incorporation into soil RNA, ¹³C:¹²C isotope analysis was performed using a method previously described (Manefield et al., 2002a). RNA (1 μg) was cut with sucrose to provide a minimum C content of 25 μg. Samples were then freeze-dried for 16 h prior to combustion to CO₂ using a Carlo-Erba N1500 Elemental Analyser (Carlo-Erba, Valencia, CA, USA). Abundances of ¹³C were expressed as δ¹³C values, using the following equation:

$$\delta^{13}\text{C} (\%) = [(R_{\text{sample}} \div R_{\text{standard}}) - 1] \times 1000$$

where R_{sample} and R_{standard} are the ¹³C:¹²C ratio of soil extracted RNA and Pee Dee Belemnite standard, respectively.

Substrate ¹³C incorporated into RNA (μg μg⁻¹ RNA) was calculated for each sampling event using the following equation:

$$^{13}\text{C substrate in RNA} = [(\text{RNA}^{13}\text{C atom\%} \div 100) \times 25] - \text{sucrose}^{13}\text{C}$$

where $\text{RNA}^{13}\text{C atom\%}$ = ¹³C atom% of RNA; $\text{sucrose}^{13}\text{C}$ = the amount of ¹³C (μg) in sucrose standard used to cut extracted RNA; and 25 = minimum C content in μg.

To investigate microbial substrate C use efficiency, we examined the amount of substrate ¹³C assimilated into RNA compared to the amount of substrate ¹³C respired, based on a previously described calculation (Frey et al., 2001; Brant et al., 2006):

$$\text{substrate C use efficiency} = [(\text{RNA}^{13}\text{C}) \div (\text{RNA}^{13}\text{C} + \sum \text{substrate}^{13}\text{C})]$$

where $\text{RNA }^{13}\text{C}$ = the amount of ^{13}C incorporated into extracted RNA ($\mu\text{g } \mu\text{g}^{-1} \text{ RNA}$), and substrate ^{13}C = cumulative substrate-specific respiration ($\mu\text{g } \text{g}^{-1} \text{ dry soil}$).

RNA STABLE ISOTOPE PROBING

SIP and denaturing gradient gel electrophoresis (DGGE) analysis were performed similarly to Manefield et al. (2002a). Extracted RNA was subjected to isopycnic density gradient centrifugation in caesium trifluoroacetate gradients containing deionised formamide. Gradients were loaded with 500 ng of extracted RNA and spun at $136,000 \times g$ for 42 h at 20°C . Following centrifugation, samples were fractionated for 30 s per fraction at a flow rate of $3.3 \mu\text{l s}^{-1}$, resulting in a total of 20 fractions per sample. RNA was precipitated from each gradient fraction by incubating at -20°C with isopropanol followed by centrifugation at $16,000 \times g$ for 30 min at 4°C . Finally, fractions were dried under vacuum and RNA dissolved in RNase-free water.

Precipitated RNA from density gradient fractions was reverse transcribed using reverse primer 519r (MWG Biotech, London, UK) and avian myeloblastosis virus reverse transcriptase (Promega, Southampton, UK). cDNA was then amplified using bacterial 16S rRNA gene primers GC338F and 519r (MWG Biotech, London, UK). DGGE analysis was performed with a 10% (wt/vol) acrylamide gel containing a denaturant gradient of 30–60%. Denaturing gradient gels were cast and run using the Ingeny PhorU2 system (Goes, The Netherlands) at 60°C and 200 V for 8 h. Approximate amounts of RT-PCR product were loaded into each lane on the gel to examine active bacterial communities. Gels were subsequently stained with SYBR gold nucleic acid gel stain (Molecular Probes, Invitrogen, Paisley, UK), then visualized by UV trans-illumination.

STATISTICAL ANALYSES

Cumulative respiration data, RNA ^{13}C incorporation and substrate C use efficiency were examined for significant differences between treatments with a One-Way analysis of variance (ANOVA) combined with Tukey's *post hoc* testing with a family error rate set at 5, using MINITAB release 14 (MINITAB Inc.). Statistical analyses of soil bacterial communities were performed with the vegan library (Oksanen et al., 2009) of the R software package (R Core Development Team, 2005). Briefly, a Bray-Curtis distance matrix of between sample dissimilarities was calculated and subsequently represented through two-dimensional non-metric multidimensional scaling (NMDS), using the metaMDS function. Differences in communities were quantified by permutational multivariate analysis of variance (PERMANOVA) using the adonis function, and group dispersions (beta diversity) were further assessed using the betadisper function. To assess differences in the relative abundances of particular terminal restriction fragments (T-RFs) between treatments, similarities of percentages (SIMPER) analysis (Clarke, 1993) was performed using the PAST statistical package (<http://folk.uio.no/ohammer/past>).

RESULTS

EFFECTS OF VEGETATION REMOVAL ON SOIL PROPERTIES AND BIOMASS

After 3 years without plant cover, soil C and N contents, and microbial biomass were significantly lower in the bare soil

treatment compared to the vegetated soil ($P < 0.05$, $F = 70.49$, $F = 63.78$, and $F = 23.27$ for soil C and N contents, and microbial biomass, respectively). The C:N ratio was less in vegetated soil, although not significantly so. Additionally, there were no significant differences in % moisture and soil pH between the two soil treatments (Table 1).

GROSS RESPIRATION IS HIGHER IN VEGETATED SOIL

Cumulative soil basal respiration was significantly higher ($P < 0.05$, $F = 315.47$) in vegetated soil compared to the bare (Figure 1), by the end of the experiment. Similarly, total respiration following substrate addition was greatest in vegetated soil regardless of which substrate was added ($P < 0.05$, glucose $F = 13.74$; glycine $F = 184.84$; phenol $F = 35.17$). By examining the shape of the respiration curves, rates were shown to differ depending on substrate added. Following glucose addition, respiration was greatest at the start of the experiment, tailing off toward the end. Yet, for glycine and phenol lower levels of respiration were sustained for a longer period of time, and displayed a biphasic respiration response (data not shown). In both treatments, mean cumulative total respiration rates were ranked in the following order: glycine > glucose > phenol. In vegetated soil, mean cumulative total respiration was significantly higher following glycine addition compared to the glucose and phenol incubations; however, there was no significant difference between glucose and phenol mean cumulative total respiration (based on confidence interval ranges following a One-Way ANOVA with Tukey's *post hoc* test). Contrastingly, in bare soil, phenol total respiration was significantly less than glucose or glycine, and there were no significant differences between the glucose and glycine treatments. Basal respiration was more than two times greater in the vegetated control treatment than the bare, though this magnitude of difference was not observed in total respiration rates. This emphasizes that C processing, in terms of total amounts of respiration following substrate addition, differed to that of native organic matter respiration between the two treatments.

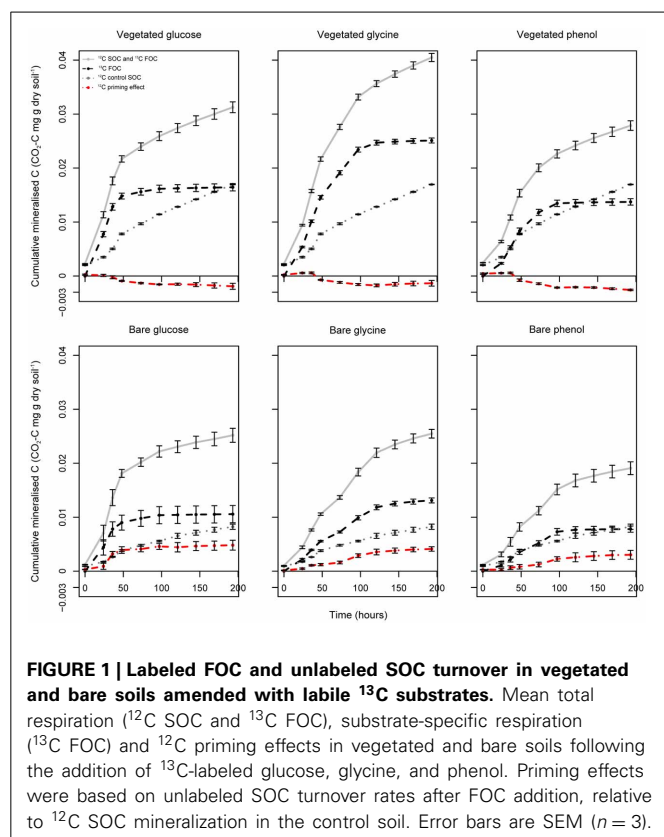
INCREASED FOC MINERALISATION IN VEGETATED SOIL

In order to specifically examine proportions of ^{13}C labeled FOC and unlabeled SOC mineralized, headspace gases were analyzed using GC-IRMS to determine the specific amount of respired $^{13}\text{CO}_2$. By the end of the incubation, cumulative substrate-specific respiration was significantly higher in vegetated soil compared to bare soil for all substrate additions ($P < 0.05$, ^{13}C -glucose $F = 11.32$; ^{13}C -glycine $F = 336.72$; ^{13}C -phenol $F = 51.14$) (Figure 1). Over the entire duration of the experiment, ^{13}C -substrates were mineralized in the following order: glycine > glucose > phenol for both vegetated and bare soils. In vegetated soil, total cumulative ^{13}C -substrate mineralization was significantly different between all substrates (based on confidence interval ranges following a One-Way ANOVA with Tukey's *post hoc* test). In bare soil, however, there were found to be no significant differences between glucose and glycine, and glucose and phenol mineralization rates. Both substrate-induced and substrate-specific respiration data revealed very similar patterns in terms of treatment differences between bare and vegetated soils, and soil respiration responses to substrate amendment.

Table 1 | Mean soil properties in vegetated and bare soils.

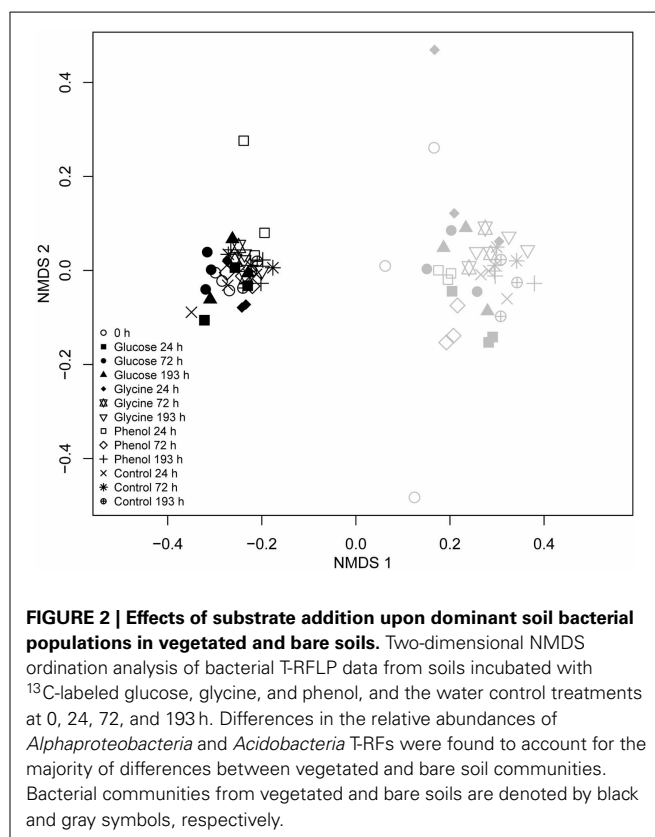
	C (g g ⁻¹ soil)	N (g g ⁻¹ soil)	C:N	Soil pH	Moisture (%)	Microbial biomass (PLFA μg g ⁻¹ dry soil)
Vegetated	0.119 (0.001)	0.0094 (0.0002)	12.70 (0.33)	4.81 (0.03)	54.00 (0.58)	102.40 (9.11)
Bare	0.097 (0.002)	0.0070 (0.0002)	13.57 (0.26)	4.77 (0.01)	53.67 (1.67)	57.47 (1.91)
<i>P</i>	0.001	0.001	0.109	0.22	0.86	0.008
<i>F</i>	70.49	63.78	4.24	0.17	0.04	23.27

Standard error of the mean (SEM) in parentheses (*n* = 3). Significant differences calculated using One-Way ANOVA.



ACCELERATED SOC TURNOVER IN BARE SOILS FOLLOWING FOC ADDITION

The difference in ¹²C respiration between substrate amended soils and the water controls was inferred to have arisen from the decomposition of SOC in response to the addition of FOC (the priming effect). The direction and intensity of unlabeled SOC turnover varied with soil treatment, FOC type and incubation time (**Figure 1**, red lines). For all FOC additions there was a significant difference in mean cumulative SOC turnover between vegetated and bare soils (*P* < 0.05, glucose *F* = 39.29; glycine *F* = 68.03; phenol *F* = 44.58). For vegetated soil, irrespective of the FOC used, addition of labile ¹³C led to a cumulative decrease in SOC mineralization compared to the control incubation (11, 7, and 15% mean decrease for glucose, glycine and phenol, respectively). Conversely, in the bare soils, addition of low molecular weight FOC brought about a cumulative increase



in SOC decomposition relative to the water control (61, 51, and 38% mean increase for glucose, glycine and phenol, respectively). Within each soil treatment, the cumulative amount of SOC mineralized over the duration of the experiment was unaffected by the type of FOC added (vegetated soil *P* = 0.21; bare soil *P* = 0.29). This shows that regardless of the nature of FOC added, the presence or absence of vegetation had significant consequences for unlabeled SOC turnover; with consistent positive priming (accelerated unlabeled SOC turnover) in bare soils and negative priming (retarded unlabeled SOC turnover) in vegetated soils.

TOTAL BACTERIAL COMMUNITY STRUCTURE

Two-dimensional NMDS analysis was performed to explore any differences in bacterial community structure between vegetated and bare soils across the incubations (**Figure 2**). The presence or absence of plants was shown to be the main factor responsible

for community differences as vegetated and bare soils were clearly separated along the first axis. The effect of the vegetated and bare treatments on soil bacterial community structure was also shown to be significant when analyzed with PERMANOVA ($R^2 = 0.53$, $P < 0.05$). NMDS analysis also showed little change in bacterial communities after exposure to FOC or at different sampling events throughout the incubation. However, in the bare treatment there were distinct differences in the bacterial communities analyzed prior to FOC addition as these samples grouped separately from all other samples. Additionally, bare soil communities across all substrate additions and time points were significantly more variable than vegetated soil communities (betadisper, $P < 0.05$).

SIMPER analysis highlighted that the T-RFs which accounted for approximately 55% of the dissimilarity between the vegetated and bare soil bacterial communities were 53, 55, 76, 77, 78, 97, 99, 110, 111, 112, 113, 227, 229, and 396 nucleotides (n.t.) in length. Using data from a previous *in silico* endonuclease restriction digest of 16S rRNA gene clone libraries from vegetated and bare soils (Thomson et al., 2010), we could confidently identify all but two (97 and 99 n.t.) of these T-RFs at the class level (Table 2). Only minor variations in the relative abundances of these taxa occurred over time and between substrate additions in vegetated and bare soils, with the main treatment differences being a significantly greater relative abundance of *Alphaproteobacteria* T-RFs in vegetated soil ($P < 0.05$, $F = 104.18$) and *Acidobacteria* T-RFs in bare soil ($P < 0.05$, $F = 16.86$) (Figure 3). Furthermore, the mean ratio of *Alphaproteobacteria* to *Acidobacteria* T-RFs was significantly higher ($P < 0.05$, $F = 51.64$) in vegetated soil than bare soil, with values of 1.69 and 1.22, respectively.

¹³C INCORPORATION INTO SOIL RNA

Dynamic measurements of ¹³C incorporation into extracted soil RNA were performed to assess microbial utilization of added

FOC. Differences in rates of ¹³C incorporation were particularly pronounced for glucose amended soils, as the total ¹³C incorporation into RNA from glucose was three- to four-fold greater than glycine or phenol ¹³C. Throughout the experiment there was no consistent difference in RNA ¹³C incorporation rates between vegetated and bare soils; significant differences were only observed 24 h after glucose addition ($P < 0.05$, $F = 8.31$), and 72 h and 193 h after phenol addition ($P < 0.05$, $F = 11.77$; $P = 0.004$, $F = 31.92$) (Figure 4A).

To further investigate FOC dynamics across treatments, we calculated and contrasted substrate ¹³C use efficiencies, determined as the ratio of RNA incorporated ¹³C to respired substrate ¹³C (Figure 4B). Here we assume RNA incorporation rates reflect the amount of FOC being assimilated into cellular components as opposed to being respired for catabolic metabolism (Manzoni et al., 2012). In terms of the differences between applied substrates, glucose ¹³C was consistently utilized more efficiently than the other two substrates independently of vegetation presence or absence. Additionally, mean utilization efficiencies were consistently higher in bare soil independent of FOC type, with significant differences 24 h after glucose addition ($P < 0.05$, $F = 12.25$); 24, 72, and 193 h following glycine addition ($P < 0.01$, $F = 22.28$; $P = 0.01$, $F = 19.81$; $P < 0.01$, $F = 33.01$); and 193 h after phenol addition ($P < 0.01$, $F = 51.12$).

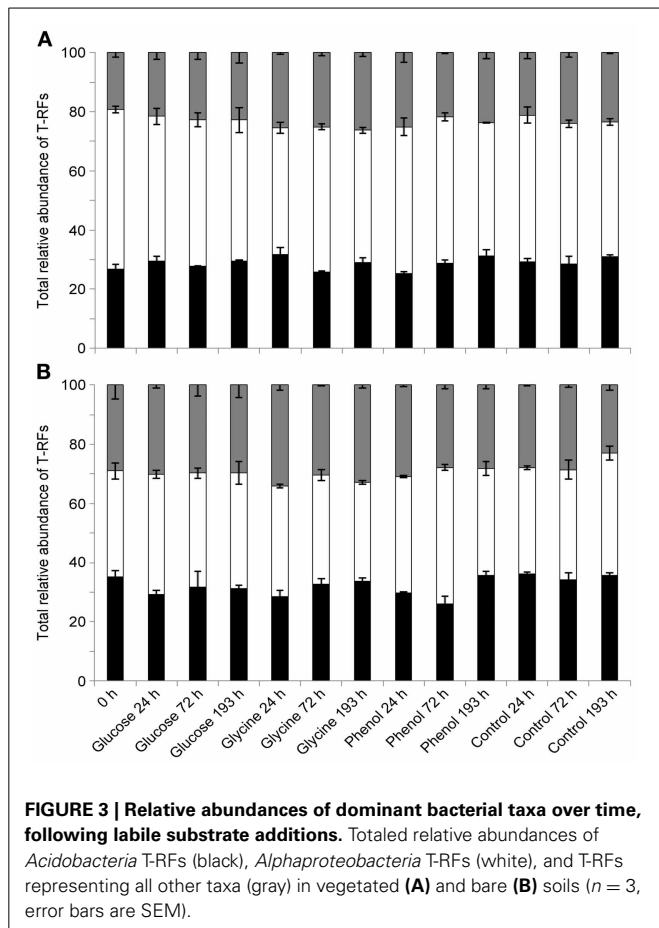
SIP REVEALS NO DIFFERENCE BETWEEN “TOTAL” AND “ACTIVE” COMMUNITIES

To examine the bacterial populations actively utilizing the added substrates, density gradient centrifugation was used to isolate ¹³C enriched RNA, prior to molecular analyses. Consistent with previous studies, reproducible linear gradients were achieved (Figure 5A) spanning a density range of approximately 1.75–1.85 g ml⁻¹ (Manefield et al., 2002b; Whiteley et al., 2007). We

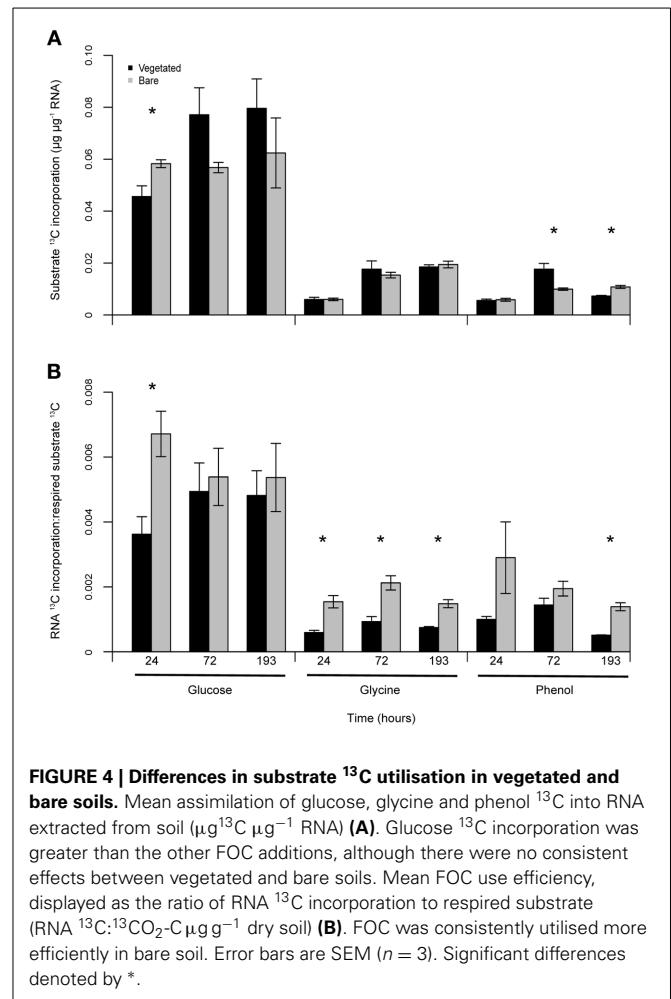
Table 2 | Analysis of dominant bacterial taxa in vegetated and bare soils.

	Contribution	Cumulative %	Vegetated soil abundance	Bare soil abundance	ID
55	3.185	8.767	0.0798	0.143	<i>Acidobacteria</i>
113	2.645	16.05	0.133	0.08	<i>Alphaproteobacteria</i>
112	2.098	21.82	0.00455	0.0457	<i>Alphaproteobacteria</i>
227	1.799	26.77	0.0632	0.0275	<i>Acidobacteria</i>
99	1.682	31.41	0.00867	0.0422	Unclassified
53	1.343	35.1	0.0595	0.0828	<i>Acidobacteria</i>
78	1.089	38.1	0.0334	0.0123	<i>Alphaproteobacteria</i>
76	1.071	41.05	0.0311	0.0104	<i>Alphaproteobacteria</i>
110	0.9677	43.71	0.11	0.0986	<i>Alphaproteobacteria</i>
111	0.9456	46.32	0.00647	0.0163	<i>Alphaproteobacteria</i>
97	0.9057	48.81	0.0033	0.0213	Unclassified
229	0.8751	51.22	0.0477	0.0314	<i>Acidobacteria</i>
77	0.8027	53.43	0.0189	0.00328	<i>Alphaproteobacteria</i>
396	0.6228	55.14	0.0332	0.0258	<i>Alphaproteobacteria</i>

Mean relative abundances of dominant T-RFs contributing to approximately 55% of the total dissimilarity ($n = 39$). Results from SIMPER analysis are listed in order of decreasing importance. Numbers highlighted in bold indicate whether a particular T-RF was more abundant in the vegetated or bare soil. The identities of T-RFs are based on 16S rRNA gene clone libraries from a previous study (Thomson et al., 2010).

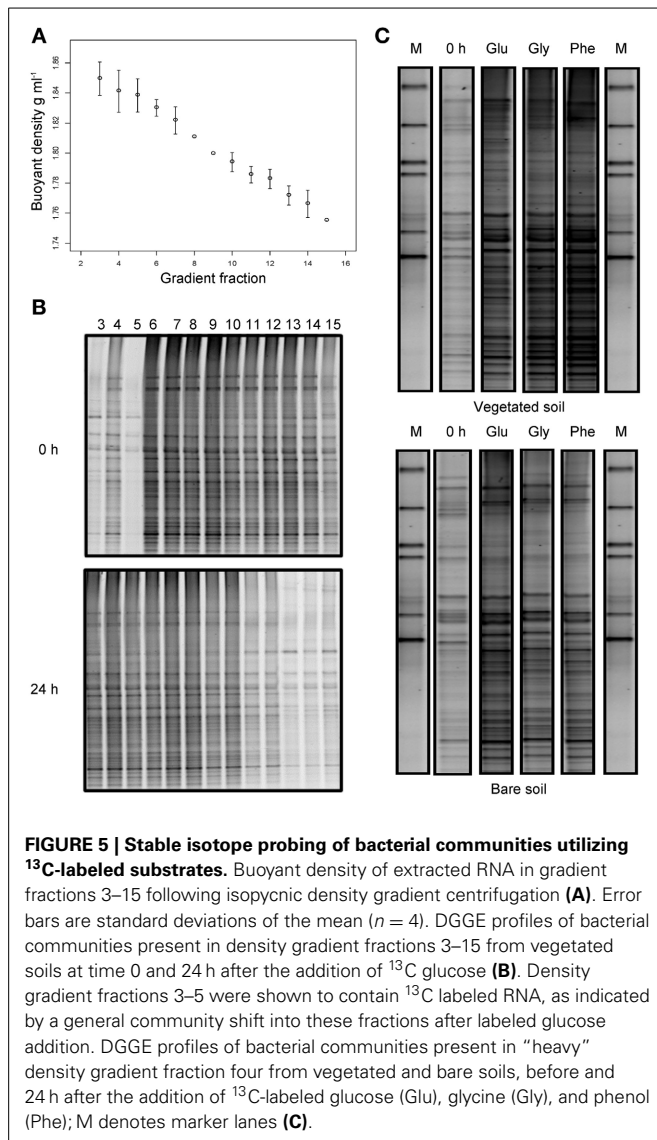


then sought to identify specific fractions containing ^{13}C labeled RNA, which could be subsequently examined for all substrate additions. All 20 gradient fractions from unlabeled (0 h) and glucose-labeled (24 h) samples from the vegetated treatment were amplified by reverse transcription PCR. Amplicons were only found in fractions 3–15 (buoyant densities of $1.75\text{--}1.85\text{ g ml}^{-1}$) and subsequent DGGE analysis revealed that, despite the presence of some weak bands in the first three “heavy” fractions (3–5) before substrate addition, 24 h after incubation with ^{13}C -labeled glucose, DGGE banding patterns were clearly more intense; indicating that ^{13}C labeled RNA had been separated into these fractions (Figure 5B). Fraction four (buoyant density $1.84\text{ g ml}^{-1} \pm 0.013$) was then selected to examine the bacterial populations actively utilizing ^{13}C -labeled glucose, glycine and phenol prior to (0 h; unlabeled RNA) and 24 h after FOC addition (labeled RNA), through 16S rRNA-SIP and DGGE analyses (Figure 5C). Despite subtle differences in band intensities, the DGGE banding patterns illustrate that most community members originally present in the soil became enriched in substrate ^{13}C after 24 h incubation. This suggests that the differences in “total” bacterial communities between vegetated and bare soils are likely indicative of the differences in “active” communities degrading the added FOC, and we found no evidence that limited sub-populations of bacteria were implicated in FOC degradation.



DISCUSSION

This study combined a ^{13}C tracer approach with molecular methodologies to examine FOC and SOC dynamics in relation to vegetation-induced changes in soil resource availability and bacterial communities. Soil C and N contents, microbial biomass and basal respiration were greater in vegetated soil which is consistent with current knowledge on how plants affect soil resource availability and stimulate microbial communities (Nguyen and Guckert, 2001; Orwin et al., 2010; Thomson et al., 2010). Substrate additions revealed that, generally, resources of differing quality were decomposed at different rates, as found in other studies (Webster et al., 1997; Schmidt et al., 2004; Brant et al., 2006; Hartley et al., 2010; Bradford et al., 2013), but rates were consistently higher in vegetated soil independent of substrate type. However, despite bare soils being much less active in degrading added FOC substrates, unlabeled SOC cycling was markedly increased, indicative of positive priming effects. In contrast, unlabeled SOC turnover was retarded in the vegetated soils following the addition of FOC exemplifying negative priming effects. The directions of these priming phenomena were therefore dependent on the presence or absence of plants and associated changes in resource availability and not the type of FOC input used in the assay.



Whilst isotope labeled substrate additions represent a useful assay exploring potential activities with regard to FOC and SOC cycling, such short-term assays can be criticized for not discriminating between “real” or “apparent” priming effects (Blagodatskaya and Kuzyakov, 2008). Additionally, it has been discussed how simple measures of function based on respired C should be used cautiously in inferring longer term dynamics of SOM (Bradford et al., 2008a; Conant et al., 2011). However, we found that the direction of unlabeled SOC turnover following FOC addition reflected longer term changes in soil carbon stocks, where increased SOC cycling in the bare soils was indicative of an overall decrease in soil C content. In the absence of plants microbes must utilize C from an extant pool; and regardless of whether this pool is within existing biomass or native SOC the end result will be C loss (assuming limited inputs from autotrophic carbon fixation). Therefore, based on our results, the short term FOC addition assays proffer a window on these processes, with the directions of priming effects being reflective of longer term changes in soil C. Supporting our findings, increased

positive priming effects have been observed in short term assays on forest soils which had reduced soil C content due to the exclusion of plant inputs for 6 years (Brant et al., 2006). More generally, other studies have also reported strongest positive priming effects in soils with comparatively reduced resource availability (Fontaine et al., 2004b; Hamer and Marschner, 2005a,b). Negative priming effects are not documented as frequently as positive ones and whilst there are several potential causes (Kuzyakov et al., 2000; Blagodatskaya et al., 2007) it is thought that they may simply arise through preferential substrate utilization, where organisms switch from degrading SOM to the fresh labile substrate (Sparling et al., 1982; Kuzyakov and Bol, 2006).

Vegetated and bare soils were dominated by *Alphaproteobacteria* and *Acidobacteria*, respectively, in agreement with previous theories that proteobacterial taxa are generally competitively adapted and dominate in soils of higher resource availability, whilst *Acidobacteria* dominate in more resource limited environments (Smit et al., 2001; Griffiths et al., 2003; Cleveland et al., 2007; Fierer et al., 2007; Philippot et al., 2009; Eilers et al., 2010; Thomson et al., 2010; Will et al., 2010). Therefore, we postulate that differences in labile FOC and native SOC processing between vegetated and bare soils may, in addition to simple changes in biomass, be also attributable to changes in dominant taxa. That is, vegetated soils, by virtue of higher resource availability, are dominated by fast growing *r* selected taxa, represented by the *Alphaproteobacteria* which are likely adapted to the fast utilization of labile substrates in the forms of root exudates and rhizodeposits. Conversely, the resource starved bare soils are dominated by *K* selected communities such as the *Acidobacteria*, which are adapted to more efficient, slow growth under limiting conditions. In support of this, a previous experiment (Fierer et al., 2007; Bradford et al., 2008b) directly manipulated forest soil resource availability through repeated sucrose additions, and found low additions generated *Acidobacteria*-dominated communities which were associated with less respiration and long term C loss; whereas high rates of addition favored *Proteobacteria* with increased respiration responses and long term C gain. We feel these findings are analogous to those in our present study, where the removal of plants lowered resource availability, decreased respiratory activity and C storage whilst selecting for an *Acidobacteria*-dominated community. We note that both studies have focused on acidic, low diversity soils which are typically dominated by *Acidobacteria* and *Proteobacteria*, highlighting the need for more distributed studies encompassing different soils and plant communities in order to generalize associations linking plant-driven resource availability, microbial communities and soil carbon storage.

Despite identifying a potentially widespread associative relationship on how resources control bacterial biodiversity and C storage, there remains a challenge to definitively link resource driven changes in communities with explicit roles in C cycling; be it the proposed role of *K* selected taxa in SOC turnover or *r* selected taxa in preferential FOC use. To further investigate the flow of labile FOC into active microbial communities a coupled molecular and isotopic tracer approach was undertaken. Such SIP approaches are believed to shed more light on the explicit mechanisms linking microbes with both FOC and SOC cycling (Bernard et al., 2007, 2012; Pascault et al., 2013). However, we

detected few differences between labeled (active) and unlabeled (total) communities 24 h after each substrate amendment indicating many community members had received substrate C, rather than specific active sub-populations. In contrast, a previous study has shown that the addition of labile substrates can shift communities favoring specific bacterial taxa (Goldfarb et al., 2011). However, nearly ten times more C was added in this study compared to ours, potentially explaining why we failed to observe any specific population “enrichment” effects either in our TRFLP or SIP data.

Enrichment of the entire bacterial community has been documented before in soil SIP studies (Rangel-Castro et al., 2005) and is likely due to the universal rapid degradability of simple substrates by soil microbes. Therefore, we found little evidence to suggest that certain discrete populations of bacteria were preferentially utilizing substrates resulting in outgrowth in either vegetated or bare soils. This, therefore, contradicts suppositions that particular members of communities with different life history strategies are exclusively utilizing FOC substrates, but suggests all members are capable of accessing these simple compounds. This is conceivable since, despite an assumed preference for growth in resource limited environments, *K*-strategists are also considered effective at scavenging different substrates (Pianka, 1970; Button, 1993; Bernard et al., 2007; Fierer et al., 2007). Indeed, such theories have been invoked to explain priming effects, whereby easily-assimilated FOC inputs are utilized by *K*-strategists to drive SOC mineralization (Fontaine et al., 2003), though based on the SIP results we were unable to prove or disprove these theories.

The quantitative analyses of ^{13}C incorporation into bulk RNA pools revealed further insights into microbial C processing resulting from different substrate additions to vegetated and bare soils. Firstly, we observed that for all soils more glucose was assimilated into RNA than the other two substrates, which is consistent with other studies contrasting glucose, glycine and phenol utilization (Brant et al., 2006; Rinnan and Baath, 2009; Bradford et al., 2013; Frey et al., 2013). Despite these studies using other biomarkers such as fatty acids and total biomass, it is apparent that relatively high glucose-C use efficiency compared to other substrates may be a general phenomenon occurring widely in soils. This may be due to glucose C being preferentially incorporated into structural components (as it requires no extracellular enzymatic breakdown), whereas glycine, as a source of both C and N, gets catabolically metabolized as an energy source (Webster et al., 1997; Hartley et al., 2010) and phenol requires extracellular enzymatic degradation before it can be accessed by the microbial community (Powlowski and Shingler, 1994; Frey et al., 2013). These findings are of wider relevance in demonstrating how different types of FOC inputs are stored or lost from soil and indicate that soil RNA is a potentially useful biomarker for assessing the metabolic fate of different FOC inputs into soil microbial communities.

Secondly, although significant ^{13}C incorporation into RNA was found for all samples post-labeling, there were few differences in rates of uptake between vegetated and bare communities. Without a full mass balance calculation these results should be interpreted with some caution; nonetheless it is clear that in vegetated soils a greater proportion of ^{13}C was respired, as opposed to assimilated, compared with the bare soils. This can be explained

simply by considering past evidence that FOC inputs will be catabolized for energy gain instead of being converted to biomass when C resources are plentiful (Bremer and Kuikman, 1994; Nguyen and Guckert, 2001) or more likely, when N is limiting with respect to plentiful C (Schimel and Weintraub, 2003). Conversely, in bare soil under assumed C limitation (or C limitation with respect to N) these theories suggest more energy from FOC inputs would be channeled into biomass generation. Our findings additionally identify that the biodiversity of soil microbes may also be a factor to consider in explaining these processes, though it is apparent that more quantitative approaches are required to assess the flow of C through specific community members in order to reveal how populations with different life history strategies process distinct C pools. In this regard, further insights into the functional roles played by specific microbial groups in degrading FOC inputs may be gleaned by assessing specific assimilation rates into separated RNA pools using more quantitative approaches such as magnetic bead capture SIP (Macgregor et al., 2002) targeted at specific taxa (e.g., hypothesized *r*- and *K*-strategist taxa). Moreover, to specifically examine SOC degradation and issues pertaining to soil priming, these methods could be coupled with novel experimental approaches to quantitatively trace C from the SOC pool directly (Blagodatskaya et al., 2011). We do, however, also identify an additional complicating issue whereby, particularly for the vegetated soils, substrate “use” may not mean substrate “assimilation” and this should be considered in future soils linking microbial communities and functionality using such isotope tracing approaches.

To conclude, in this upland grassland ecosystem, the field removal of plants for several years decreased soil resources resulting in differential rates of FOC and SOC cycling which are potentially explainable by considering emerging theories on the life histories of microbial taxa. Our results suggest that baseline soil resource availability, controlled by plants, can have a pronounced effect on soil bacterial communities and associated activities in cycling different soil carbon pools. An additional finding of note was that the direction of the priming effect was independent of the type of substrate used, therefore future studies investigating a wider range of soils could perhaps focus on using a single substrate. Coupling isotope and tracer methodologies was useful in providing new insights into the microbial cycling of FOC inputs but further developments are required to quantitatively assess flows into different taxa, and notably trace SOC pools directly. We feel these results provide a strong case for considering microbial biodiversity and the development of further molecular approaches in future studies seeking to understand the differential cycling of different soil carbon pools, be they FOC inputs of different quality or native SOC.

ACKNOWLEDGMENTS

We thank Darren Sleep, Helen Grant, and Andy Stott at the NERC/CEH stable isotope facility for their assistance in carrying out soil C and N, and ^{13}C RNA and $^{13}\text{CO}_2$ analyses. We appreciate the anonymous reviewers' constructive comments which improved the manuscript. Thanks also to Ashish Malik for helpful discussions. This work was partly funded by the European Commission project, EcoFINDERS (FP7-264465) and a NERC UK studentship.

REFERENCES

- Bardgett, R. D. (2005). "Linkages between plant and soil biological communities," in *The Biology of Soil: A Community and Ecosystem Approach*, ed R. D. Bardgett (Oxford: Oxford University Press), 119–139.
- Bardgett, R. D., Freeman, C., and Ostle, N. J. (2008). Microbial contributions to climate change through carbon cycle feedbacks. *ISME J.* 2, 805–814. doi: 10.1038/ismej.2008.58
- Bardgett, R. D., Hobbs, P. J., and Frostegard, A. (1996). Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. *Biol. Fert. Soils* 22, 261–264. doi: 10.1007/BF00382522
- Bell, J. M., Smith, J. L., Bailey, V. L., and Bolton, H. (2003). Priming effect and C storage in semi-arid no-till spring crop rotations. *Biol. Fert. Soils* 37, 237–244.
- Bernard, L., Chapuis-Lardy, L., Razafimbelo, T., Razafindrakoto, M., Pablo, A.-L., Legname, E., et al. (2012). Endogeic earthworms shape bacterial functional communities and affect organic matter mineralization in a tropical soil. *ISME J.* 6, 213–222. doi: 10.1038/ismej.2011.87
- Bernard, L., Mougél, C., Maron, P. A., Nowak, V., Leveque, J., Henault, C., et al. (2007). Dynamics and identification of soil microbial populations actively assimilating carbon from ¹³C-labelled wheat residue as estimated by DNA- and RNA-SIP techniques. *Envir. Microbiol.* 9, 752–764. doi: 10.1111/j.1462-2920.2006.01197.x
- Bingeman, C. W., Varner, J. E., and Martin, W. P. (1953). The effect of the addition of organic materials on the decomposition of an organic soil. *Soil Sci. Soc. Am. Pro.* 29, 692–696.
- Blagodatskaya, E., and Kuzyakov, Y. (2008). Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biol. Fert. Soils* 45, 115–131. doi: 10.1007/s00374-008-0334-y
- Blagodatskaya, E., Yuyukina, T., Blagodatsky, S., and Kuzyakov, Y. (2011). Three-source-partitioning of microbial biomass and of CO₂ efflux from soil to evaluate mechanisms of priming effects. *Soil Biol. Biochem.* 43, 778–786. doi: 10.1016/j.soilbio.2010.12.011
- Blagodatskaya, E. V., Blagodatsky, S. A., Anderson, T. H., and Kuzyakov, Y. (2007). Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. *Appl. Soil Ecol.* 37, 95–105. doi: 10.1016/j.apsoil.2007.05.002
- Bradford, M. A., Fierer, N., Jackson, R. B., Maddox, T. R., and Reynolds, J. F. (2008a). Nonlinear root-derived carbon sequestration across a gradient of nitrogen and phosphorous deposition in experimental mesocosms. *Glob. Change Biol.* 14, 1113–1124. doi: 10.1111/j.1365-2486.2008.01564.x
- Bradford, M. A., Fierer, N., and Reynolds, J. F. (2008b). Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. *Funct. Ecol.* 22, 964–974. doi: 10.1111/j.1365-2435.2008.01404.x
- Bradford, M. A., Keiser, A. D., Davies, C. A., Mersmann, C. A., and Strickland, M. S. (2013). Empirical evidence that soil carbon formation from plant inputs is positively related to microbial growth. *Biogeochemistry* 113, 271–281. doi: 10.1007/s10533-012-9822-0
- Brant, J. B., Sulzman, E. W., and Myrold, D. D. (2006). Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. *Soil Biol. Biochem.* 38, 2219–2232. doi: 10.1016/j.soilbio.2006.01.022
- Bremer, E., and Kuikman, P. (1994). Microbial utilization of ¹⁴C[U]glucose in soil is affected by the amount and timing of glucose additions. *Soil Biol. Biochem.* 26, 511–517. doi: 10.1016/0038-0717(94)90184-8
- Button, D. K. (1993). Nutrient limited microbial growth kinetics: overview and recent advances. *Antonie Van Leeuwenhoek* 63, 225–235. doi: 10.1007/BF00871220
- Clarke, K. R. (1993). Nonparametric multivariate analyses of changes in community structure. *Aust. J. Ecol.* 18, 117–143. doi: 10.1111/j.1442-9993.1993.tb00438.x
- Cleveland, C. C., Nemergut, D. R., Schmidt, S. K., and Townsend, A. R. (2007). Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. *Biogeochemistry* 82, 229–240. doi: 10.1007/s10533-006-9065-z
- Conant, R. T., Ryan, M. G., Agren, G. I., Birge, H. E., Davidson, E. A., Eliasson, P. E., et al. (2011). Temperature and soil organic matter decomposition rates – synthesis of current knowledge and a way forward. *Glob. Change Biol.* 17, 3392–3404. doi: 10.1111/j.1365-2486.2011.02496.x
- Dalenberg, J. W., and Jager, G. (1981). Priming effect of small glucose additions to ¹⁴C-labelled soil. *Soil Biol. Biochem.* 13, 219–223. doi: 10.1016/0038-0717(81)90024-9
- De Deyn, G. B., Cornelissen, J. H. C., and Bardgett, R. D. (2008). Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecol. Lett.* 11, 516–531. doi: 10.1111/j.1461-0248.2008.01164.x
- De Graaff, M.-A., Classen, A. T., Castro, H. F., and Schadt, C. W. (2010). Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. *New Phytol.* 188, 1055–1064. doi: 10.1111/j.1469-8137.2010.03427.x
- De Nobili, M., Contin, M., Mondini, C., and Brookes, P. C. (2001). Soil microbial biomass is triggered into activity by trace amounts of substrate. *Soil Biol. Biochem.* 33, 1163–1170. doi: 10.1016/S0038-0717(01)00020-7
- Eilers, K. G., Lauber, C. L., Knight, R., and Fierer, N. (2010). Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. *Soil Biol. Biochem.* 42, 896–903. doi: 10.1016/j.soilbio.2010.02.003
- Falchini, L., Naumova, N., Kuikman, P. J., Bloem, J., and Nannipieri, P. (2003). CO₂ evolution and denaturing gradient gel electrophoresis profiles of bacterial communities in soil following addition of low molecular weight substrates to simulate root exudation. *Soil Biol. Biochem.* 35, 775–782. doi: 10.1016/S0038-0717(03)00105-6
- Fierer, N., Bradford, M. A., and Jackson, R. B. (2007). Toward an ecological classification of soil bacteria. *Ecology* 88, 1354–1364. doi: 10.1890/05-1839
- Fierer, N., Strickland, M. S., Liptzin, D., Bradford, M. A., and Cleveland, C. C. (2009). Global patterns in below-ground communities. *Ecol. Lett.* 12, 1238–1249. doi: 10.1111/j.1461-0248.2009.01360.x
- Fontaine, S., Bardoux, G., Abbadie, L., and Mariotti, A. (2004a). Carbon input to soil may decrease soil carbon content. *Ecol. Lett.* 7, 314–320. doi: 10.1111/j.1461-0248.2004.00579.x
- Fontaine, S., Bardoux, G., Benest, D., Verdier, B., Mariotti, A., and Abbadie, L. (2004b). Mechanisms of the priming effect in a savannah soil amended with cellulose. *Soil Sci. Soc. Am. J.* 68, 125–131. doi: 10.2136/sssaj2004.0125
- Fontaine, S., and Barot, S. (2005). Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. *Ecol. Lett.* 8, 1075–1087. doi: 10.1111/j.1461-0248.2005.00813.x
- Fontaine, S., Mariotti, A., and Abbadie, L. (2003). The priming effect of organic matter: a question of microbial competition? *Soil Biol. Biochem.* 35, 837–843. doi: 10.1016/S0038-0717(03)00123-8
- Frey, S. D., Gupta, V., Elliott, E. T., and Paustian, K. (2001). Protozoan grazing affects estimates of carbon utilization efficiency of the soil microbial community. *Soil Biol. Biochem.* 33, 1759–1768. doi: 10.1016/S0038-0717(01)00101-8
- Frey, S. D., Lee, J., Melillo, J. M., and Six, J. (2013). The temperature response of soil microbial efficiency and its feedback to climate. *Nat. Clim. Change* 3, 395–398. doi: 10.1038/nclimate1796
- Goldfarb, K. C., Karaoz, U., Hanson, C. A., Santee, C. A., Bradford, M. A., Treseder, K. K., et al. (2011). Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. *Front. Microbiol.* 2:94. doi: 10.3389/fmicb.2011.00094
- Griffiths, R. I., Whiteley, A. S., O'donnell, A. G., and Bailey, M. J. (2000). Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. *Appl. Environ. Microbiol.* 66, 5488–5491. doi: 10.1128/AEM.66.12.5488-5491.2000
- Griffiths, R. I., Whiteley, A. S., O'donnell, A. G., and Bailey, M. J. (2003). Influence of depth and sampling time on bacterial community structure in an upland grassland soil. *FEMS Microbiol. Ecol.* 43, 35–43. doi: 10.1111/j.1574-6941.2003.tb01043.x
- Guenet, B., Leloup, J., Raynaud, X., Bardoux, G., and Abbadie, L. (2010). Negative priming effect on mineralization in a soil free of vegetation for 80 years. *Eur.*

- J. Soil Sci.* 61, 384–391. doi: 10.1111/j.1365-2389.2010.01234.x
- Hamer, U., and Marschner, B. (2005a). Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. *Soil Biol. Biochem.* 37, 445–454. doi: 10.1016/j.soilbio.2004.07.037
- Hamer, U., and Marschner, B. (2005b). Priming effects in soils after combined and repeated substrate additions. *Geoderma* 128, 38–51. doi: 10.1016/j.geoderma.2004.12.014
- Hartley, I. P., Hopkins, D. W., Sommerkorn, M., and Wookey, P. A. (2010). The response of organic matter mineralisation to nutrient and substrate additions in sub-arctic soils. *Soil Biol. Biochem.* 42, 92–100. doi: 10.1016/j.soilbio.2009.10.004
- Hopkins, D. W., and Gregorich, E. G. (2005). “Carbon as a substrate for soil organisms,” in *Biological Diversity and Function in Soils*, eds R. D. Bardgett, M. B. Usher, and D. W. Hopkins (Cambridge: Cambridge University Press), 57–82. doi: 10.1017/CBO9780511541926.005
- Johnson, D., Booth, R. E., Whiteley, A. S., Bailey, M. J., Read, D. J., Grime, J. P., et al. (2003). Plant community composition affects the biomass, activity and diversity of microorganisms in limestone grassland soil. *Eur. J. Soil Sci.* 54, 671–677. doi: 10.1046/j.1351-0754.2003.0562.x
- Kemmitt, S. J., Lanyon, C. V., Waite, I. S., Wen, Q., Addiscott, T. M., Bird, N. R. A., et al. (2008). Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass – a new perspective. *Soil Biol. Biochem.* 40, 61–73. doi: 10.1016/j.soilbio.2007.06.021
- Kuzakov, Y. (2002). Separating microbial respiration of exudates from root respiration in non-sterile soils: a comparison of four methods. *Soil Biol. Biochem.* 34, 1621–1631. doi: 10.1016/S0038-0717(02)00146-3
- Kuzakov, Y., and Bol, R. (2006). Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. *Soil Biol. Biochem.* 38, 747–758. doi: 10.1016/j.soilbio.2005.06.025
- Kuzakov, Y., and Demin, V. (1998). CO₂ efflux by rapid decomposition of low molecular organic substances in soils. *Sci. Soils* 3, 1–12. doi: 10.1007/s10112-998-0002-2
- Kuzakov, Y., Friedel, J. K., and Stahr, K. (2000). Review of mechanisms and quantification of priming effects. *Soil Biol. Biochem.* 32, 1485–1498. doi: 10.1016/S0038-0717(00)00084-5
- Landi, L., Valori, F., Ascher, J., Renella, G., Falchini, L., and Nannipieri, P. (2006). Root exudate effects on the bacterial communities, CO₂ evolution, nitrogen transformations and ATP content of rhizosphere and bulk soils. *Soil Biol. Biochem.* 38, 509–516. doi: 10.1016/j.soilbio.2005.05.021
- Lane, D. J. (1991). “16S/23S rRNA Sequencing,” in *Nucleic Acid Techniques in Bacterial Systematics*, eds E. Stackenbrandt and M. Goodfellow (New York, NY: John Wiley and Sons), 115–175.
- Macgregor, B. J., Bruchert, V., Fleischer, S., and Amann, R. (2002). Isolation of small-subunit rRNA for stable isotopic characterization. *Envir. Microbiol.* 4, 451–464. doi: 10.1046/j.1462-2920.2002.00324.x
- Manefield, M., Whiteley, A. S., Griffiths, R. I., and Bailey, M. J. (2002a). RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. *Appl. Environ. Microbiol.* 68, 5367–5373. doi: 10.1128/AEM.68.11.5367-5373.2002
- Manefield, M., Whiteley, A. S., Ostle, N., Ineson, P., and Bailey, M. J. (2002b). Technical considerations for RNA-based stable isotope probing: an approach to associating microbial diversity with microbial community function. *Rapid Commun. Mass Spectrom.* 16, 2179–2183. doi: 10.1002/rcm.782
- Manzoni, S., Taylor, P., Richter, A., Porporato, A., and Agren, G. I. (2012). Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytol.* 196, 79–91. doi: 10.1111/j.1469-8137.2012.04225.x
- Marchesi, J. R., Sato, T., Weightman, A. J., Martin, T. A., Fry, J. C., Hiom, S. J., et al. (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Appl. Environ. Microbiol.* 64, 795–799.
- Nannipieri, P., Ascher, J., Ceccherini, M. T., Landi, L., Pietramellara, G., and Renella, G. (2003). Microbial diversity and soil functions. *Eur. J. Soil Sci.* 54, 655–670. doi: 10.1046/j.1351-0754.2003.0556.x
- Nguyen, C., and Guckert, A. (2001). Short-term utilisation of ¹⁴C-glucose by soil microorganisms in relation to carbon availability. *Soil Biol. Biochem.* 33, 53–60. doi: 10.1016/S0038-0717(00)00114-0
- Nottingham, A. T., Griffiths, H., Chamberlain, P. M., Stott, A. W., and Tanner, E. V. J. (2009). Soil priming by sugar and leaf-litter substrates: a link to microbial groups. *Appl. Soil Ecol.* 42, 183–190. doi: 10.1016/j.apsoil.2009.03.003
- Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., O'hara, R. G., Simpson, G. L., et al. (2009). *Vegan: community ecology package*. R package version 1.16-33.
- Orwin, K. H., Buckland, S. M., Johnson, D., Turner, B. L., Smart, S., Oakley, S., et al. (2010). Linkages of plant traits to soil properties and the functioning of temperate grassland. *J. Ecol.* 98, 1074–1083. doi: 10.1111/j.1365-2745.2010.01679.x
- Pascual, N., Ranjard, L., Kaisermann, A., Bachar, D., Christen, R., Terrat, S., et al. (2013). Stimulation of different functional groups of bacteria by various plant residues as a driver of soil priming effect. *Ecosystems* 16, 810–822. doi: 10.1007/s10021-013-9650-7
- Paterson, E., Midwood, A. J., and Millard, P. (2009). Through the eye of the needle: a review of isotope approaches to quantify microbial processes mediating soil carbon balance. *New Phytol.* 184, 19–33. doi: 10.1111/j.1469-8137.2009.03001.x
- Philippot, L., Bru, D., Saby, N. P. A., Cuhel, J., Arrauays, D., Simek, M., et al. (2009). Spatial patterns of bacterial taxa in nature reflect ecological traits of deep branches of the 16S rRNA bacterial tree. *Environ. Microbiol.* 11, 3096–3104. doi: 10.1111/j.1462-2920.2009.02014.x
- Pianka, E. R. (1970). On r selection and K selection. *Am. Nat.* 104, 592–597. doi: 10.1086/282697
- Powlowski, J., and Shingler, V. (1994). Genetics and biochemistry of phenol degradation by *Pseudomonas* sp. CF600. *Biodegradation* 5, 219–236. doi: 10.1007/BF00696461
- R Core Development Team. (2005). “R: a language and environment for statistical computing,” in *R Foundation for Statistical Computing* (Vienna, Austria), ISBN 3-900051-07-0. Available online at: <http://www.R-project.org>
- Rangel-Castro, J. I., Killham, K., Ostle, N., Nicol, G. W., Anderson, I. C., Scrimgeour, C. M., et al. (2005). Stable isotope probing analysis of the influence of liming on root exudate utilization by soil microorganisms. *Environ. Microbiol.* 7, 828–838. doi: 10.1111/j.1462-2920.2005.00756.x
- Rinnan, R., and Baath, E. (2009). Differential utilization of carbon substrates by bacteria and fungi in tundra soil. *Appl. Environ. Microbiol.* 75, 3611–3620. doi: 10.1128/AEM.02865-08
- Schimel, J. P., and Schaeffer, S. M. (2012). Microbial control over carbon cycling in soil. *Front. Microbiol.* 3:348. doi: 10.3389/fmicb.2012.00348
- Schimel, J. P., and Weintraub, M. N. (2003). The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biol. Biochem.* 35, 549–563. doi: 10.1016/S0038-0717(03)00015-4
- Schmidt, S. K., Lipson, D. A., Ley, R. E., Fisk, M. C., and West, A. E. (2004). Impacts of chronic nitrogen additions vary seasonally and by microbial functional group in tundra soils. *Biogeochemistry* 69, 1–17. doi: 10.1023/B:BI0G.0000031028.53116.9b
- Smit, E., Leeflang, P., Gommans, S., Van Den Broek, J., Van Mil, S., and Wernars, K. (2001). Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. *Appl. Environ. Microbiol.* 67, 2284–2291. doi: 10.1128/AEM.67.5.2284-2291.2001
- Sparling, G. P., Cheshire, M. V., and Mundie, C. M. (1982). Effect of barley plants on the decomposition of ¹⁴C labelled soil organic matter. *J. Soil Sci.* 33, 89–100. doi: 10.1111/j.1365-2389.1982.tb01750.x
- Thomson, B. C., Ostle, N., McNamara, N., Bailey, M. J., Whiteley, A. S., and Griffiths, R. I. (2010). Vegetation affects the relative abundances of dominant soil bacterial taxa and soil respiration rates in an upland grassland soil. *Microb. Ecol.* 59, 335–343. doi: 10.1007/s00248-009-9575-z
- Webster, E. A., Chudek, J. A., and Hopkins, D. W. (1997). Fates of ¹³C from enriched glucose and glycine in an organic soil determined by solid-state NMR. *Biol. Fert. Soils* 25, 389–395. doi: 10.1007/s003740050330
- Whiteley, A. S., Thomson, B., Lueders, T., and Manefield, M. (2007). RNA stable-isotope probing. *Nat. Protoc.* 2, 838–844. doi: 10.1038/nprot.2007.115
- Will, C., Thuermer, A., Wollherr, A., Nacke, H., Herold, N., Schrumpp, M., et al. (2010). Horizon-specific bacterial community composition

of German grassland soils, as revealed by pyrosequencing-based analysis of 16S rRNA genes. *Appl. Environ. Microbiol.* 76, 6751–6759. doi: 10.1128/AEM.01063-10

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships

that could be construed as a potential conflict of interest.

Received: 22 April 2013; accepted: 07 August 2013; published online: 10 September 2013.

Citation: Thomson BC, Ostle NJ, McNamara NP, Oakley S, Whiteley AS, Bailey MJ and Griffiths RI (2013) Plant soil interactions alter carbon cycling

in an upland grassland soil. *Front. Microbiol.* 4:253. doi: 10.3389/fmicb.2013.00253

This article was submitted to *Terrestrial Microbiology*, a section of the journal *Frontiers in Microbiology*.

Copyright © 2013 Thomson, Ostle, McNamara, Oakley, Whiteley, Bailey and Griffiths. This is an open-access article distributed under the terms of the

Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Off-season biogenic volatile organic compound emissions from heath mesocosms: responses to vegetation cutting

Riikka Rinnan^{1,2*}, Diana Gierth^{1,3}, Merete Bilde^{4†}, Thomas Rosenørn⁴ and Anders Michelsen^{1,2}

¹ Terrestrial Ecology Section, Department of Biology, University of Copenhagen, Copenhagen, Denmark

² Center for Permafrost, University of Copenhagen, Copenhagen, Denmark

³ Department of Physiology and Cell Biology, Molecular Plant Nutrition, Leibniz Institute of Plant Genetics and Crop Plant Research, Stadt Seeland, Gatersleben, Germany

⁴ Department of Chemistry, University of Copenhagen, Copenhagen, Denmark

Edited by:

Johannes Rousk, Lund University, Sweden

Reviewed by:

Kim Yrjölä, University of Helsinki, Finland

Christopher Gray, University of Colorado at Boulder, USA

*Correspondence:

Riikka Rinnan, Terrestrial Ecology Section, Department of Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark
e-mail: riikka@bio.ku.dk

†Present address:

Merete Bilde, Department of Chemistry, Aarhus University, Aarhus C, Denmark

Biogenic volatile organic compounds (BVOCs) affect both atmospheric processes and ecological interactions. Our primary aim was to differentiate between BVOC emissions from above- and belowground plant parts and heath soil outside the growing season. The second aim was to assess emissions from herbivory, mimicked by cutting the plants. Mesocosms from a temperate *Deschampsia flexuosa*-dominated heath ecosystem and a subarctic mixed heath ecosystem were either left intact, the aboveground vegetation was cut, or all plant parts (including roots) were removed. For 3–5 weeks, BVOC emissions were measured in growth chambers by an enclosure method using gas chromatography-mass spectrometry. CO₂ exchange, soil microbial biomass, and soil carbon and nitrogen concentrations were also analyzed. Vegetation cutting increased BVOC emissions by more than 20-fold, and the induced compounds were mainly eight-carbon compounds and sesquiterpenes. In the *Deschampsia* heath, the overall low BVOC emissions originated mainly from soil. In the mixed heath, root, and soil emissions were negligible. Net BVOC emissions from roots and soil of these well-drained heaths do not significantly contribute to ecosystem emissions, at least outside the growing season. If insect outbreaks become more frequent with climate change, ecosystem BVOC emissions will periodically increase due to herbivory.

Keywords: induced volatiles, BVOC, sesquiterpenes, soil, plant wounding, grazing, *Deschampsia flexuosa*, arctic

INTRODUCTION

Ecosystem-level emissions of biogenic volatile organic compounds (BVOC) are considered to mainly originate from plant leaves (Laothawornkitkul et al., 2009). Some studies have identified that stems including bark (Sallas et al., 1999; Amin et al., 2012), plant roots and the rhizosphere (Bais et al., 2004; Lin et al., 2007), decomposing litter (Warneke et al., 1999; Leff and Fierer, 2008), and even microorganisms (Schulz and Dickschat, 2007; Korpi et al., 2009; Insam and Seewald, 2010) also release BVOCs contributing to the blend of compounds emitted from natural ecosystems. However, the emissions from soil and belowground plant parts (including roots and rhizomes), are still poorly characterized (Lin et al., 2007; Insam and Seewald, 2010).

The contribution of BVOCs to the carbon loss from soil is minimal relative to the respiratory CO₂ effluxes (Aaltonen et al., 2011; Faubert et al., 2012). However, BVOCs are important as reactive atmospheric trace gases, and BVOC oxidation products contribute to secondary organic aerosol formation and may even be involved in new particle formation (see e.g., Fuentes et al., 2000; Jimenez et al., 2009; Riipinen et al., 2012). In addition to their role in atmospheric chemistry, BVOCs also play an important part in many biological interactions (Lehnhin et al., 1999; Dicke and Bruin, 2001). In the soil atmosphere, BVOCs serve as a carbon source for some microorganisms, but they also have adverse

effects on biogeochemical cycles (White, 1994; Smolander et al., 2006) and influence microbial activity, which can have important implications for ecosystem processes. For instance, monoterpenes have been observed to inhibit nitrogen mineralization, nitrification and methane oxidation, and stimulate carbon mineralization in soil (White, 1991; Amaral and Knowles, 1998; Smolander et al., 2006).

Separation of soil and vegetation emissions has been attempted in a few field studies. One of the first studies was conducted by Hayward et al. (2001), who used a dynamic chamber technique to measure monoterpene emissions from the forest floor and the foliage of a *Picea sitchensis* forest. Most of the forest floor emissions were reported to stem from needle litter and roots rather than from bulk soil (Hayward et al., 2001), although the potential soil emissions could not be separated from those of belowground plant material with the applied experimental strategy (removing soil layers). A field study conducted in a mountain birch forest in Abisko, northern Sweden compared emissions from vegetated forest floor plots to emissions from plots where aboveground vegetation had been removed by cutting (Faubert et al., 2012). The removal of the aboveground vegetation reduced the number of different BVOCs emitted whilst having no significant effects on the total quantity emitted, but again, it was not possible to separate emissions from soil and belowground plant parts.

Past research has temporally concentrated on the growing season period when biological activity is at its highest. However, recent studies have revealed that boreal forest floor BVOC emissions peak during early summer and autumn (Aaltonen et al., 2011) and not at midsummer even though the green plant biomass is peaking at midsummer. BVOC emissions can even be measured from the snowpack during winter (Helmig et al., 2009; Aaltonen et al., 2012). In this work we focus on BVOC emissions both from soil and the whole ecosystem in a period of the year which has hitherto been largely neglected, namely the shoulder periods between summer and winter.

Results from laboratory studies assessing BVOCs emissions from root-free soil and litter samples indicate that soil emissions are controlled by both microbial activity and substrate quality. Stahl and Parkin (1996) measured contrasting BVOC emission spectra from soils amended with different substrates and selective inhibitors. Leff and Fierer (2008) detected 100 different compounds, 70 of which were identified, in emissions from 40 different soil and litter samples. The emissions from the soil samples appeared to be related to the overall level of microbial activity in soil, while those from the litter samples were best predicted by the organic carbon quality (Leff and Fierer, 2008).

The main aim of this work was to differentiate between BVOC emissions from above- and belowground plant parts and soil outside of the growing season. We compared emissions from intact vegetation-soil mesocosms to emissions from mesocosms with belowground plant parts plus soil and further to emissions from root-free soil mesocosms. The mesocosms originated from two different heath ecosystems: (1) a subarctic heath with mixed vegetation dominated by evergreen dwarf shrubs and soil characterized by high soil organic matter content and (2) a semi-natural temperate heath with monospecific stands of the grass *Deschampsia flexuosa* and sandy soil. In both systems, the experiments were conducted with largely inactive vegetation to elucidate off-season BVOC emissions.

While many BVOCs are constitutively emitted by plants and other living organisms, their production can also be induced by abiotic (Loreto and Schnitzler, 2010) or biotic stresses (Holopainen and Gershenzon, 2010). In the experimental setup of the present study, we cut the aboveground vegetation to obtain mesocosms with only belowground plant material. This allowed us to estimate how mechanical damage affected the BVOC emissions from heath ecosystems. In nature, mechanical damage similar to that caused by cutting can occur via grazing, freezing or drying of plants. The *Deschampsia* heath of this work belongs to semi-natural ecosystem types that have been traditionally managed by grazing. Subarctic heaths are browsed by both large grazers, such as reindeer (*Rangifer tarandus*), and small rodents, like voles (*Clethrionomys rufocanus*) and lemmings (*Lemmus lemmus*). In addition, insect outbreaks shape the vegetation community.

With the help of the vegetation removal treatments we aimed to answer the following questions: What fraction of total BVOC emissions from heath ecosystems originates from belowground plant parts and what fraction originates from the soil alone outside of the growing season? Which compounds are emitted

from vegetation and which from soil? Does vegetation cutting induce BVOC emissions from plants outside the main growing season?

METHODS

COLLECTION OF MESOCOSM

Material for the experiment originated at a subarctic heath located in Abisko, Sweden (68°20'N, 18°50'E) and a temperate heath located in Brandbjerg, Denmark (55°53'N; 11°58'E). In both locations, nine mesocosms, quadrants of 20 × 20 cm with intact vegetation on top, were cut with a knife to the soil depth of 10 cm and mounted into an aluminium frame, which rested on a metal base in the growth chambers. The upper 0–10 cm soil contains ca. 76% of the total fine root biomass at the mixed heath (Rinnan, unpublished data) and ca. 71% of the total fine root biomass at the *Deschampsia* heath (Arndal, unpublished data).

The vegetation in the mesocosms from Abisko was dominated by *Empetrum nigrum* ssp. *hermaphroditum* and *Rhododendron lapponicum* and accompanied with *Andromeda polifolia*, *Vaccinium uliginosum*, *Arctostaphylos alpina*, *Tofieldia pusilla*, and *Carex vaginata* as minor components. The soil was highly organic (organic matter content $89 \pm 1\%$), 10–15 cm deep, overlying stones or bedrock, and had a pH of 6.8. These mesocosms were collected in late growing season, at the end of August 2010.

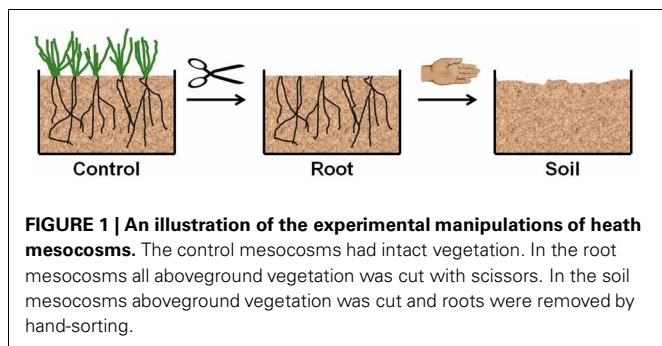
The mesocosms from Brandbjerg were collected from areas dominated by the perennial grass *Deschampsia flexuosa* in early November 2010. The soil consisted of 70% sand, 20% coarse sand, 6% silt, and 2% clay, and had a pH of about 4.2 in the organic layer and 3.5 in the upper mineral soil, and the soil organic matter content was $5.7 \pm 0.4\%$.

All the mesocosms were transported to the University of Copenhagen campus by air freight (Abisko, 7 h) or surface transport (Brandbjerg, 1 h), where they were stored outdoors until the start of the experiment and watered if needed. The storage conditions in Copenhagen, with a mean temperature of 13.4°C in September 2010, exposed the mesocosms from Abisko to a longer than normal but fairly natural transition from growing season to autumn.

VEGETATION MANIPULATIONS AND ANALYSES

The mesocosms from Abisko and Brandbjerg were treated in a similar manner (Figure 1): The vegetation in three mesocosms was left intact and these served as control mesocosms. In three mesocosms all aboveground vegetation was cut with scissors (from here on “root mesocosms”) and in another three the cutting of aboveground vegetation was followed by removal of roots and rhizomes from the soil (“soil mesocosms”). The belowground plant parts were removed from each mesocosm by hand-sorting through the soil for an equal amount of time.

At the end of the growth chamber experiment, above- and belowground vegetation from all mesocosms was collected and the soil was sorted as described above. The aboveground plant parts were separated into species and into leaves and stems when applicable. All vegetation samples were dried for 72 h at 70°C to obtain dry biomass.



GROWTH CHAMBER CONDITIONS

Two separate experiments were carried out, one with the mesocosms from Abisko on October 18–November 22, 2010 and one with the mesocosms from Brandbjerg on December 3–23, 2010. The mesocosms were divided into three growth chambers with air circulation, each with one mesocosm from each of the three treatments, yielding three replicate mesocosms per treatment. The conditions in the growth chambers were set to represent a shoulder season between summer and winter, taking into account that the temperature at the level of vegetation in low-stature systems is up to 10°C higher than air temperature at 2 m height (Scherrer and Körner, 2010). During the experiment, the mean 24-h air temperature at the level of vegetation was 13.6 and 18.0°C for the Abisko and Brandbjerg mesocosms, respectively. The daylight lasted 16 h and the darkness 8 h, and the daylight PAR at the level of vegetation was 200–300 $\mu\text{E s}^{-1} \text{ m}^{-2}$. The soil water content was maintained at 40%.

VOC SAMPLING AND ANALYSIS

The mesocosms were sampled for VOC emissions before the vegetation manipulation treatments, right after performing the treatments and at intervals after that. During the sampling, the growth chamber door was closed, which ensured that light, temperature, and humidity stayed constant.

VOCs were sampled by a push-pull system described by Faubert et al. (2010, 2012) for 30 min. A transparent polycarbonate chamber (23 × 23 cm, height 25 cm), equipped with a fan to mix the headspace air, was placed on top of a water-filled groove in the aluminum frame holding each mesocosm. The water-filled groove sealed up the connection from the chamber to the aluminum frame. Pumps (12 V, Rietschle Thomas, Puchheim, Germany) pushed air through a charcoal filter and a MnO_2 scrubber, to remove hydrocarbon impurities and ozone, respectively, into the chamber with a flow rate of 215 ml min^{-1} . At the same time, air was pulled from the chamber with a flow rate of 200 ml min^{-1} through a stainless steel adsorbent cartridge filled with 150 mg Tenax TA and 200 mg Carbograph 1TD (Markes International Limited, Llantrisant, UK). In addition, blank samples were collected to obtain an estimate of the potential VOC emissions from the metal base, aluminium frames and the polycarbonate chamber inside the growth chambers. Using filtered, VOC-free incoming air may cause an increased diffusion gradient between soil and the chamber headspace, which would lead to a slight overestimation of the emissions. In addition, the potential

uptake of VOCs cannot be detected by the used measurement system.

VOCs were analyzed by gas chromatography-mass spectrometry (6850 Network GC system and a 5975C VL MSD with triple axis detector, Agilent, Santa Clara, CA, USA) after thermodesorption at 250°C and cryofocusing at −10°C with a UNITY 2 thermal desorber (Markes, Llantrisant, UK) coupled with a ULTRA 2 autosampler. The compounds were separated using an HP-5 capillary column (50 m × 0.2 mm, film thickness 0.33 μm). Helium was used as the carrier gas. The oven temperature was held at 40°C for 1 min, raised to 210°C at a rate of 5°C min^{-1} , and then raised to 250°C at a rate of 20°C min^{-1} .

The compounds were identified using standard compounds and the NIST library, and those present in blank samples were omitted from further analysis. The quantification was done using pure standards solutions for α -pinene, borneol, β -myrcene, coprene, trans- β -farnesene, humulene, aromadendrene, δ -cadinene, trans-2-hexenal, cis-3-hexenol, cis-3-hexenyl acetate, 1-octen-3-ol, cis-3-hexenyl butyrate, cis-3-hexenyl isovalerate, and nonanal (Fluka, Buchs, Switzerland). To quantify the compounds without a specific standard, we used a pure standard for as similar compound as possible.

The emission rates were calculated following the procedure outlined in Faubert et al. (2012) taking into account the different soil surface microtopographies in each mesocosm and the additional air volume due to the slightly higher flow rate into than out from the chamber.

CO₂ EXCHANGE MEASUREMENTS

Carbon dioxide exchange was measured in conjunction with the VOC sampling using an EGM-4 gas monitor (PP Systems, Hitchin, UK). A transparent chamber equipped with a fan was placed on top of the water-filled groove of the aluminium frame and the headspace CO_2 concentration was recorded for max. 5 min. The chamber was lifted up to air it before repeating the measurement with a darkened chamber. A linear regression of the change in the CO_2 concentration in light was used as an estimate of net ecosystem exchange (NEE) and that in the dark of the dark ecosystem respiration (R_{TOT}). The gross photosynthesis (P_G) could be derived by subtracting R_{TOT} from NEE.

SOIL MICROBIAL BIOMASS AND C AND N ANALYSES

After the last gas exchange measurements, the hand-sorted and well mixed soil was sampled and analyzed for NH_4^+ , NO_3^- , dissolved organic nitrogen (DON), dissolved organic carbon (DOC), microbial biomass carbon (C_{MIC}), and microbial biomass nitrogen (N_{MIC}) following standard extraction and fumigation-extraction procedures (Jenkinson and Powlson, 1976; Vance et al., 1987; Rinnan et al., 2008).

Shortly, a 10-g subsample of soil was fumigated with chloroform for 24 h. These and another set of 10-g subsamples were extracted with 50 ml water in a rotary shaker for 1 h following filtration through Whatman GF/D filters. Then, the non-fumigated samples were analyzed for NH_4^+ and NO_3^- on a Fiastar 5000 flow injection analyzer (FOSS Tecator, Höganäs, Sweden) and both the fumigated (to determine C_{MIC} and N_{MIC}) and non-fumigated (to determine DOC and DON) samples were analyzed for total

organic N on the FOSS Fiastar 5000 and for total organic C on the total organic carbon analyzer TOC 5000A (Shimadzu, Kyoto, Japan).

C_{MIC} and N_{MIC} were calculated as the difference in dissolved C and N in the fumigated and the non-fumigated samples. The values were corrected for incomplete extractability by a factor 0.45 for microbial C (Joergensen, 1996) and a factor 0.40 for microbial N (Jonasson et al., 1996).

STATISTICAL ANALYSES

The data were analyzed for differences between the vegetation manipulation treatments by univariate (single variables, e.g., total BVOC emissions) or multivariate (e.g., vegetation cover percentages) analysis of variance in which the vegetation manipulation and time (when appropriate) were set as fixed factors and the growth chamber as a random factor. When the effect of vegetation manipulation was significant, the data were subjected to Tukey's HSD post hoc tests to identify significant differences between the three treatment levels. The *Deschampsia* and mixed heath mesocosms were analyzed separately, as the data was derived from two separate experiments, and the soil and ecosystem types clearly differed from each other.

RESULTS

BVOC EMISSIONS AND VEGETATION IN THE HEATH MESOCOSMS

The BVOC emissions from the mixed heath mesocosms with intact vegetation cover were composed of sesquiterpenes and non-terpenoid compounds (Table 1). In total, 20 compounds were detected and of these 16 could be identified. The most emitted individual compounds were methyl-2-ethylhexanoate, β -selinene, and 2-methylfuran (Table 1).

The vegetation cover in the mixed heath mesocosms was not significantly different before the manipulations were performed ($P > 0.5$, MANOVA), so any differences between the mesocosm types should not be due to different vegetation. Averaged across all mesocosms, the cover percentages of the different species were $69 \pm 3\%$ for *Empetrum hermaphroditum*, $10 \pm 5\%$ for *Rhododendron lapponicum*, $3 \pm 1\%$ for *Andromeda polifolia*, and *Vaccinium uliginosum*, $2 \pm 1\%$ for graminoids, and $4 \pm 1\%$ for bryophytes. Furthermore, the total belowground biomass was similar in all mesocosms types ($P > 0.35$, ANOVA; that for soil mesocosms harvested when manipulations started and for the other treatments at the end of the experiment). Leaf and stem biomass for each species is shown in Table S1 in Supplementary Material.

The BVOC emissions from the *Deschampsia* mesocosms consisted of non-terpenoid compounds, in addition to the low emission of the monoterpene cis-ocimene (Table 1). The compound that was emitted in the highest quantity was 3-hexenyl acetate.

The total *Deschampsia* shoot biomass was $1.2 \pm 0.1 \text{ kg m}^{-2}$ in the root and soil mesocosms at the time of vegetation manipulations, and $1.8 \pm 0.2 \text{ kg m}^{-2}$ in the control mesocosms at the end of the experiment ($P < 0.01$, ANOVA followed by Tukey's HSD). The total belowground biomass was $3.2 \pm 1.0 \text{ kg m}^{-2}$ in the control mesocosms and not significantly different in the other mesocosms types ($P > 0.2$, ANOVA).

Table 1 | Emission rates (mean \pm SE) of biogenic volatile organic compounds emitted from the mixed heath and *Deschampsia* mesocosms with intact vegetation.

Site	Compound	Emission rate ($\mu\text{g m}^{-2} \text{ h}^{-1}$)
Mixed heath^a		
	α -Copaene	0.30 ± 0.08
	α -Bourbonene	0.01 ± 0.01
	trans-Caryophyllene	0.08 ± 0.02
	γ -curcumene	0.09 ± 0.03
	Aromadendrene	0.07 ± 0.02
	Humulene	0.22 ± 0.05
	Valencene	0.10 ± 0.04
	β -Selinene	1.94 ± 0.57
	α -Selinene	0.81 ± 0.28
	δ -Cadinene	0.02 ± 0.01
	Selina-3,7(11)-diene	0.14 ± 0.06
	Unidentified SQT	0.01 ± 0.01
	Unidentified SQT	0.04 ± 0.01
	Unidentified SQT	0.10 ± 0.05
	Total sesquiterpenes	3.94 ± 1.05
	2-Methylfuran	1.63 ± 0.40
	Methoxy-phenyl-oxime	0.39 ± 0.14
	Styrene	0.21 ± 0.07
	Methyl 2-ethylhexanoate	2.36 ± 0.37
	Benzenepropanol	0.04 ± 0.02
	Unidentified compound	1.76 ± 0.43
	Total other compounds	6.39 ± 1.10
	Total BVOCs	10.33 ± 1.39
<i>Deschampsia</i>		
	cis-ocimene	0.05 ± 0.05
	Total monoterpenes	0.05 ± 0.05
	Methoxy-phenyl-oxime	0.55 ± 0.27
	Phenol	0.09 ± 0.09
	3-Hexenyl acetate	2.35 ± 1.50
	Methyl 2-ethylhexanoate	0.18 ± 0.07
	3-methylheptylacetate	0.02 ± 0.02
	Total other compounds	3.19 ± 1.49
	Total BVOCs	3.24 ± 1.48

^aThe mixed heath was dominated by evergreen and deciduous dwarf shrubs (see text for details). SQT, sesquiterpene.

EFFECTS OF VEGETATION MANIPULATIONS ON BVOC EMISSIONS

Cutting of aboveground vegetation caused high emission bursts from the mesocosms (Figures 1, 2). For mixed heath mesocosms, the total emission of sesquiterpenes increased from $3.0 \pm 1.7 \mu\text{g m}^{-2} \text{ h}^{-1}$ (mean \pm SE, root mesocosms) right before cutting to $114 \pm 71 \mu\text{g m}^{-2} \text{ h}^{-1}$ measured within an hour after cutting. The following day the total sesquiterpene emissions decreased to $1.6 \pm 1.0 \mu\text{g m}^{-2} \text{ h}^{-1}$. The total emission of other compounds changed from $6.5 \pm 1.7 \mu\text{g m}^{-2} \text{ h}^{-1}$ before to $94 \pm 56 \mu\text{g m}^{-2} \text{ h}^{-1}$ immediately following cutting, and then to $5.8 \pm 5.3 \mu\text{g m}^{-2} \text{ h}^{-1}$ the day after cutting (Figure 2). The increase in emission rates

owed mainly to the induced emission of various C8-compounds (e.g., 1-octene, 1,3-octadiene, 2-octen-1-ol, and 1-octanone) and sesquiterpenes, most of which could not be identified to compound level (Table 2; Table S2 in Supplementary Material).

In *Deschampsia* mesocosms, the constitutive emissions were composed of non-terpenoid compounds, and therefore the grouping of compounds was omitted. The total BVOC emissions increased from $1.4 \pm 0.8 \mu\text{g m}^{-2} \text{h}^{-1}$ prior to cutting to $167 \pm 139 \mu\text{g m}^{-2} \text{h}^{-1}$ after the cutting of aboveground vegetation (Figure 3). Cutting induced an emission of 24 compounds, various mono- and sesquiterpenes and C8 compounds, of which ten had an emission rate above $1 \mu\text{g m}^{-2} \text{h}^{-1}$ (Table 2; Table S3 in Supplementary Material).

The effects of vegetation cutting on the emissions had ceased by the following day (Figure 2, Tables S2, S3 in Supplementary Material). During the rest of the experiment, the emissions from the control mixed heath mesocosms were considerably higher than the emissions from root and soil mesocosms (Figure 2). Sesquiterpenes were solely emitted from the mesocosms with aboveground vegetation at the rate of $5.4 \mu\text{g m}^{-2} \text{h}^{-1}$ averaged

across the measurement dates (Figure 2A). The emissions of other compounds were 4 and 18 times higher from the control mesocosms than from the root and soil mesocosms, respectively (Figure 2B).

In *Deschampsia* mesocosms, there were no significant differences between the vegetation treatments in the period 4–16 days after cutting (Figure 3).

Table 2 | List of biogenic volatile organic compounds induced by cutting of aboveground vegetation.

Mixed heath mesocosms	<i>Deschampsia</i> mesocosms
1-Octene	1,3-Octadiene
1,3-Octadiene	1,3,5-Octatriene
2-Octen-1-ol	2-Octen-1-ol
2-Octanone	2-Octanone
3-Octanol	β -Myrcene
Pentyl propanate	2-methylenebornane
trans-Caryophyllene	3-Octanol
γ -curcumene	Pentyl propanate
Aromadendrene	Geosmin
α -Elemene	Unidentified MT
β -Selinene	
α -Selinene	
α -Guaiane	
Selina-3,7(11)-diene	
Unidentified SQT	

The emission of the listed compounds increased at least by one order of magnitude directly after cutting. See Tables S2, S3 in Supplementary Material for emission rates.
SQT, sesquiterpene; MT, monoterpene.

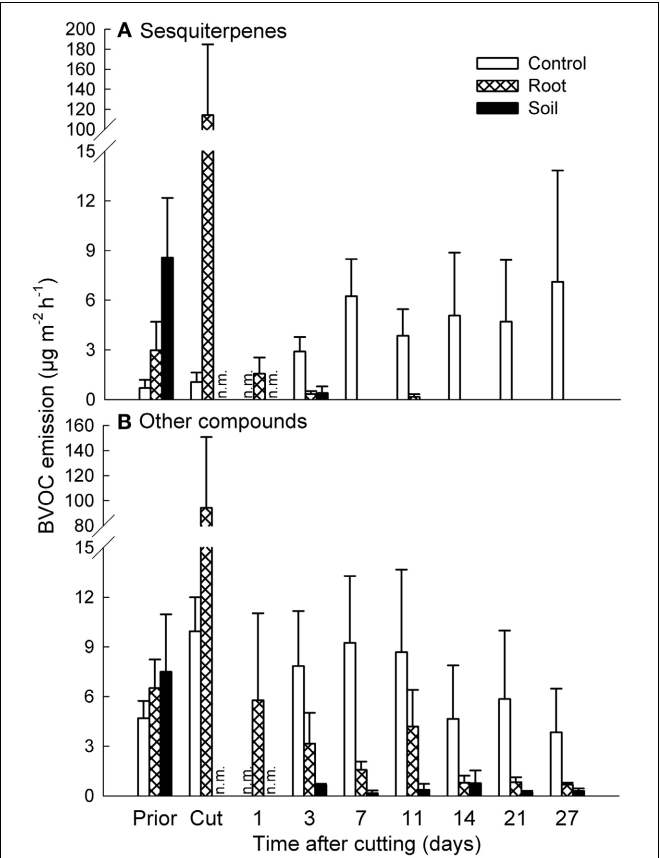


FIGURE 2 | Effect of experimental manipulations on biogenic volatile organic compound (BVOC) emissions from mixed heath mesocosms. The total emissions of (A) sesquiterpenes and (B) other BVOCs (mean + SE, $n = 3$) with intact vegetation (control), cut aboveground vegetation (root mesocosms), and all vegetation removed (soil mesocosms) prior to cutting (Prior), directly after cutting (Cut) and after cutting at different time intervals. n.m., not measured.

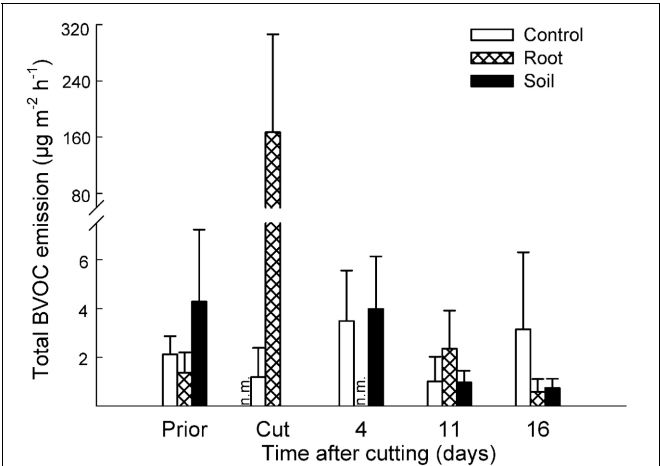


FIGURE 3 | Effect of experimental manipulations on biogenic volatile organic compound (BVOC) emissions from *Deschampsia* mesocosms. The total emissions of BVOCs (mean + SE, $n = 3$) with intact vegetation (control), cut aboveground vegetation (root mesocosms) and all vegetation removed (soil mesocosms) prior to cutting (Prior), directly after cutting (Cut) and after cutting at different time intervals. n.m., not measured.

CO₂ EXCHANGE

The mesocosms from both heath locations were net sources of CO₂ to the atmosphere with lower gross photosynthesis (P_G) rate than dark ecosystem respiration (R_{TOT}) rate during the experiment. Cutting of the aboveground vegetation or removing the belowground biomass did not cause any immediate alterations in CO₂ exchange, and only the data from after the established treatments are shown.

There were no statistically significant differences between the vegetation treatments in NEE, P_G , or R_{TOT} of the mixed heath mesocosms, although the control mesocosms showed some gross carbon assimilation while the root and soil mesocosms did not (Figure 4). In contrast, for *Deschampsia* mesocosms the NEE was significantly lower in the soil mesocosms

than in the control and root mesocosms ($P < 0.001$; Figure 5). For R_{TOT} , all treatments differed significantly from each other, with highest total respiration in the control mesocosms followed by root and soil mesocosms. Carbon uptake into the system as a result of P_G was naturally highest in the control mesocosms and significantly lower in the root and soil mesocosms.

CONCENTRATIONS OF C AND N IN SOIL AND MICROBIAL BIOMASS

In the mixed heath soil, the concentrations of NH_4^+ , NO_3^- , DOC, and DON in the soil mesocosms were 43–67% lower than the concentrations in the control mesocosms, although the difference for NH_4^+ was only marginally significant ($0.05 < P < 0.1$, Tukey's HSD; Table 3). There were no significant differences in

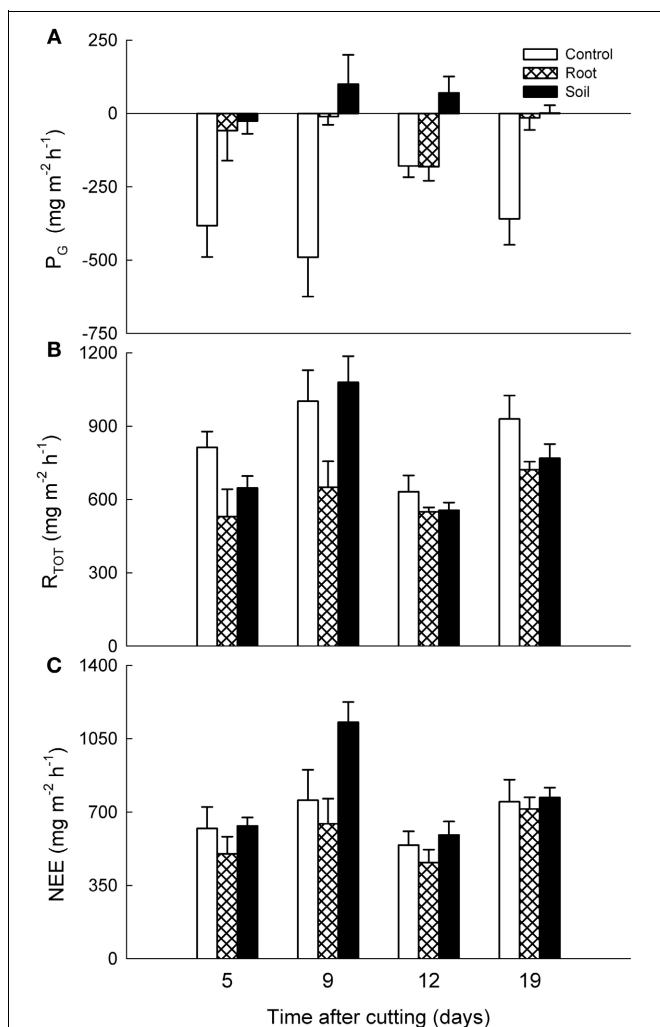


FIGURE 4 | Effects of experimental manipulations on CO₂ exchange in mixed heath mesocosms. Mean (\pm SE, $n = 3$) (A) gross photosynthesis (P_G), (B) dark ecosystem respiration (R_{TOT}) and (C) net ecosystem exchange (NEE) with intact vegetation (control), cut aboveground vegetation (root mesocosms), and all vegetation removed (soil mesocosms) as function of time after cutting. Positive values denote carbon loss and negative values carbon uptake into the system.

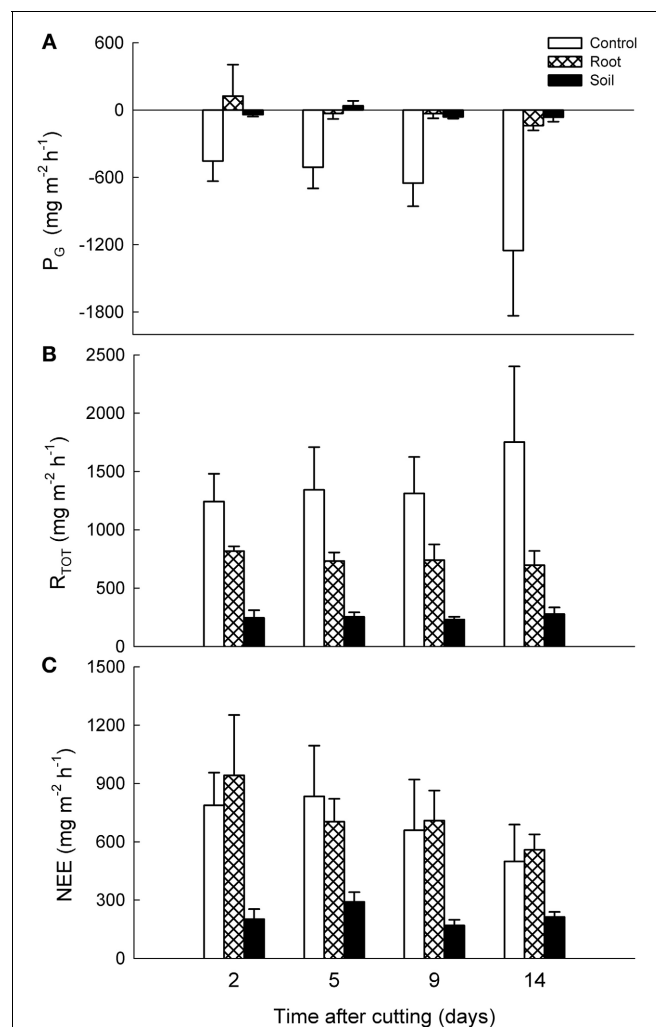


FIGURE 5 | Effects of experimental manipulations on CO₂ exchange in *Deschampsia* mesocosms. Mean (\pm SE, $n = 3$) (A) gross photosynthesis (P_G), (B) dark ecosystem respiration (R_{TOT}) and (C) net ecosystem exchange (NEE) with intact vegetation (control), cut aboveground vegetation (root mesocosms) and all vegetation removed (soil mesocosms) as function of time after cutting. Positive values denote carbon loss and negative values carbon uptake into the system.

Table 3 | Concentrations of NH_4^+ , NO_3^- , dissolved organic carbon (DOC), dissolved organic nitrogen (DON), microbial biomass carbon (C_{MIC}), and microbial biomass nitrogen (N_{MIC}) in soil from the mixed heath and *Deschampsia* mesocosms.

	Mixed heath mesocosms			<i>Deschampsia</i> mesocosms		
	Control	Root	Soil	Control	Root	Soil
$\text{NH}_4^+\text{-N}$ ($\mu\text{g g}^{-1}$ SOM)	3.6 ± 0.6	3.2 ± 0.3	1.8 ± 0.1	31 ± 16	137 ± 41	103 ± 54
$\text{NO}_3^-\text{-N}$ ($\mu\text{g g}^{-1}$ SOM)	0.81 ± 0.16^a	$0.61 \pm 0.10^{a, b}$	0.27 ± 0.06^b	80 ± 23	260 ± 67	159 ± 134
DOC (mg g^{-1} SOM)	1.6 ± 0.3^a	$1.4 \pm 0.1^{a, b}$	0.9 ± 0.1^b	2.6 ± 0.3^a	2.5 ± 0.1^a	4.1 ± 0.4^b
DON ($\mu\text{g g}^{-1}$ SOM)	125 ± 23^a	$110 \pm 9^{a, b}$	65 ± 4^b	565 ± 62^a	843 ± 92^b	1255 ± 58^c
C_{MIC} (mg g^{-1} SOM)	5.8 ± 0.7	5.1 ± 0.3	5.3 ± 0.2	6.4 ± 1.1	5.8 ± 0.7	5.9 ± 0.6
N_{MIC} ($\mu\text{g g}^{-1}$ SOM)	442 ± 57	397 ± 18	430 ± 28	569 ± 183	548 ± 119	570 ± 30

The mesocosms had intact vegetation (control), had cut aboveground vegetation (root mesocosms) or all vegetation removed (soil mesocosms). Soil was analysed after the last gas exchange measurements.

The values are mean \pm SE.

The different letters (a, b, c) indicate statistically significant differences between the mesocosms types within a location at 95% significance level (Tukey's HSD test).

C_{MIC} and N_{MIC} concentrations between the mesocosms types ($P > 0.3$, ANOVA).

In *Deschampsia* soil, the variation was higher, and the only statistically significant differences were the higher concentrations of DOC and DON in the soil mesocosms than in the control and root mesocosms ($P < 0.05$, Tukey's HSD; Table 3). For DON, the concentration was lowest in the control, 50% higher in the root mesocosms ($P < 0.01$, Tukey's HSD; Table 3) and more than doubled in the soil mesocosms ($P < 0.001$, Tukey's HSD).

DISCUSSION

CONSTITUTIVE BVOC EMISSIONS FROM HEATH ECOSYSTEMS OUTSIDE THE GROWING SEASON

The two heath ecosystems under investigation emitted contrasting BVOCs from different sources. While the *Deschampsia*-dominated temperate heath was characterized by low BVOC emissions from vegetation, the subarctic mixed heath had clearly vegetation-dominated emissions, despite the vegetation being less active due to onset of autumn, as shown by the low gross photosynthesis rates. The gross photosynthesis rate in the mixed heath mesocosms was in the same range as that measured *in situ* before the growing season start in May (Nielsen, et al., unpublished data). Despite the plants being less active, both vegetation types reacted with strongly induced emissions to cutting of the aboveground vegetation. This is most likely because the BVOCs released upon cutting originate from storage structures, such as resin ducts or glandular trichomes, and not from *de novo* synthesis (Holopainen and Gershenson, 2010).

A characteristic feature for the emissions from the mixed heath mesocosms with nearly 70% *E. hermaphroditum* cover was the lack of isoprene and monoterpenes, and the higher amount and variety of sesquiterpene emissions. This finding is in line with the results of Faubert et al. (2012) who found that *E. hermaphroditum* was an important sesquiterpene source for the mountain birch forest floor emissions measured during the growing season. The total BVOC emission rate of the mixed heath mesocosms, $10.33 \mu\text{g m}^{-2} \text{h}^{-1}$, was in the same range as the emission rates during the growing season from *in situ*

measurements in Abisko: $10.9\text{--}14.61 \mu\text{g m}^{-2} \text{h}^{-1}$ for a mixed wet heath (Tiiva et al., 2008; Faubert et al., 2010) and $3.5\text{--}45 \mu\text{g m}^{-2} \text{h}^{-1}$ for an *E. hermaphroditum*-dominated forest floor (Faubert et al., 2012), depending on the year. The emission rates of the present experiment may be somewhat understated due to the light intensity in the growth chambers being lower than for open ecosystems under field conditions in full sunlight. However, the light intensity was similar to shaded or clouded conditions in a forest understory (Olsrud and Michelsen, 2009). The lacking isoprene and monoterpene emissions are likely due to that the vegetation has been without active *de novo* synthesis of BVOCs in the photosynthesizing green leaves. It appears, however, that despite lacking photosynthetic activity, the evergreen plants are releasing BVOCs at a temperature of about 13.6°C .

The constitutive emissions from the *Deschampsia* mesocosms lacked all terpenoids. This is in agreement with an earlier qualitative assessment of emissions from *D. flexuosa*, which indicated no isoprene or monoterpene emissions (Hewitt and Street, 1992). In fact, there were no significant differences in emissions from the *Deschampsia* mesocosms among control, root and soil mesocosms, suggesting that the plants were a minor source for the ecosystem-level emissions in these off-season measurements, except immediately after cutting.

SOURCE OF THE EMITTED BVOCs

Most BVOCs emitted from the mixed heath mesocosms were clearly produced by plants as their emission ceased after the aboveground vegetation was removed by cutting. Further, the net emissions originating below soil surface appeared to be mainly derived from roots and rhizomes. It should be taken into account that by cutting the vegetation also the transport of assimilates into belowground plant parts ceases and root exudation rates are likely to decrease, thereby reducing potential BVOC release both from belowground plant parts and soil microbial activity fuelled by root exudates. However, we assume that assimilate transport within the plants is minimal outside the main growth period.

The emissions of 2-methylfuran and methyl-2-ethylhexanoate, both of which can be used as indicators of microbial growth on construction materials (e.g., Korpi et al., 2009), were halved by

cutting and reduced to below the detection limit when below-ground plant parts were removed from the soil. This suggests that these compounds could originate from decomposition of root exudates released from living plants. Different furans are widely emitted from soils (Leff and Fierer, 2008; McNeal and Herbert, 2009), and therefore their absence in the soil mesocosm emissions was surprising. However, these compounds were also not detected in the emissions from a mountain birch forest floor with removed aboveground vegetation cover (Faubert et al., 2012).

The only compound emitted from all the mesocosm types in both heath ecosystems, including the soil mesocosms, was methoxy-phenyl-oxime. This N-containing aromatic compound has been previously observed to be emitted from soil (McNeal and Herbert, 2009). It is likely to originate from microbial activity as it has been reported to be an antifungal volatile emitted by *Bacillus subtilis* bacteria isolated from soil (Liu et al., 2008), to be released from myxobacteria during fermentation (Xu et al., 2011) and to be synthesized by an *in vitro* system consisting of the ectomycorrhizal fungus *Tuber borchii* and the *Tilia americana* L. plant roots (Menotta et al., 2004).

The lack of net emissions from soil may owe to a lack of microbial BVOC production in the absence of decaying belowground plant biomass and root exudates or stimulated uptake of BVOCs by soil microorganisms (Owen et al., 2007; Ramirez et al., 2010) as a result of increased aeration of soil due to the hand-sorting of the soil upon removal of roots and rhizomes. The hand-sorting received by the soil mesocosms caused a side-effect to this treatment in the form of broken physical structure and increased aeration of microsites within soil. We estimate that the stimulative effect on microbial uptake of BVOCs is more important than the suppressive effect on gross microbial BVOC production, but these two processes cannot be separated in the present study.

In the mixed heath mesocosms, the removal of belowground plant parts significantly reduced the concentrations of DOC and DON in the soil, while there was no difference in the CO₂ exchange rates between the vegetation manipulation treatments. In *Deschampsia* mesocosms, in contrast, the concentrations of DOC and DON were significantly higher and the loss of CO₂ decreased to less than half of the other mesocosm types after the removal of belowground plant parts. Hence, in the more sandy *Deschampsia* dominated system, mineralization of dissolved organic compounds was partially dependent upon the presence of roots, while microbial turnover of dissolved organic compounds continued in the absence of roots in the mixed heath, possibly due to higher fungal dominance (microbial C/N ratio) and higher soil organic matter content.

Other BVOCs potentially emitted are carbonyls, but our measurement setup could not be used to measure these compounds, because of breakthrough of these light molecules through the used adsorbents. Carbonyls were the compounds emitted at highest rates by cultured boreal forest soil fungi, as highlighted by a proton transfer reaction-mass spectrometer (PTR-MS) study by Bäck et al. (2010). Other PTR-MS studies have also shown that the short-chain carbonyls, such as methanol and acetone, are compounds emitted at high rates from litter and soil (Ramirez et al., 2010; Gray and Fierer, 2012).

EMISSIONS INDUCED BY VEGETATION CUTTING

Cutting the aboveground vegetation caused large emission bursts, which is a common observation in response to mechanical wounding of plants (Fall et al., 1999; Loreto et al., 2006; Brilli et al., 2011). However, in contrast with the earlier studies reporting high emissions of C₆ compounds (so called green leaf volatiles, GLVs) (Fall et al., 1999; Loreto et al., 2006; Brilli et al., 2011), the induced emissions here mainly consisted of C₈-ketones, C₈-alcohols and sesquiterpenes. The C₈-compounds such as 1-octen-3-ol, 3-octanol, 3-octanone have common precursors with the commonly observed GLVs, namely linoleic and linolenic acid (Wurzenberger and Grosch, 1982; Hatanaka, 1993).

Some C₈-compounds have been reported to be emitted from cabbage leaves in response to mechanical damage and herbivore wounding (Mattiacci et al., 1994). However, *Deschampsia cespitosa* has been shown to emit these compounds both with and without mechanical damage or jasmonic acid application (Watkins et al., 2006). Another possibility is that the C₈-compounds were released from fungal endophytes living within the leaves (Rosa et al., 2009) and roots (Tejesvi et al., 2012) of *Deschampsia*. Both C₈-compounds and geosmin, which was also induced by cutting of *Deschampsia*, are common volatiles produced by microorganisms, especially fungi (Korpi et al., 2009). Also cyanobacteria are known to emit C₈-compounds (Schulz and Dickschat, 2007), and it is possible that the cyanobacteria living within mosses are a source of part of the induced emissions especially in the mixed heath mesocosms from which the moss cover was removed and the emissions increased by cutting.

Studies using online monitoring by PTR-MS of BVOCs produced in connection with mechanical wounding of plants have shown that the instantaneous release of C₆ aldehydes originating from membrane rupture and the consequent oxidation of unsaturated fatty acids shifts to the production of alcohols and acetates within a couple of minutes from wounding (Fall et al., 1999; Brilli et al., 2011). In this study, the BVOC measurement was started within 10 min from finishing the cutting, which means that the compounds immediately released from the ruptured plant tissues were most probably not caught by our measurements.

To conclude, the net BVOC emissions from the belowground part of these well-drained heath ecosystems do not significantly contribute to the ecosystem emissions, at least during late autumn when the vegetation is not fully active. We recommend that future studies attempting to connect soil BVOC exchange with soil microbiology would use methods that can separate production and uptake of BVOCs, for example by PTR-MS measurements, and combine these with analyses of microbial community structure and function e.g., by DNA- and RNA-based techniques. In general, the total BVOC emissions from the mixed heath were in the same range as the emissions measured in a similar ecosystem with active vegetation and peaking biomass. This finding highlights the importance of taking into account the off-season period, also for the northern areas. The ecosystem emissions are likely to be momentarily or periodically increased by the production of induced volatiles as a result of grazing by mammals or insect herbivory. Especially the latter can have a significant impact on subarctic heath BVOC emissions as large-scale insect

outbreaks are expected to become more frequent in a warmer climate (Arneth and Niinemets, 2010).

ACKNOWLEDGMENTS

This work was financially supported by the Danish Council for Independent Research | Natural Sciences and the Villum Foundation. We also thank the Danish National Research Foundation for supporting the activities within the Center for Permafrost (CENPERM DNRF100). We acknowledge the Nordic

Center of Excellence on Cryosphere-Atmosphere Interactions in a Changing Arctic Climate (CRAICC). We thank Gosha Sylvester and Esben V. Nielsen for laboratory assistance, and Prof. Jarmo K. Holopainen for insightful discussions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/Terrestrial_Microbiology/10.3389/fmicb.2013.00224/abstract

REFERENCES

- Aaltonen, H., Pumpanen, J., Hakola, M., Vesala, T., Rasmus, S., and Bäck, J. (2012). Snowpack concentrations and estimated fluxes of volatile organic compounds in a boreal forest. *Biogeosciences* 9, 2033–2044. doi: 10.5194/bg-9-2033-2012
- Aaltonen, H., Pumpanen, J., Pihlatie, M., Hakola, H., Hellén, H., Kulmala, L., et al. (2011). Boreal pine forest floor biogenic volatile organic compound emissions peak in early summer and autumn. *Agric. For. Meteorol.* 151, 682–691. doi: 10.1016/j.agrformet.2010.12.010
- Amaral, J. A., and Knowles, R. (1998). Inhibition of methane consumption in forest soils by monoterpenes. *J. Chem. Ecol.* 24, 723–734. doi: 10.1023/A:1022398404448
- Amin, H., Atkins, P. T., Russo, R. S., Brown, A. W., Sive, B., Hallar, A. G., et al. (2012). Effect of bark beetle infestation on secondary organic aerosol precursor emissions. *Environ. Sci. Technol.* 46, 5696–5703. doi: 10.1021/es204205m
- Arneth, A., and Niinemets, Ü. (2010). Induced BVOCs: how to bug our models? *Trends Plant Sci.* 15, 118–125.
- Bäck, J., Aaltonen, H., Hellén, H., Kajos, M. K., Patokoski, J., Taipale, R., et al. (2010). Variable emissions of microbial volatile organic compounds (MVOCs) from root-associated fungi isolated from Scots pine. *Atmos. Environ.* 44, 3651–3659. doi: 10.1016/j.atmosenv.2010.06.042
- Bais, H. P., Park, S.-W., Weir, T. L., Callaway, R. M., and Vivanco, J. M. (2004). How plants communicate using the underground superhighway. *Trends Plant Sci.* 9, 26–32. doi: 10.1016/j.tplants.2003.11.008
- Brilli, F., Ruuskanen, T. M., Schnitzhofer, R., Müller, M., Breitenlechner, M., Bittner, V., et al. (2011). Detection of plant volatiles after leaf wounding and darkening by proton transfer reaction “Time-of-Flight” mass spectrometry (PTR-TOF). *PLoS ONE* 6:e20419. doi: 10.1371/journal.pone.0020419
- Dicke, M., and Bruin, J. (2001). Chemical information transfer between plants: back to the future. *Biochem. Syst. Ecol.* 29, 981–994. doi: 10.1016/S0305-1978(01)00045-X
- Fall, R., Karl, T., Hansel, A., Jordan, A., and Lindinger, W. (1999). Volatile organic compounds emitted after leaf wounding: on-line analysis by proton-transfer-reaction mass spectrometry. *J. Geophys. Res.* 104, 15963–15974. doi: 10.1029/1999JD900144
- Faubert, P., Tiiva, P., Michelsen, A., Rinnan, Å., Ro-Poulsen, H., and Rinnan, R. (2012). The shift in plant species composition in a subarctic mountain birch forest floor due to climate change would modify the biogenic volatile organic compound emission profile. *Plant Soil* 352, 199–215. doi: 10.1007/s11104-011-0989-2
- Faubert, P., Tiiva, P., Rinnan, Å., Michelsen, A., Holopainen, J. K., and Rinnan, R. (2010). Doubled volatile organic compound emissions from subarctic tundra under simulated climate warming. *New Phytol.* 187, 199–208. doi: 10.1111/j.1469-8137.2010.03270.x
- Fuentes, J. D., Gu, L., Lerdau, M., Atkinson, R., Baldocchi, D., Bottenheim, J. W., et al. (2000). Biogenic hydrocarbons in the atmospheric boundary layer: a review. *Bull. Am. Meteorol. Soc.* 81, 1537–1575
- Gray, C. M., and Fierer, N. (2012). Impacts of nitrogen fertilization on volatile organic compound emissions from decomposing plant litter. *Global Change Biol.* 18, 739–748. doi: 10.1111/j.1365-2486.2011.02569.x
- Hatanaka, A. (1993). The biogeneration of green odour by green leaves. *Phytochemistry* 34, 1201–1218. doi: 10.1016/0031-9422(91)80003-J
- Hayward, S., Muncey, R. J., James, A. E., Halsall, C. J., and Hewitt, C. N. (2001). Monoterpene emissions from soil in a Sitka spruce forest. *Atmos. Environ.* 35, 4081–4087. doi: 10.1016/S1352-2310(01)00213-8
- Helmig, D., Apel, E., Blake, D., Ganzeveld, L., Lefer, B. L., Meinardi, S., et al. (2009). Release and uptake of volatile inorganic and organic gases through the snowpack at Niwot Ridge, Colorado. *Biogeochemistry* 95, 167–183. doi: 10.1007/s10533-009-9326-8
- Hewitt, C. N., and Street, R. A. (1992). A qualitative assessment of the emission of non-methane hydrocarbon compounds from the biosphere to the atmosphere in the U.K.: present knowledge and uncertainties. *Atmos. Environ.* 26, 3069–3077. doi: 10.1016/0960-1686(92)90463-U
- Holopainen, J. K., and Gershenson, J. (2010). Multiple stress factors and the emission of plant VOCs. *Trends Plant Sci.* 15, 176–184
- Insam, H., and Seewald, M. (2010). Volatile organic compounds (VOCs) in soils. *Biol. Fert. Soils* 46, 199–213.
- Jenkinson, D. S., and Powlson, D. S. (1976). The effect of biocidal treatments on metabolism in soil. V. A method of measuring soil biomass. *Soil Biol. Biochem.* 8, 209–213
- Jimenez, J. L., Canagaratna, M. R., Donahue, N. M., Prevot, A. S. H., Zhang, Q., Kroll, J. H., et al. (2009). Evolution of organic aerosols in the atmosphere. *Science* 326, 1525–1529. doi: 10.1126/science.1180353
- Joergensen, R. G. (1996). The fumigation-extraction method to estimate soil microbial biomass: calibration of the kEC value. *Soil Biol. Biochem.* 28, 25–31. doi: 10.1016/0038-0717(95)00102-6
- Jonasson, S., Michelsen, A., Schmidt, I. K., Nielsen, E. V., and Callaghan, T. V. (1996). Microbial biomass C, N and P in two arctic soils and the responses to addition of NPK fertilizer and carbon: implications for plant nutrient uptake. *Oecologia* 106, 507–515.
- Korpi, A., Järnberg, J., and Pasanen, A.-L. (2009). Microbial volatile organic compounds. *Crit. Rev. Toxicol.* 39, 139–193. doi: 10.1080/10408440802291497
- Laothawornkitkul, J., Taylor, J. E., Paul, N. D., and Hewitt, C. N. (2009). Biogenic volatile organic compounds in the Earth system. *New Phytol.* 183, 27–51. doi: 10.1111/j.1469-8137.2009.02859.x
- Leff, J. W., and Fierer, N. (2008). Volatile organic compound (VOC) emissions from soil and litter samples. *Soil Biol. Biochem.* 40, 1629–1636. doi: 10.1016/j.soilbio.2008.01.018
- Lehnhin, A., Zimmer, I., Steinbrecher, R., Brüggemann, N., and Schnitzler, J.-P. (1999). Isoprene synthase activity and its relation to isoprene emission in *Quercus robur* L. leaves. *Plant Cell Environ.* 22, 495–504. doi: 10.1046/j.1365-3040.1999.00425.x
- Lin, C., Owen, S. M., and Peñuelas, J. (2007). Volatile organic compounds in the roots and rhizosphere of *Pinus* spp. *Soil Biol. Biochem.* 39, 951–960.
- Liu, W., Mu, W., Zhu, B., and Liu, F. (2008). Antifungal activities and components of VOCs produced by *Bacillus subtilis* G8. *Curr. Res. Bact.* 1, 28–34. doi: 10.3923/crb.2008.28.34
- Loreto, F., Barta, C., Brilli, F., and Nogue, I. (2006). On the induction of volatile organic compound emissions by plants as consequence of wounding or fluctuations of light and temperature. *Plant Cell Environ.* 29, 1820–1828. doi: 10.1111/j.1365-3040.2006.01561.x
- Loreto, F., and Schnitzler, J.-P. (2010). Abiotic stresses and induced BVOCs. *Trends Plant Sci.* 15, 154–166. doi: 10.1016/j.tplants.2009.12.006
- Mattiacci, L., Dicke, M., and Posthumus, M. A. (1994). Induction of parasitoid attracting synomone in brussels sprouts plants by feeding of *Pieris brassicae* larvae: role of mechanical damage and herbivore elicitor. *J. Chem. Ecol.* 20, 2229–2247. doi: 10.1007/BF02033199
- McNeal, K. S., and Herbert, B. E. (2009). Volatile organic

- metabolites as indicators of soil microbial activity and community composition shifts. *Soil Sci. Soc. Am. J.* 73, 579–588. doi: 10.2136/sssaj2007.0245
- Menotta, M., Gioacchini, A. M., Amicucci, A., Buffalini, M., Sisti, D., and Stocchi, V. (2004). Headspace solid-phase microextraction with gas chromatography and mass spectrometry in the investigation of volatile organic compounds in an ectomycorrhizae synthesis system. *Rapid Commun. Mass Sp.* 18, 206–210. doi: 10.1002/rcm.1314
- Olsrud, M., and Michelsen, A. (2009). Effects of shading on photosynthesis, plant organic nitrogen uptake, and root fungal colonization in a subarctic mire ecosystem. *Botany* 87, 463–474. doi: 10.1139/B09-021
- Owen, S. M., Clark, S., Pompe, M., and Semple, K. T. (2007). Biogenic volatile organic compounds as potential carbon sources for microbial communities in soil from the rhizosphere of *Populus tremula*. *FEMS Microbiol. Lett.* 268, 34–39. doi: 10.1111/j.1574-6968.2006.00602.x
- Ramirez, K., Lauber, C., and Fierer, N. (2010). Microbial consumption and production of volatile organic compounds at the soil-litter interface. *Biogeochemistry* 99, 97–107. doi: 10.1007/s10533-009-9393-x
- Riipinen, I., Yli-Juuti, T., Pierce, J. R., Petäjä, T., Worsnop, D. R., Kulmala, M., et al. (2012) The contribution of organics to atmospheric nanoparticle growth. *Nat. Geosci.* 5, 453–458.
- Rinnan, R., Michelsen, A., and Jonasson, S. (2008). Effects of litter addition and warming on soil carbon, nutrient pools and microbial communities in a subarctic heath ecosystem. *Appl. Soil Ecol.* 39, 271–281. doi: 10.1016/j.apsoil.2007.12.014
- Rosa, L., Vaz, A., Caligiorne, R., Campolina, S., and Rosa, C. (2009). Endophytic fungi associated with the Antarctic grass *Deschampsia antarctica* Desv. (*Poaceae*). *Polar Biol.* 32, 161–167. doi: 10.1007/s00300-008-0515-z
- Sallas, L., Vuorinen, M., Kainulainen, P., and Holopainen, J. K. (1999). Effects of planting on concentrations of terpenes, resin acids and total phenolics in *Pinus sylvestris* seedlings. *Scand. J. For. Res.* 14, 218–226. doi: 10.1080/02827589950152737
- Scherrer, D., and Körner, C. (2010). Infra-red thermometry of alpine landscapes challenges climatic warming projections. *Global Change Biol.* 16, 2602–2613.
- Schulz, S., and Dickschat, J. S. (2007). Bacterial volatiles: the smell of small organisms. *Nat. Prod. Rep.* 24, 814–842. doi: 10.1039/b507392h
- Smolander, A., Ketola, R. A., Kotiaho, T., Kanerva, S., Suominen, K., and Kitunen, V. (2006). Volatile monoterpenes in soil atmosphere under birch and conifers: effects on soil N transformations. *Soil Biol. Biochem.* 38, 3436–3442. doi: 10.1016/j.soilbio.2006.05.019
- Stahl, P. D., and Parkin, T. B. (1996). Microbial production of volatile organic compounds in soil microcosms. *Soil Sci. Soc. Am. J.* 60, 821–828. doi: 10.2136/sssaj1996.03615995006000030020x
- Tejesvi, M. V., Sauvola, T., Pirttilä, A. M., and Ruotsalainen, A. L. (2012). Neighboring *Deschampsia flexuosa* and *Trientalis europaea* harbor contrasting root fungal endophytic communities. *Mycorrhiza* 23, 1–10. doi: 10.1007/s00572-012-0444-0
- Tiiva, P., Faubert, P., Michelsen, A., Holopainen, T., Holopainen, J. K., and Rinnan, R. (2008). Climatic warming increases isoprene emission from a subarctic heath. *New Phytol.* 180, 853–863. doi: 10.1111/j.1469-8137.2008.02587.x
- Vance, E. D., Brookes, P. C., and Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703–707. doi: 10.1016/0038-0717(87)90052-6
- Warneke, C., Karl, T., Judmaier, H., Hansel, A., Jordan, A., Lindinger, W., et al. (1999). Acetone, methanol, and other partially oxidized volatile organic emissions from dead plant matter by abiological processes: Significance for atmospheric HO_x chemistry. *Global Biogeochem. Cycles* 13, 9–17. doi: 10.1029/98GB02428
- Watkins, E., Gianfagna, T. J., Sun, R., and Meyer, W. A. (2006). Volatile compounds of tufted hairgrass. *Crop. Sci.* 46, 2575–2580. doi: 10.2135/cropsci2006.02.0094
- White, C. S. (1991). The role of monoterpenes in soil nitrogen cycling processes in ponderosa pine: results from laboratory bioassays and field studies. *Biogeochemistry* 12, 43–68. doi: 10.1007/BF00002625
- White, C. S. (1994). Monoterpenes: their effects on ecosystem nutrient cycling. *J. Chem. Ecol.* 20, 1381–1406. doi: 10.1007/BF02059813
- Wurzenberger, M., and Grosch, W. (1982). The enzymic oxidative breakdown of linoleic acid in mushrooms (*Psalliota bispora*). *Z. Lebensm. Unters. F.* A 175, 186–190. doi: 10.1007/BF01139769
- Xu, F., Tao, W., and Sun, J. (2011). Identification of volatile compounds released by myxobacteria *Sorangium cellulosum* AHB103-101. *Afr. J. Microbiol. Res.* 5, 353–358.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 15 April 2013; accepted: 17 July 2013; published online: 15 August 2013.
Citation: Rinnan R, Gierth D, Bilde M, Rosenørn T and Michelsen A (2013) Off-season biogenic volatile organic compound emissions from heath mesocosms: responses to vegetation cutting. *Front. Microbiol.* 4:224. doi: 10.3389/fmicb.2013.00224

This article was submitted to *Frontiers in Terrestrial Microbiology*, a specialty of *Frontiers in Microbiology*.

Copyright © 2013 Rinnan, Gierth, Bilde, Rosenørn and Michelsen. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Effects of *Spartina alterniflora* invasion on the communities of methanogens and sulfate-reducing bacteria in estuarine marsh sediments

Jemaneh Zeleke¹, Qiang Sheng², Jian-Gong Wang¹, Ming-Yao Huang², Fei Xia¹, Ji-Hua Wu² and Zhe-Xue Quan^{1*}

¹ Department of Microbiology and Microbial Engineering, School of Life Sciences, Fudan University, Shanghai, China

² Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, Department of Ecology and Evolutionary Biology, Fudan University, Shanghai, China

Edited by:

Per Bengtson, Lund University, Sweden

Reviewed by:

Hongchen Jiang, Miami University, USA

Xiuzhu Dong, Chinese Academy of Sciences, China

*Correspondence:

Zhe-Xue Quan, Department of Microbiology and Microbial Engineering, School of Life Sciences, Fudan University, 220 Handan Road, Shanghai 200433, China
e-mail: quanxz@fudan.edu.cn

The effect of plant invasion on the microorganisms of soil sediments is very important for estuary ecology. The community structures of methanogens and sulfate-reducing bacteria (SRB) as a function of *Spartina alterniflora* invasion in *Phragmites australis*-vegetated sediments of the Dongtan wetland in the Yangtze River estuary, China, were investigated using 454 pyrosequencing and quantitative real-time PCR (qPCR) of the methyl coenzyme M reductase A (*mcrA*) and dissimilatory sulfite reductase (*dsrB*) genes. Sediment samples were collected from two replicate locations, and each location included three sampling stands each covered by monocultures of *P. australis*, *S. alterniflora* and both plants (transition stands), respectively. qPCR analysis revealed higher copy numbers of *mcrA* genes in sediments from *S. alterniflora* stands than *P. australis* stands (5- and 7.5-fold more in the spring and summer, respectively), which is consistent with the higher methane flux rates measured in the *S. alterniflora* stands (up to $8.01 \pm 5.61 \text{ mg m}^{-2} \text{ h}^{-1}$). Similar trends were observed for SRB, and they were up to two orders of magnitude higher than the methanogens. Diversity indices indicated a lower diversity of methanogens in the *S. alterniflora* stands than the *P. australis* stands. In contrast, insignificant variations were observed in the diversity of SRB with the invasion. Although *Methanomicrobiales* and *Methanococcales*, the hydrogenotrophic methanogens, dominated in the salt marsh, *Methanomicrobiales* displayed a slight increase with the invasion and growth of *S. alterniflora*, whereas the later responded differently. *Methanosarcina*, the metabolically diverse methanogens, did not vary with the invasion of, but *Methanosaeta*, the exclusive acetate utilizers, appeared to increase with *S. alterniflora* invasion. In SRB, sequences closely related to the families *Desulfobacteraceae* and *Desulfobulbaceae* dominated in the salt marsh, although they displayed minimal changes with the *S. alterniflora* invasion. Approximately $11.3 \pm 5.1\%$ of the *dsrB* gene sequences formed a novel cluster that was reduced upon the invasion. The results showed that in the sediments of tidal salt marsh where *S. alterniflora* displaced *P. australis*, the abundances of methanogens and SRB increased, but the community composition of methanogens appeared to be influenced more than did the SRB.

Keywords: dissimilatory sulfite reductase B (*dsrB*), methyl coenzyme M reductase A (*mcrA*), *spartina alterniflora*, *phragmites australis*, estuarine marsh

INTRODUCTION

Estuarine wetlands are among the most productive ecosystems on the Earth and provide many key ecosystem services. These environments are highly vulnerable to the invasion of exotic plant species, and ecosystem functions may be altered as a consequence of the invasion (Williams and Grosholz, 2008). For example, the native *Phragmites australis* and *Scirpus maritimus* communities of the Dongtan tidal flats, comprising approximately 32,600 ha of the Yangtze River estuary, are currently being invaded by the aggressive exotic *Spartina alterniflora*. Furthermore, *S. alterniflora* was introduced to the Yangtze River estuary in 1990s to increase

the protection of coastal banks and to accelerate sedimentation and land formation (Chung, 1993; Liao et al., 2007). However, due to its extensive expansion (approximately 43% of the vegetated part as of 2004) (Chen et al., 2008) and displacement of the native species, a number of ecological impacts have occurred. The impacts of *S. alterniflora* invasion on the aboveground flora and fauna of Dongtan tidal salt marsh have been described by several studies (Jiang et al., 2009; Zhang et al., 2010). Recently, a study by Cheng and his colleagues (2007) demonstrated that methane flux rates in *S. alterniflora* stands are higher than in areas dominated by *P. australis*, which is consistent with several

studies that indicate higher methane flux rates in the stands covered by *S. alterniflora* than in those covered by other salt marsh plants (Cheng et al., 2007; Wang et al., 2009; Zhang et al., 2010; Tong et al., 2012). The root exudates and litter decomposition of *S. alterniflora* can alter the nutritional contents of soils, which in turn may affect the functioning of soil microbial communities. Hence, parallel to the impacts that *S. alterniflora* may pose to the aboveground ecology, it has the potential to affect belowground microbial communities and processes. In tidal marshes of estuaries where large amounts of vegetation and river-derived organic matter are deposited, nutrients are typically mineralized through anaerobic processes, predominantly via methanogenesis and sulfate reduction.

Methanogenesis and dissimilatory sulfate reduction are the two key terminal electron-scavenging processes in the anaerobic decomposition of organic matter. These anaerobic processes are known to compete for hydrogen and acetate (Abram and Nedwell, 1978; King, 1984), but the latter has higher affinity and can outcompete with methanogenesis in sediments having high sulfate concentration, such as marine and salt marsh surface sediments (Oremland et al., 1982; Winfrey and Ward, 1983; Purdy et al., 2003). Although competition is one of the major factors controlling the distribution and activity of methanogenesis, land and tide-derived depositions and autochthonous production by marsh plants and freshwater dilutions of sea water in tidal marshes may create conditions for methanogenesis to be a major terminal electron accepting process (Senior et al., 1982; Schubauer and Hopkinson, 1984; Holmer and Kristensen, 1994; Kaku et al., 2005). As the type of marsh plants may determine the availability and level of nutrients in sediments and, hence, microbial activities (Andersen and Hargrave, 1984; Ravit et al., 2003; Hawkes et al., 2005; Batten et al., 2006), the structure and function of methanogens and SRB in salt marshes may be influenced by the invasion and displacement of native plants by exotic species.

Methanogens are strictly anaerobic microorganisms. They are composed of six well-established archaeal orders: *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales*, *Methanocellales*, and *Methanopyrales*. Moreover, recent culture independent techniques have discovered novel methanogen members such as Zoige cluster-I (ZC-I) (Zhang et al., 2008) and the anaerobic methanotrophs (ANME-1, 2 and 3) (Knittel et al., 2005). Most methanogens are hydrogenotrophic, utilizing carbon dioxide as a source of carbon and hydrogen or formate as electron sources. However, members of the genus *Methanosarcina* are physiologically versatile, additionally utilizing acetate and methylated compounds such as methanol, monomethylamine, dimethylamine and trimethylamine, whereas genus *Methanosaeta* is restricted to acetate (Liu and Whitman, 2008). Similarly, sulfate-reducing microbes are strictly anaerobic microorganisms. They reduce sulfate to sulfide using several substrates such as hydrogen, formate, acetate, butyrate, propionate and ethanol as electron sources (Barton and Fauque, 2009). These microbes are found within several phylogenetic lines: in the class *Deltaproteobacteria* (*Desulfobacteriales*, *Desulfovibrionales*, and *Syntrophobacteriales*), phylum *Firmicutes* (*Desulfotomaculum*,

Desulfosporomusa, and *Desulfosporosinus*), phylum *Nitrospirae* (*Thermodesulfobrevibrio*), phylum *Thermodesulfobacteria* and archaeal genera (*Archaeoglobus*, *Thermocladium*, and *Calditerrivirga*). Diverse phylogenetic lines generally present major challenges to specifically targeting these organisms in environmental samples using the universal 16S rRNA gene as a marker. Therefore, targeting group-specific functional genes, such as those encoding methyl coenzyme M reductase A (*mcrA*) and dissimilatory sulfite reductase (*dsrB*), are commonly used as alternatives to the 16S rRNA gene to investigate methanogens and SRB, respectively (Luton et al., 2002; Dar et al., 2006; Geets et al., 2006).

In this study, 454 pyrosequencing and quantitative real-time PCR (qPCR) were used to investigate the diversity and abundance of methanogens and SRB in the sediments vegetated by *P. australis*, *S. alterniflora* and transition stands where both plants are available. Investigations were conducted in two seasons: before growth (April) and during the full growth stage (August).

MATERIALS AND METHODS

SAMPLING AND *in situ* MEASUREMENTS

This study was conducted in the tidal salt marsh of Dongtan, where the intentionally introduced *S. alterniflora* is aggressively displacing the native *P. australis*. Two replicate sampling locations (approximately 60 m apart) were selected, each with three distinct sampling stands (approximately 20 m apart) covered by monocultures of *P. australis* (non-invaded), transition stands (both plants available) and *S. alterniflora* (completely displaced). Investigations were conducted in two seasons: before growth (April) and at full growth stage (August). In both sampling seasons, the methane and carbon dioxide flux rates were determined from each location by collecting the gases using the enclosed static chamber technique (Hirota et al., 2004) and gas chromatographic analysis using a 6890N gas chromatograph (Agilent Technologies, Ltd. USA).

Soil temperatures and conductivities were directly measured using a Field Scout™ direct soil EC meter with a jab probe (2265FS, USA), whereas pH was measured using a IQ150 portable pH meter (IQ Scientific Instruments, USA). From each sampling stand, five replicate sediment samples (surface to 5 cm) were collected within a radius of approximately 2 m. Soil samples were immediately sealed in polyethylene bags and transported to the laboratory on ice. Within one hour of collection, replicate samples were homogenized and stored at −20°C for downstream analyses.

Sediment samples for chemical analysis were dried completely in an oven at 50°C. Then, all non-decomposed plant litter and root materials were removed easily from the gently crushed sediments. The sediments were then ground into powder and passed through #10 meshes where part of the powder was used for determination of total carbon and total nitrogen using an NC analyzer (FlashEA1112 Series, Thermo Inc., Italy), and the remainder was used for analysis of sulfate ions using an ion chromatograph (ICS-1000; Dionex, USA).

DNA EXTRACTION AND PCR AMPLIFICATION

The total genomic DNA of each sample was extracted in duplicate tubes from 0.25 g of sediment (wet weight) using a

PowerSoil DNA Kit (Mo Bio Laboratories, USA) following the manufacturer's instructions. Amplicons for 454 pyrosequencing were prepared following the 2-step barcoded PCR method (Berry et al., 2011). In the first-step PCR, the 50 μ l reaction contained 2 μ l of template DNA (5–10 ng), 25 μ l of Taq PCR Master Mix (TianGen, China), 2 μ l (10 μ M) of each primer (Table 1), 2 μ l of bovine serum albumin (BSA) (0.8 μ g μ l⁻¹ final concentration) and 17 μ l of distilled water. Amplifications were conducted in a thermal cycler (PCR Thermal Cycler Dice; Takara, Japan) for 25 cycles. For the second-step PCR, the forward and reverse primers were modified as follows: 5'-Adapter A + 8 bp barcode + TC + forward primer—3' and 5'-Adapter B + CA + reverse primer -3', respectively. Whereas Adaptor A and B represent the 30 bp that were used as sequencing primers, the 8-bp barcode sequences were used to identify individual samples, and TC and CA were used as linkers. For each sample, 2 μ l of the first-step PCR product was used as template DNA in the second-step PCR. Except for the annealing temperatures, which were raised by 2°C, all the PCR conditions were the same in the second-step PCR as in the first-step PCR. Amplifications were conducted for 10 cycles. The PCR products were then purified using the UltraClean PCR Clean-Up kit (MoBio, USA) and then quantified using the PicoGreen reagent (Invitrogen, USA) for dsDNA on a ND3300 Fluorospectrometer (NanoDrop Technologies, USA). Lastly, the amplicons of all samples were pooled in an equivalent concentration for 454 pyrosequencing. Pyrosequencing was conducted using a Roche/454 (GS FLX Titanium System). For 454 pyrosequencing, the barcoded and pooled amplicons were rechecked for the absence of primer dimers, and emulsion PCR was set up according to Roche's protocols.

QUANTITATIVE REAL-TIME PCR

SYBR Green I-based qPCR was conducted for both the 16S rRNA gene (bacteria and archaea) and functional genes (*mcrA* and *dsrB*) using the primers presented in Table 1. The coverage and specificity of the functional gene primers were validated through clone library construction before application to qPCR. The reaction mixes were prepared as previously described (Zelege et al., 2013), and triplicate reaction tubes were used for each sample. Known copy numbers of linearized plasmid DNA with the respective gene inserted from pure clones were used as

standards for the quantifications. The linearization of the plasmid DNA of each gene was performed using the *EcoRI* restriction enzyme (Fermentas, USA) following the recommended protocol. The amplification efficiency and R^2 values were between 90–99% and 0.98–1, respectively. qPCR of all genes were conducted using a MX3000P QPCR thermocycler (Stratagene, USA). The annealing temperatures are described in Table 1. Melting curves were analyzed to detect the presence of primer dimers. The results were analyzed using MxPro QPCR software version 3.0 (Stratagene, USA).

DATA ANALYSIS

The raw 454 pyrosequencing data of the 16S rRNA and functional genes were trimmed using the sample-specific barcodes, in which the forward primers and sequences with ambiguous nucleotides were removed. For the functional genes, BLASTx analyses were performed against the known sequences of the NCBI database. The sequences that did not match the target gene were excluded from further analysis. The remaining sequences were translated by the RDP's functional gene and repository FrameBot tool (<http://fungene.cme.msu.edu/FunGenePipeline/framebot/form.spr>), which detects and corrects the likely frameshift errors. After removing amino acid sequences with stop codons and/or unknown amino acids, the remaining sequences were aligned by the RDP's functional gene and repository aligner tool. A few (0.5–1%) poorly aligned amino acid sequences were also removed, and the names of the remaining quality amino acid sequences were used to recover the corresponding original nucleotide sequences using Mothur software, version 1.8 (Schloss et al., 2009). Moreover, potential chimeric sequences were also removed (<1%) using the chimera.uchime command in Mothur (Schloss et al., 2009). The final quality nucleotide sequences were used to define operational taxonomic units (OTUs) and downstream analyses. Before calculating the diversity indices, the sequence numbers of each sample were normalized to an equal number. Sequence information was also used for principal component analysis (PCA) using the UniFrac online tool (<http://bmf.colorado.edu/unifrac/>). For 16S rRNA genes of the total bacteria and archaea, raw data sequences were treated through the Mothur program (Schloss et al., 2009). Briefly, after trimming, pre-clustering and removing the potential

Table 1 | Primers used for pyrosequencing and/or qPCR.

Primer	Sequence (5' – 3') ^a	Target	Ta (°C)	References
Mlas	GGTGGTGTGGGDTTCACMCARTA	<i>mcrA</i> gene	55	Steinberg and Regan, 2008
mcrA-rev	CGTTCATBGCGTAGTTVGGRTAGT			
DSRp2060F	CAACATCGTYCAYACCCAGGG	<i>dsrB</i> gene	53	Dar et al., 2006; Geets et al., 2006
Dsr-4Rdeg	GTGTARCAAGTTDCCRCA			
27f	AGAGTTGATYMTGGCTCAG	Bacterial 16S rDNA (for pyrosequencing)	52	Giovannoni et al., 1988
536r	GTATTACCGCGGCKGCTG			
338f	ACTCCTACGGGAGGCAGC	Bacterial 16S rDNA (for qPCR)	52	Giovannoni, 1991
536r	GTATTACCGCGGCKGCTG			
Arch340F	CCCTAYGGGGYGCASCAG	Archaeal 16S rDNA	57	Gantner et al., 2011
Arch1000R	GAGARGWRGTGCATGGCC			

^aM, A/C; D, A/G/T; R, A/G; B, C/G/T; V, A/C/G; Y, C/T; K, G/T; S, G/C; W, A/T.

chimeric sequences, the remaining purified sequences were used for phylogenetic affiliation, which was performed through BLAST analysis against the Silva taxonomy files at an 80% threshold value.

SEQUENCE ACCESSION NUMBERS

All the *mcrA*, *dsrB* and 16S rRNA genes of the total bacterial and archaeal sequences recovered from the estuarine marsh of Dongtan were deposited in the NCBI's sequence read archives with the accession numbers of SRP021055, SRP021326, SRP021327 and SRP021329, respectively.

RESULTS

METHANE FLUX RATES AND SEDIMENT CHARACTERISTICS

Methane flux rates differed both with the invasion and growth of *S. alterniflora* (Figure 1). In spring, the mean flux rates in the *P. australis*, transition, and *S. alterniflora* stands were approximately 0.51 ± 0.31 , 0.93 ± 0.37 , and 0.99 ± 0.35 $\text{mg m}^{-2} \text{h}^{-1}$, respectively. This indicates an approximately 97% increase of flux

rate with *S. alterniflora* invasion. When the plants were fully grown, these flux rates were increased to 1.63 ± 0.34 , 4.11 ± 2.49 , and 8.01 ± 5.61 $\text{mg m}^{-2} \text{h}^{-1}$, respectively, in the *P. australis*, transition and *S. alterniflora* stands and the impact of *S. alterniflora* was significant in the summer (152 and 391% higher in the transition and *S. alterniflora* strands, respectively, than in *P. australis* stands). The gas flux rates also displayed significant increases in the summer (225, 343, and 709% in the *P. australis*, transition and *S. alterniflora* stands, respectively). These increases were positively correlated with the change in temperatures ($R^2 = 0.2$, $\alpha = 0.05$), although not significant. Similarly, carbon dioxide flux rates were relatively high in the *S. alterniflora* stands. When the mean flux ratios of methane to carbon dioxide were compared, higher values were observed in the *S. alterniflora* stands than in the *P. australis* stands. In spring, the ratios were approximately 9×10^{-4} and 3×10^{-3} , in the *P. australis* and *S. alterniflora* stands, respectively, representing an approximate 3.5-fold increase in the *S. alterniflora* stands (Figure 1). In summer, these ratios were approximately 1.74×10^{-3} and 3.6×10^{-3} , in the *P. australis* and *S. alterniflora* stands, respectively, representing an increase of approximately 2.1-fold in the *S. alterniflora* stands.

As expected, soil temperature, pH, conductivity and sulfate levels did not vary across the sampling stands (Table 2). However, there was a marked increase in the mean soil temperature from spring to summer (from approximately 15–28°C). In both seasons, the total carbon (TC) and total nitrogen (TN) levels of sediments were higher in the *S. alterniflora*-influenced sediments than the *P. australis* sediments. However, minor reductions of TC and TN were observed in all stands during the summer (Table 2).

ABUNDANCES OF METHANOGENS AND SRB

To understand the overall relative abundances of methanogens and SRB, SYBR Green I-based quantification of total archaeal and bacterial 16S rRNA genes were determined from the samples used to investigate methanogens and SRB. As revealed from the copy numbers of *mcrA* and *dsrB* genes, the abundances of methanogens and SRB varied with the invasion and growth of *S. alterniflora* (Figures 2A,B). In spring, the mean

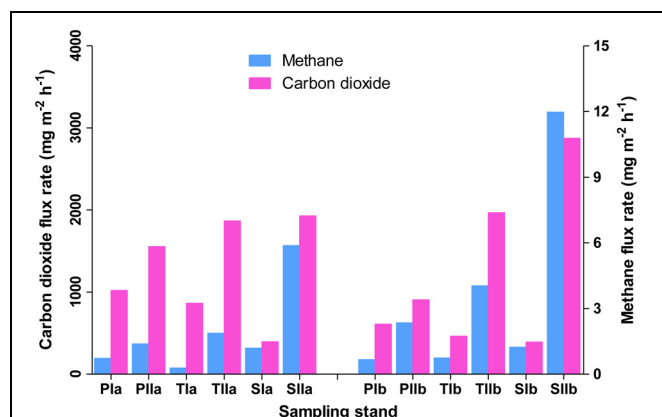


FIGURE 1 | Methane and carbon dioxide flux rates in the *P. australis* (P), *S. alterniflora* (S) and transition (T) stands in the Dongtan salt marsh located in the Yangtze River estuary. In the sample names, l and ll represent the spring and summer samples, respectively, whereas “a” and “b” indicate the replicate locations.

Table 2 | Characteristics of sediments from *P. australis* (P), *S. alterniflora* (S) and transition (T) stands in the Dongtan salt marsh in the Yangtze estuary.

Sample	pH		Temperature (°C)		Conductivity (mS)		Sulfate (mg kg ⁻¹)		TC (% of dry soil)		TN (% of dry soil)	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
Pa	6.1 ± 0.1	7.1 ± 0.1	15.4 ± 0.9	27.9 ± 0.1	ND	7.8 ± 0.6	242.4	272.2	2.1	2	0.14	0.14
Pb	5.9 ± 0.1	7.1 ± 0.1	14.8 ± 0.2	28.1 ± 0.1	7.5 ± 1.2	7.3 ± 0.2	224.7	275.4	2.4	2	0.21	0.14
Ta	6.2 ± 0.2	7.1 ± 0.1	15.9 ± 0.4	27.5 ± 0.2	ND	8.2 ± 1.1	287.3	331	2.7	2.5	0.19	0.18
Tb	5.9 ± 0.1	7.3 ± 0.4	16.3 ± 0.3	28.1 ± 0.1	6.6 ± 0.5	7.7 ± 0.5	245.3	254.7	3.1	2.6	0.22	0.18
Sa	5.8 ± 0.2	6.9 ± 0.1	15.5 ± 0.4	27.8 ± 0.2	ND	9.4 ± 0.7	263.4	331.8	3.3	3.5	0.25	0.23
Sb	6.2 ± 0.2	7.4 ± 0.4	13.5 ± 0.4	27.9 ± 0.1	8.2 ± 0.8	7.4 ± 0.3	326.6	325	3.5	3.3	0.24	0.22

In the sample names, ‘a’ and ‘b’ represent the replicate sampling locations.

Values given for pH, temperature and conductivity are the means ± standard deviations of 5 replicate measurements, but sulfate was measured once (from the mixed samples) in the lab. ND, not determined.

abundance of methanogens were approximately $2.4 \pm 1.3 \times 10^5$, $1.1 \pm 0.9 \times 10^6$ and $1.2 \pm 0.4 \times 10^6$ copies per g of dried soil in the *P. australis*, transition and *S. alterniflora* stands, respectively, indicating there were approximately 5 times more methanogens in the *S. alterniflora* stands than in the *P. australis* stands. Furthermore, higher abundances of methanogens were observed in the summer ($4.8 \pm 0.1 \times 10^5$, $1.2 \pm 0.1 \times 10^6$ and $3.6 \pm 0.6 \times 10^6$ copies per g of dried soil in the *P. australis*, transition and *S. alterniflora* stands, respectively), representing a dramatic increase of methanogens in the *S. alterniflora* stands (approximately 7.5-fold higher than the *P. australis* stands). In terms of the changes associated with *S. alterniflora* invasion, similar trends were observed for the abundance of archaeal 16S rRNA gene copies in both sampling seasons (Figure 2A). The mean abundance proportions of methanogens (copies of *mcrA* to 16S rRNA gene of total archaea) were also higher in the *S. alterniflora*-impacted stands than in the *P. australis* stands. In the spring, the mean abundance proportions of

methanogens were approximately 31, 53, and 63% in the *P. australis*, transition and *S. alterniflora* stands, respectively, whereas they slightly changed to 30, 32 and 71%, respectively, in the summer.

Similarly, the invasion and growth of *S. alterniflora* increased the abundance of SRB in both sampling seasons (Figure 2B). In the spring, the mean abundances of SRB were approximately $5.99 \pm 4.06 \times 10^6$, $1.28 \pm 0.71 \times 10^7$, and $1.72 \pm 0.12 \times 10^7$ per g of dried soil, respectively, for *P. australis*, transition and *S. alterniflora* stands, indicating 2.2- and 2.9-times more copies in the transition and *S. alterniflora* stands than in the *P. australis* stands, respectively. Although the abundance of SRB was greater than that of the methanogens (up to 2 orders of magnitude), the increases in the mean abundances of SRB associated with *S. alterniflora* invasion in the summer were relatively lower (approximately 3.0-fold lower than the abundances in the spring). When compared with the total bacterial abundance, the mean abundance proportions of the SRB ranged from 17 to 34%. Unlike most of the sediment characteristics measured at the salt marsh, TC was positively correlated with the abundance of methanogens ($R^2 = 0.54$, $\alpha = 0.05$) and SRB ($R^2 = 0.70$, $\alpha = 0.05$).

METHANOGENS AND SRB DIVERSITY

Purified *mcrA* sequences were used to define OTUs at the genus (89% similarity cutoff) and family (79% similarity cutoff) levels (Steinberg and Regan, 2008). At the genus level, maximum numbers of *mcrA* OTUs were observed in sediments collected from *P. australis* stands, whereas the minimum numbers were observed in sediments from *S. alterniflora* stands (Table 3). Rarefaction curves at both similarity cutoffs leveled off horizontally between 30 and 58 OTUs (data not shown here), indicating the use of acceptable numbers of sequences for good representation of the methanogen communities from the study site. Despite the greater abundance of methanogens in the *S. alterniflora* stands of both sampling seasons (Figure 2A), richness and diversity indices (Chao1, ACE and Shannon) indicated lower diversity and richness of methanogens in the *S. alterniflora* stands compared with the *P. australis* stands (Table 3).

However, *dsrB* OTUs were defined at three sequence similarity cutoffs (90, 80, and 70%). Unlike *mcrA*, significantly large numbers of *dsrB* OTUs were observed at 90 and 80% similarity cutoffs (Table 4). At the 70% similarity cutoff, the numbers of OTUs were reduced by more than half (approximately 160 OTUs), which was used in the construction of a phylogenetic tree. Despite the diversity of SRB, the rarefaction curves indicated good representation of the SRB, particularly at the 80% similarity cutoff (data not shown here). In both seasons, the diversity indices (Chao1, ACE and Shannon) did not vary significantly with *S. alterniflora* invasion (Table 4), suggesting its minimal impact on the diversity of SRB.

PHYLOGENETIC ANALYSES OF *mcrA* AND *dsrB* OTUs

To determine the identity and phylogenetic positions of the collected sequences, trees were constructed for both the methanogens and SRB. OTU representatives of both genes (79 and 70% similarity OTUs for *mcrA* and *dsrB*, respectively) were

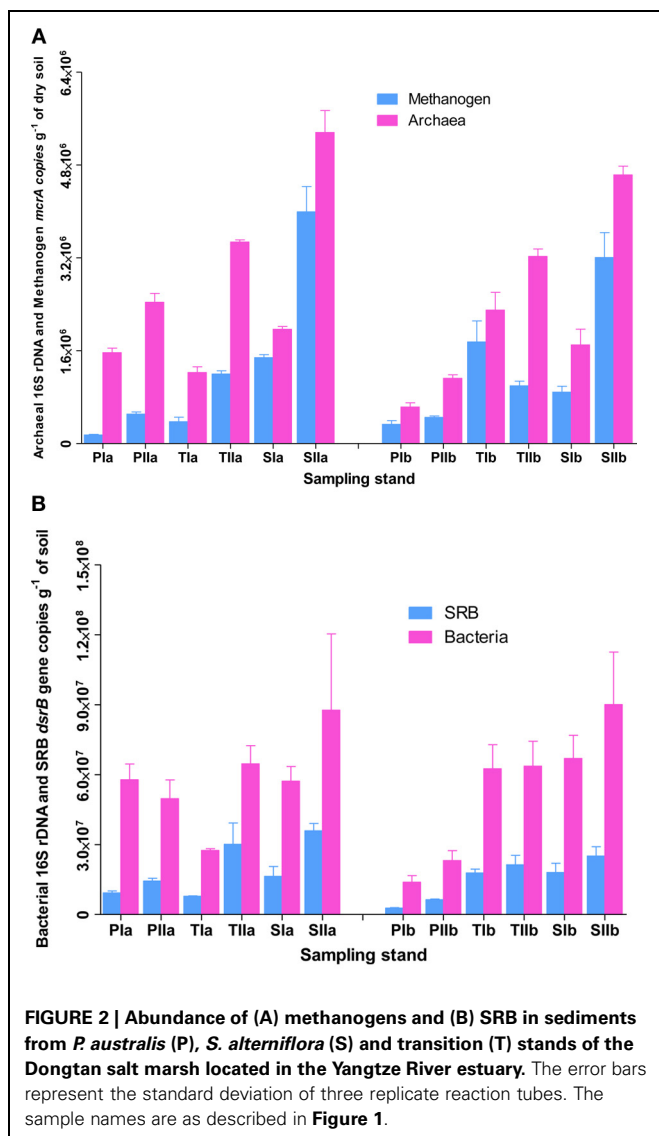


Table 3 | Summary of the diversity indices of *mcrA* genes amplified from sediment samples of the *S. alterniflora* (S), transition (T) and *P. australis* (P) stands.

Season	Sample	NS ^a	89% Similarity cutoff				79% Similarity cutoff			
			NO ^b	Chao1	ACE	Shannon	NO ^b	Chao1	ACE	Shannon
Spring	Pla	162	58	128	188	3.6	33	46	65	2.8
	Plb	176	39	65	115	2.6	22	23	24	2
	Tla	504	39	58	82	2.5	22	31	39	1.9
	Tlb	479	32	56	60	2.3	20	33	41	1.7
	Sla	1455	33	60	61	2.4	21	30	32	1.8
	Slb	1035	25	51	148	2	20	31	33	1.6
Summer	Plla	493	50	95	182	3.3	36	113	163	2.7
	Pllb	319	50	347	371	3.2	26	61	111	2.3
	Tlla	598	34	111	130	2.5	26	44	72	2.1
	Tllb	516	35	50	96	2.7	22	31	41	2.1
	Slla	763	35	98	97	2.7	20	56	41	2.1
	Sllb	586	41	96	93	2.9	26	37	68	2.3

The diversity indices were calculated at 89 and 79% similarity cutoffs using normalized sequence numbers. The sample names are as described in **Figure 1**. NS^a, number of sequences; NO^b, number of OTUs.

Table 4 | Summary of the diversity indices of *dsrB* genes amplified from sediment samples in the *S. alterniflora* (S), transition (T), and *P. australis* (P) stands.

Season	Sample	NS ^a	90% Similarity cutoff				80% Similarity cutoff			
			NO ^b	Chao1	ACE	Shannon	NO ^b	Chao1	ACE	Shannon
Spring	Pla	414	187	421	600	4.7	144	306	356	4.3
	Plb	704	310	1096	2137	5.4	202	456	846	4.8
	Tla	778	294	1277	2605	5.3	203	530	847	4.8
	Tlb	706	307	1000	2179	5.3	215	493	874	4.8
	Sla	971	165	331	460	4.7	138	199	215	4.5
	Slb	701	303	1558	3092	5.3	214	584	1073	4.9
Summer	Plla	523	274	591	989	5.3	193	302	463	4.9
	Pllb	675	285	979	2650	5.3	191	466	1093	4.7
	Tlla	740	311	883	1349	5.5	209	483	680	4.9
	Tllb	686	279	882	1923	5.3	184	455	705	4.7
	Slla	704	285	1015	2283	5.2	185	407	570	4.6
	Sllb	890	140	324	368	4.6	116	163	164	4.3

The diversity indices were calculated at 90 and 80% similarity cutoffs using normalized sequence numbers. Sample names are as described in **Figure 1**. NS^a, number of sequences; NO^b, Number of OTUs.

translated into amino acid sequences, and their closest relatives were searched for using the NCBI amino acid non-redundant database (BLASTp). For SRB, a 70% similarity cutoff was selected because the numbers of OTUs observed at 90 and 80% similarity cutoffs were relatively large (up to 300) with small variation (<6.5%) among their relative composition.

McrA OTUs were dominated by OTU1, 2 and 3, representing approximately 32.5, 21.5, and 16.5% of the total sequences (**Figure 3A**). OTU1 was closely related to the *mcrA* of *Methanococcus*, whereas OTU2 and 3 were related to the *mcrA* of *Methanomicrobiales* and *Methanosarcina*, respectively.

Most of the *mcrA* gene sequences were closely related to the methanogen orders *Methanomicrobiales*, *Methanosarcinales* and *Methanococcales*, although other methanogens such as *Methanobacteriales*, ANME (1 and 3) and ZC-I were also detected in the salt marsh (**Figure 3A**). As the primers used in this study (mlas/*mcrA*-rev) can amplify *mcrA* and *mrtA* genes (Steinberg and Regan, 2009), the presence of large number of sequences (32.5%) related to *Methanococcales* (Luton et al., 2002), methanogens carrying *mcrA* and *mrtA* genes, might overestimate the abundance and diversity of the total methanogens. Interestingly, at both similarity

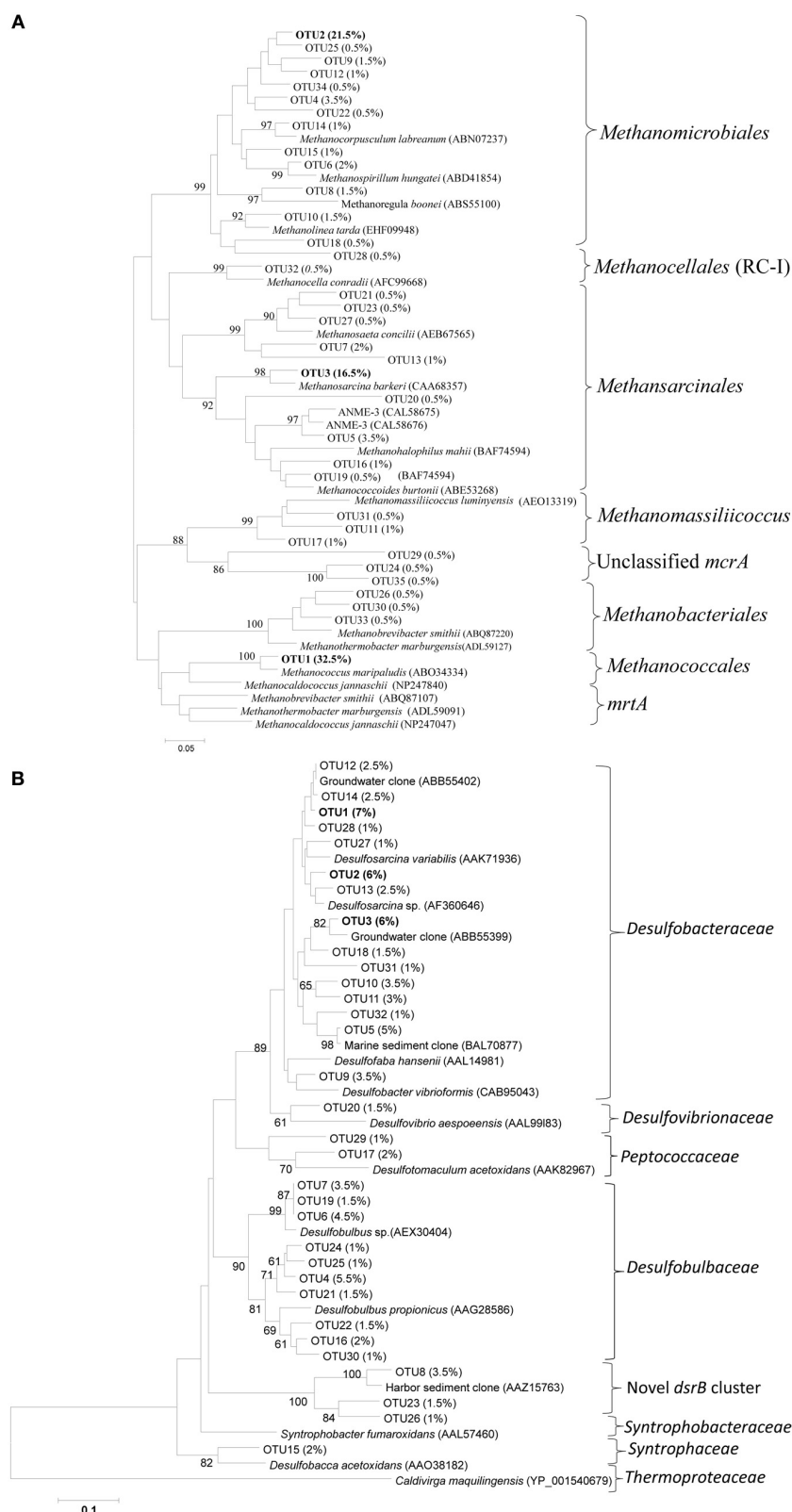


FIGURE 3 | Neighbor-joining phylogenetic trees of (A) *mcrA* and (B) *dsrB* gene OTUs recovered from the Dongtan tidal salt marsh located in the Yangtze estuary. The trees were constructed based on the inferred amino acids of the OTU-representative nucleotide

sequences. Percentages in the parenthesis indicate the percent of sequences included in that specific OTU. Only OTUs containing at least 0.5 and 1% of the total sequences *mcrA* and *dsrB* OTUs, respectively, are presented here.

cutoffs, *Methanococcales* was represented by a single OTU that is closely related (approximately 95% at the amino-acid level) to *Methanococcus maripaludis*. The occurrences of *Methanomicrobiales*, *Methanosarcinales* and *Methanococcales* in the *mcrA* sequences were also supported by the results from the analysis of archaeal 16S rRNA gene sequences where the above three methanogenic orders were also the most abundant in the phylum *Euryarchaeota*, which itself represented approximately 35% of the total archaeal sequences (data not shown here).

Among the SRB, the variations among the proportions of OTUs were very low. For instance, the most dominant 70% similarity OTUs (OTU 1, 2, and 3) represented approximately 7, 6 and 6% of the total sequences, respectively (Figure 3B). Phylogenetic analysis indicated that more than 85% of the sequences were related to *Deltaproteobacteria*, suggesting their significant role in the marsh. In the *dsrB* gene sequences, the most frequently detected families of SRB appeared to be *Desulfobacteraceae* ($49.2 \pm 8.5\%$) and *Desulfobulbaceae* ($29.6 \pm 7.8\%$), whereas sequences related to *Desulfovibrionaceae*, *Peptococcaceae*, *Syntrophaceae* and *Syntrophobacteraceae* were detected, but at much lower proportions (total <8%). The dominance of *Desulfobacteraceae* was mainly contributed to by three dominant *dsrB* OTUs that were either closely related to the genus *Desulfosarcina* or uncultured family members (Figure 3B). Interestingly, we detected relatively a large proportion of OTUs ($11.3 \pm 5.1\%$) clustered distinctly from the previously isolated *dsrB* phylotypes. This novel cluster formed a distinct deep branch between *Desulfobulbaceae* (with approximately 56.6% amino-acid sequence similarities) and *Syntrophobacteraceae* (with approximately 61% amino-acid sequence similarities) (Figure 3B). An analysis of the total bacterial 16S rRNA gene sequences indicated consistent results with the *dsrB* gene sequence results in that sequences related to the order *Desulfobacteriales* were dominant (Figure 7).

COMMUNITY DISTRIBUTION PATTERNS WITH *S. alterniflora* INVASION

Both invasion- and growth-associated variations in the relative proportions of *mcrA* and *dsrB* phylotypes were analyzed. For methanogens, the orders *Methanomicrobiales*, *Methanococcales* and *Methanosarcinales* together represented 85–90% of the total *mcrA* sequences (Figure 4). However, their proportion responded differently to the invasion of *S. alterniflora*. For instance, the mean proportions of *Methanomicrobiales*, the most dominant methanogens detected in the salt marsh (representing approximately 33.1 and 44% of the sequences in the spring and summer, respectively), were greater in the *S. alterniflora* stands than in the *P. australis* stands by approximately 58 and 28% in the spring and summer samples, respectively (Figure 4). This might demonstrate the effect of *S. alterniflora* invasion in promoting the proliferation of *Methanomicrobiales*. Almost a reverse phenomenon was observed for the order *Methanococcales* (Figure 4). In the spring, *Methanococcales* represented approximately 38% of the total *mcrA* sequences, but its mean proportions indicated approximately 10% reductions from *P. australis* to *S. alterniflora* stands. In the summer, not only were the *mcrA* sequences related to *Methanococcales* reduced

(approximately 20% of the total *mcrA* gene sequences), but higher reductions (approximately 37%) were observed from *P. australis* to *S. alterniflora* stands. Hence, *S. alterniflora* growth appeared to favor *Methanomicrobiales* over *Methanococcales*. However, the two main genera of the order *Methanosarcinales* (*Methanosarcina* and *Methanosaeta*) represented approximately 20 and 24% of the *mcrA* sequences, respectively, although they did not display similar trends with *S. alterniflora* invasion. The mean proportions of *Methanosaeta* increased with *S. alterniflora* invasion. In contrast, the mean proportions of the genus *Methanosarcina* did not display significant variations with either the invasion or growth of *S. alterniflora* (Figure 4). With the exception of ANME-3, most of the rare *mcrA* phylotypes, such as *Methanobacteriales*, *Methanocellales* and ZC-I, detected in this study dominated the *P. australis* stands. This is consistent with the diversity index results (Table 3) that indicated lower diversity of methanogens in the *S. alterniflora* stands compared with the transition or *P. australis* stands.

Trends in the distribution patterns of the dominant methanogen orders revealed that the *mcrA* analysis results were generally consistent with the results of the 16S rRNA gene analysis results, except that *Methanococcales* represented relatively lower proportions of the 16S rRNA gene sequences (Figure 5). The higher proportions of *Methanococcales* in the *mcrA* sequences compared with the 16S rRNA gene sequences might be explained by the likely amplification of both *mcrA* and *mrtA* genes. Hence, methanogens might be slightly overestimated, particularly in the spring samples where *Methanococcales* represented a relatively large proportion of the sequences.

Based on the frequency of nucleotide sequences recovered from the salt marsh, *dsrB* phylotypes were dominated by *Deltaproteobacteria*, particularly the families of the order *Desulfobacteriales* (Figure 6), which represented more than 85% of the sequences. Except for the small increase of its relative proportion at PIa and reduction observed at TIIB, *Desulfobacteraceae* (the most dominant family in the order *Desulfobacteriales*) did not display significant change from the spring to the summer. On the other hand, at the *S. alterniflora* invaded sediments the mean proportion of *Desulfobacteraceae* increased from

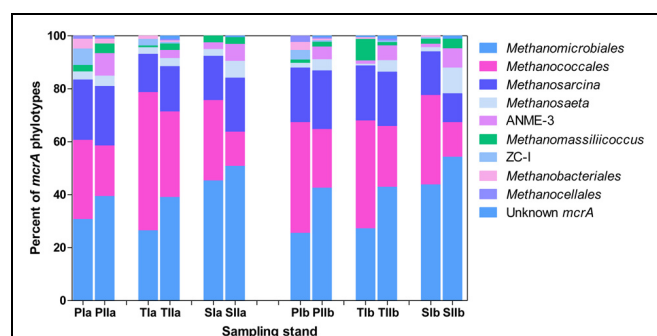


FIGURE 4 | Proportions of the major *mcrA* phylotypes detected in sediments from *P. australis* (P), *S. alterniflora* (S) and transition (T) stands. The sample names are as described in Figure 1.

approximately 61 to 54% at locations “a” and “b”, respectively (Figure 6). In contrast, the proportion of *Desulfobulbaceae*, the second dominant family in the order *Desulfobacterales*, decreased in the summer (by approximately 45%). However, insignificant change was observed with *S. alterniflora* invasion. Despite the lower proportions ($4.0 \pm 2.0\%$), similar trends were observed for the family *Peptococcaceae* (Figure 6) within *Desulfobulbaceae*. Approximately 50% increases were observed from the spring to the summer in the proportions of the novel *dsrB* cluster (Figure 6).

The abundance patterns of *dsrB* phylotypes were generally consistent with 16S rRNA gene patterns of *Deltaproteobacteria*-related phylotypes (Figure 7). *Desulfobacterales* was represented by 4.5–10% of the total bacterial 16S rRNA gene sequences, whereas *Desulfuromonadales* was represented by 1–5% of the sequences. Similar to *dsrB*, some orders of SRB, such as *Desulfovibrionales*, were represented by very low proportions among the 16S rRNA gene sequences (Figure 7). Generally, insignificant variations in the proportions of

SRB phylotypes were observed with the invasion of *S. alterniflora*, although members of *Desulfobacterales* and *Desulfovibrionales* were slightly increased with the growth of *S. alterniflora*.

COMPARISON OF COMMUNITIES IN DIFFERENT STANDS

Sediment samples from the different stands were clustered using PCA and environment clustering through weighted normalized UniFrac analysis. With a few exceptions (PIa, TIb and TIIa), PC1 indicated a clear distinction between the methanogen communities of the spring and summer samples (Figure 8). Moreover,

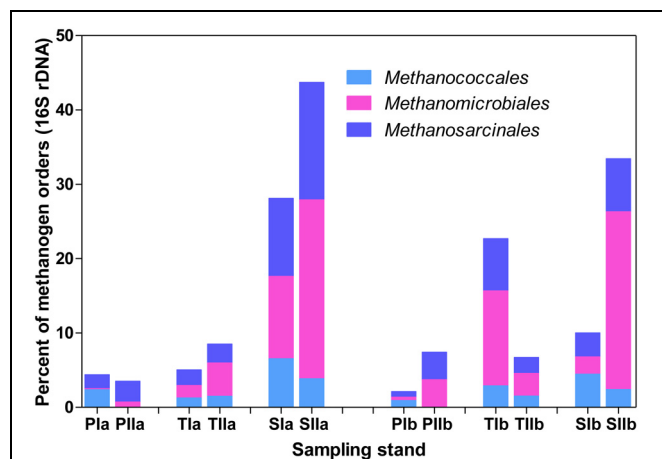


FIGURE 5 | Proportions of the dominant methanogen orders detected from 16S rRNA gene sequences of *Archaea*. The samples were collected from *P. australis* (P), *S. alterniflora* (S) and transition (T) stands. The sample names are as described in Figure 1.

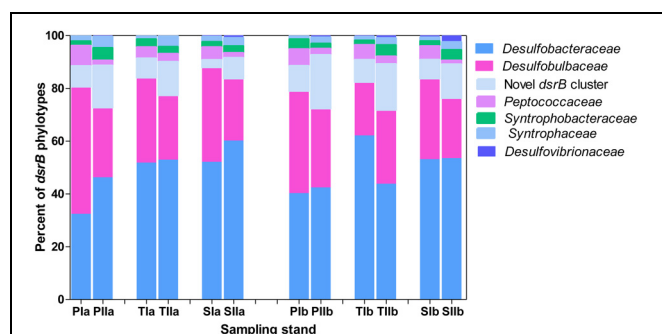


FIGURE 6 | Proportions of major *dsrB* families detected in sediments from *P. australis* (P), *S. alterniflora* (S) and transition (T) stands. The sample names are as described as Figure 1.

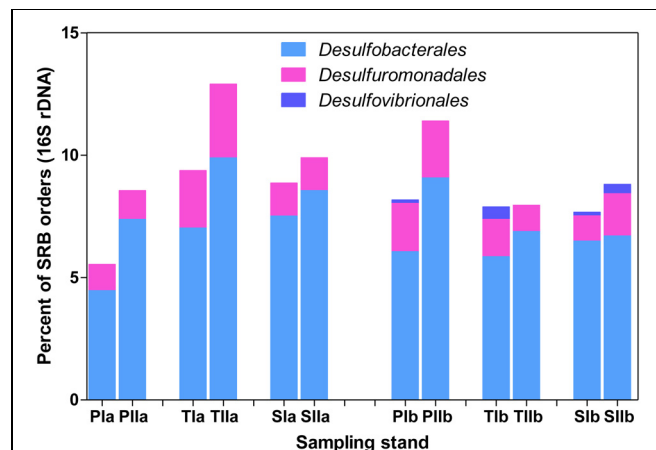


FIGURE 7 | Proportions of the dominant orders of SRB analyzed from 16S rRNA gene sequences of *Bacteria*. The samples were collected from *P. australis* (P), *S. alterniflora* (S) and transition (T) stands. The sample names are as described in Figure 1.

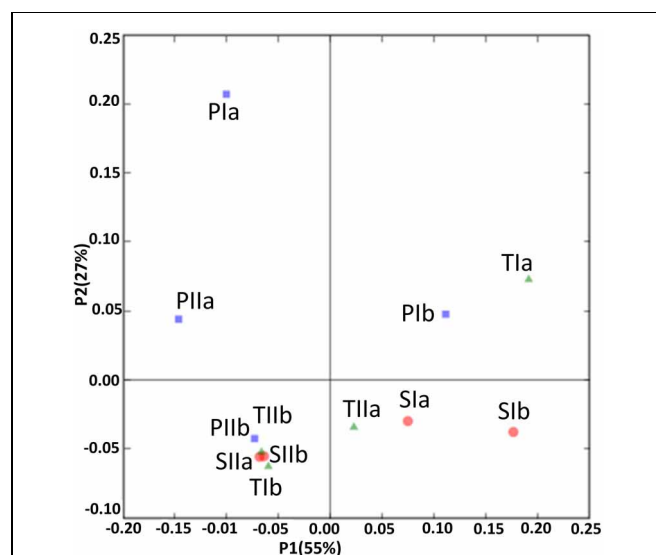


FIGURE 8 | UniFrac analyses showing the PCA plots of methanogen communities based on sequence abundance data in the *P. australis* (P), *S. alterniflora* (S) and transition (T) stands. The sample names are as described in Figure 1.

most of the communities from the *S. alterniflora*-influenced samples were also clustered distinctly from non-influenced samples in PC2, signifying the variation in the structures of methanogens in the *S. alterniflora* and *P. australis* stands. These variations were clearly supported by the dendrogram clustering of environments where samples from *S. alterniflora* influenced stands, and different growing seasons clustered separately (data not shown).

The SRB communities of the spring and summer samples were clustered distinctly along PC1 (Figure 9), which is consistent with the abundance data. The SRB community structures in the samples from the *P. australis* stands and *S. alterniflora* stands distributed separately along PC2 (Figure 9). These results were also supported by the dendrogram of the environment cluster (data not shown).

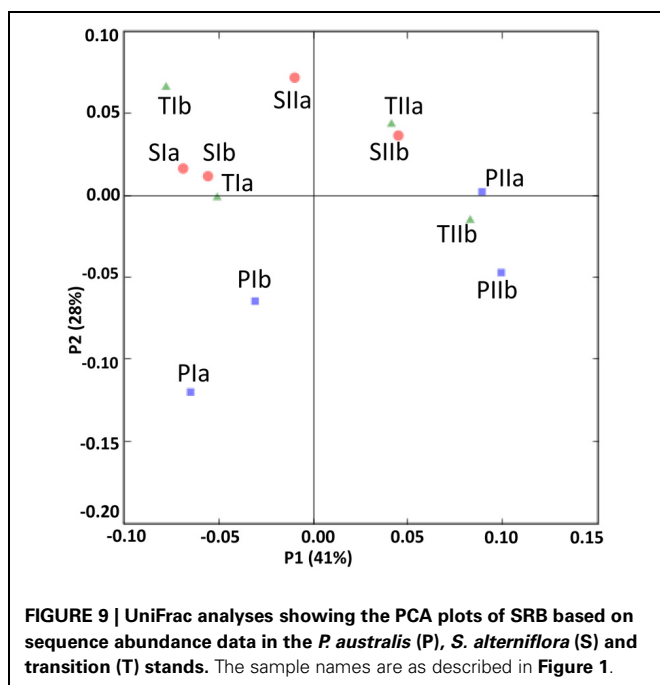
DISCUSSION

The extensive expansion of *S. alterniflora* is recognized as one of the major threats to the natural ecology of salt marshes, because the plant can alter the carbon and nitrogen contents of sediments (Turner, 1993; Liao et al., 2007; Page et al., 2010). This, in turn, may influence the carbon and nitrogen cycles in the sediments and the microbes that provide these functions. To understand the impact of *S. alterniflora* invasion on the community structure of methanogens and SRB in the salt marsh sediments of Dongtan, functional gene (*mcrA* and *dsrB*)-based investigations were conducted using samples from stands covered by monocultures of *P. australis*, *S. alterniflora* and a transition zone where both plants were available.

The higher methane flux rates detected in the *S. alterniflora* stands, particularly during the summer, were not surprising because many studies have already determined the potential of

S. alterniflora to increase the gas flux rates of environments. This might be associated with the higher density of *S. alterniflora* living biomass, greater gas transportation capacity and substrate stimulation of methane-producing microorganisms (Cheng et al., 2007; Tong et al., 2012). Substrate stimulation of methanogens might be likely, as sediments from *S. alterniflora* stands displayed higher TC and TN levels compared with the native *P. australis* stands. Moreover, increases in the ratio of methane to carbon dioxide in the *S. alterniflora* stands might suggest the invasion of the *S. alterniflora* resulted in greater methane production. This, in turn, could explain the stimulation of methanogens after the invasion. The relatively high soil temperatures detected during the summer could favor the activity of sediment microbes, which might explain the slight reductions of the TC and TN levels of the sediments.

Abundances of methanogens and SRB were increased with the invasion and growth of *S. alterniflora*, which could be related to their available nutrients in the *S. alterniflora* stands. (Schubauer and Hopkinson, 1984; Peng et al., 2011). For instance, Liao and his colleagues (Liao et al., 2008) indicated that the annual litter mass in *S. alterniflora* stands is approximately 22.8% higher than in *P. australis* stands. This abundant *S. alterniflora*-derived organic matter is hydrolyzed and fermented by heterotrophic microorganisms, which can release excess substrates for both methanogens and SRB. The higher abundance of methanogens and SRB in the *S. alterniflora* stands is also consistent with a report that identified higher TC mineralization capabilities of dissolved organic matter derived from *S. alterniflora* compared with *P. australis*-derived matter (Bushaw-Newton et al., 2008). Indeed, the enrichment of sediments with *S. alterniflora* detritus has been shown to fuel the activity of anaerobic microbial communities (Andersen and Hargrave, 1984; Kepkay and Andersen, 1985). Hines and his colleagues (Hines et al., 1999) detected a high number of active sulfate-reducing bacteria (SRB) during the active growth stage of *S. alterniflora* in salt marsh sediments, which may be associated with substrate stimulation of sulfate reducers from root exudates. In contrast, a study on Jiuduansha Island, in the Yangtze River estuary, indicated that the senescence stage of *S. alterniflora* favors the richness and abundance of SRB (Nie et al., 2009), which is likely associated with litter decomposition-related substrate stimulation of SRB. Despite contrasting reports, it is possible to argue that the presence of *S. alterniflora* in environments can alter the natural abundance and activity of SRB. In addition to the relative contribution of *S. alterniflora* to the total carbon and nitrogen levels of sediments (Moran and Hodson, 1990; Liao et al., 2007; Peng et al., 2011), *S. alterniflora* tissues are important sources of trimethylamine (Cavaliere, 1983). Trimethylamine can be a noncompetitive substrate for methanogens. Moreover, acetate might be released from the root exudates or tissue decompositions could be used by SRB and methanogens (King, 1984; Watkins et al., 2012). Temperature is also one of the important factors controlling the growth of methanogens (Zeikus and Winfrey, 1976; Turetsky et al., 2008; Liu et al., 2010), so it is reasonable to conclude that higher abundances of methanogens and higher methane flux rates were detected



during the summer and positively correlated with the soil temperature.

Although the abundances of methanogens were higher in the *S. alterniflora* stands of both seasons, their diversities were lower in the *S. alterniflora* stands compared with the *P. australis* stands. Hence, *S. alterniflora* invasion-related abundance increases might not have contributions from all members of the methanogens, and *S. alterniflora* might select methanogen communities. Although the proportions of *Methanomicrobiales* were increased with *S. alterniflora* invasion, *Methanococcales* and many of other rare *mcrA* phylotypes (e.g., *Methanobacteriales*, *Methanocellales* and ZC-I) were reduced, which might explain the lower diversity of methanogens in the *S. alterniflora* stands. *Methanomicrobiales* and *Methanococcales* (hydrogenotrophic methanogens) unexpectedly dominated the salt marsh (>60%), although they can be easily outcompeted by SRB (Oremland et al., 1982). However, the availability of excess substrates in such productive environments (Schubauer and Hopkinson, 1984; Peng et al., 2011) might reduce the competition and support the growth of both microbial groups. Interestingly, *Methanococcales* was represented by the most dominant OTU (OTU1, approximately 32.5%). Phylogenetic analysis indicated that it is closely related (96%) to *Methanococcus maripaludis*, a methanogen that is commonly distributed in marine and salt marsh sediments (Jones et al., 1983). The reasons for such dominance by a single methanogen species are not clear, but the extremely fast-growing nature of this mesophilic methanogen (Jones et al., 1983) could be triggered by the availability of excess substrates in sediments. On the other hand, the proportion of *Methanosarcina* did not display significant variation with the invasion and growth of *S. alterniflora*, which could be associated with their metabolic flexibility to utilize different substrates (King, 1984; Lyimo et al., 2000). However, the proportion of the genus *Methanosaeta*, strict acetate utilizers, was much lower in the spring but significantly increased with the growth of plants in the summer, indicating the availability of acetate increased with the growth of plants, particularly

in the *S. alterniflora* stands where the microbes displayed marked increases.

The current study also revealed that more than 80% of the *dsrB* phylotypes that were detected were members of the order *Desulfobacteriales*. These microbes are nutritionally versatile and can oxidize acetate and other organic compounds (Muyzer and Stams, 2008). The SRBA spectrum of substrates from the root exudates and decomposition of *S. alterniflora* and *P. australis* tissues could be available for these diverse SRB (Nie et al., 2009). Except for the small changes in *Desulfobacteraceae*, the proportions of *dsrB* families in both sampling seasons generally displayed minor variations with *S. alterniflora* invasion. The reason for such insignificant variation was not clear; however, nutrients that could be released from *S. alterniflora* tissue decomposition or root exudates might support most SRB phylotypes. The large numbers of novel *dsrB* OTUs that were clustered distinctly between the families *Syntrophobacteraceae* and *Desulfobulbaceae* might offer a clue into the presence of novel SRB types in the tidal salt marsh. Although the physiology of these novel sulfate-reducing phylotypes cannot be speculated about at this time, they could grow with both plant types, particularly in the stands of *P. australis*.

In conclusion, the invasion of *S. alterniflora* in the salt marsh sediments of Dongtan might support the proliferation of methanogens and SRB. However, significant impacts were only observed on the diversity of methanogens.

ACKNOWLEDGMENTS

The authors are grateful to Professor Andrew Ogram of University of Florida for reading the draft manuscript and providing us with important comments. This project was partly supported by the National Natural Science Foundation of China (31070097 and 30930019), National Key Technology R & D Program of China (2010BAK69B14) and Major Program of Science and Technology Department of Shanghai (10DZ1200700).

REFERENCES

- Abram, J. W., and Nedwell, D. B. (1978). Hydrogen as a substrate for methanogenesis and sulphate reduction in anaerobic saltmarsh sediment. *Arch. Microbiol.* 117, 93–97. doi: 10.1007/BF00689357
- Andersen, F. O., and Hargrave, B. T. (1984). Effects of *Spartina* detritus enrichment on aerobic/ anaerobic benthic metabolism in an intertidal sediment. *Mar. Ecol. Prog. Ser.* 16, 161–171. doi: 10.3354/meps016161
- Barton, L. L., and Fauque, G. D. (2009). Biochemistry, physiology and biotechnology of sulfate-reducing bacteria. *Adv. Appl. Microbiol.* 68, 41–98. doi: 10.1016/S0065-2164(09)01202-7
- Batten, K. M., Scow, K. M., Davies, K. F., and Harrison, S. P. (2006). Two invasive plants alter soil microbial community composition in serpentine grasslands. *Biol. Invasions* 8, 217–230. doi: 10.1007/s10530-004-3856-8
- Berry, D., Ben Mahfoudh, K., Wagner, M., and Loy, A. (2011). Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. *Appl. Environ. Microbiol.* 77, 7846–7849. doi: 10.1128/AEM.05220-11
- Bushaw-Newton, K. L., Kreeger, D. A., Doaty, S., and Velinsky, D. J. (2008). Utilization of *Spartina*- and *Phragmites*-derived dissolved organic matter by bacteria and Ribbed Mussels (*Geukensia demissa*) from Delaware bay salt marshes. *Estuar. Coast.* 31, 694–703. doi: 10.1007/s12237-008-9061-8
- Cavaliere, A. J. (1983). Proline and glycinebetaine accumulation by *Spartina alterniflora* Loisel. in response to NaCl and nitrogen in a controlled environment. *Oecologia* 57, 20–24. doi: 10.1007/BF00379556
- Chen, J., Zhao, B., Ren, W., Saunders, S. C., Ma, Z., Li, B., et al. (2008). Invasive *Spartina* and reduced sediments: shanghai's dangerous silver bullet. *J. Plant Ecol.* 1, 79–84. doi: 10.1093/jpe/rtn007
- Cheng, X., Peng, R., Chen, J., Luo, Y., Zhang, Q., An, S., et al. (2007). CH₄ and N₂O emissions from *Spartina alterniflora* and *Phragmites australis* in experimental mesocosms. *Chemosphere* 68, 420–427. doi: 10.1016/j.chemosphere.2007.01.004
- Chung, C. H. (1993). Thirty years of ecological engineering with *Spartina* plantations in China. *Ecol. Eng.* 2, 261–289. doi: 10.1016/0925-8574(93)90019-C
- Dar, S. A., Yao, L., Van Dongen, U., Kuenen, J. G., and Muyzer, G. (2006). Analysis of diversity and activity of sulfate-reducing bacterial communities in sulfidogenic bioreactors using 16S rRNA and *dsrB* genes as molecular markers. *Appl. Environ. Microbiol.* 73, 594–604. doi: 10.1128/AEM.01875-06
- Gantner, S., Andersson, A. F., Alonso-Saez, L., and Bertilsson, S. (2011). Novel primers for 16S rRNA-based archaeal community analyses in environmental samples. *J. Microbiol. Methods* 84, 12–18. doi: 10.1016/j.mimet.2010.10.001
- Geets, J., Borremans, B., Diels, L., Springael, D., Vangronsveld, J.,

- Van Der Lelie, D., et al. (2006). *DsrB* gene-based DGGE for community and diversity surveys of sulfate-reducing bacteria. *J. Microbiol. Methods* 66, 194–205. doi: 10.1016/j.mimet.2005.11.002
- Giovannoni, S. J. (1991). “The polymerase chain reaction,” in *Nucleic Acid Techniques in Bacterial Systematics*, eds E. Stackebrandt and M. Goodfellow (New York, NY: John Wiley and Sons, Inc.), 177–203.
- Giovannoni, S. J., Delong, E. F., Olsen, G. J., and Pace, N. R. (1988). Phylogenetic group-specific oligodeoxynucleotide probes for identification of single microbial Cells. *J. Bacteriol.* 170, 720–726.
- Hawkes, C. V., Wren, I. E., Herman, D. J., and Firestone, M. K. (2005). Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. *Ecol. Lett.* 8, 976–985. doi: 10.1111/j.1461-0248.2005.00802.x
- Hines, M. E., Evans, R. S., Sharak Gentner, B. R., Willis, S. G., Friedman, S., Rooney-Varga, J. N., et al. (1999). Molecular phylogenetic and biogeochemical studies of sulfate-reducing bacteria in the rhizosphere of *Spartina alterniflora*. *Appl. Environ. Microbiol.* 65, 2209–2216.
- Hirota, M., Tang, Y., Hu, Q., Hirata, S., Kato, T., Mo, W., et al. (2004). Methane emissions from different vegetation zones in a Qinghai-Tibetan plateau wetland. *Soil Biol. Biochem.* 36, 737–748. doi: 10.1016/j.soilbio.2003.12.009
- Holmer, M., and Kristensen, E. (1994). Coexistence of sulfate reduction and methane production in an organic-rich sediment. *Mar. Ecol. Prog. Ser.* 107, 77–184. doi: 10.3354/meps107177
- Jiang, L. F., Luo, Y. Q., Chen, J. K., and Li, B. (2009). Ecophysiological characteristics of invasive *Spartina alterniflora* and native species in salt marshes of Yangtze River estuary, China. *Estuar. Coast. Shelf Sci.* 81, 74–82. doi: 10.1016/j.ecss.2008.09.018
- Jones, W. J., Paynter, M. J. B., and Gupta, R. (1983). Characterization of *Methanococcus maripaludis* sp. nov., a new methanogen isolated from salt marsh sediment. *Arch. Microbiol.* 135, 91–97. doi: 10.1007/BF00408015
- Kaku, N., Ueki, A., Ueki, K., and Watanabe, K. (2005). Methanogenesis as an important terminal electron accepting process in estuarine sediment at the mouth of Orikasa river. *Microbes. Environ.* 20, 41–52. doi: 10.1264/jsme2.20.41
- Kepkay, P. E., and Andersen, F. O. (1985). Aerobic and anaerobic metabolism of a sediment enriched with *Spartina* detritus. *Mar. Ecol. Prog. Ser.* 21, 53–161. doi: 10.3354/meps021153
- King, G. M. (1984). Utilization of hydrogen, acetate, and “noncompetitive”; substrates by methanogenic bacteria in marine sediments. *J. Geomicrobiol.* 3, 275–306. doi: 10.1080/01490458409377807
- Knittel, K., Losekann, T., Boetius, A., Kort, R., and Amann, R. (2005). Diversity and distribution of methanotrophic archaea at cold seeps. *Appl. Environ. Microbiol.* 71, 467–479. doi: 10.1128/AEM.71.1.467-479.2005
- Liao, C., Luo, Y., Jiang, L., Zhou, X., Wu, X., Fang, C., et al. (2007). Invasion of *Spartina alterniflora* enhanced ecosystem carbon and nitrogen stocks in the Yangtze estuary, China. *Ecosystems* 10, 1351–1361. doi: 10.1007/s10021-007-9103-2
- Liao, C. Z., Luo, Y. Q., Fang, C. M., Chen, J. K., and Li, B. (2008). Litter pool sizes, decomposition, and nitrogen dynamics in *Spartina alterniflora*-invaded and native coastal marshlands of the Yangtze Estuary. *Oecologia* 156, 589–600. doi: 10.1007/s00442-008-1007-0
- Liu, D., Ding, W., Jia, Z., and Cai, Z. (2010). Influence of niche differentiation on the abundance of methanogenic archaea and methane production potential in natural wetland ecosystems across China. *Biogeosci. Discuss* 7, 7629–7655. doi: 10.5194/bgd-7-7629-2010
- Liu, Y., and Whitman, W. B. (2008). Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Ann. N.Y. Acad. Sci.* 1125, 171–189. doi: 10.1196/annals.1419.019
- Luton, P. E., Wayne, J. M., Sharp, R. J., and Riley, P. W. (2002). The *mcrA* gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. *Microbiology* 148, 3521–3530.
- Lyimo, T. J., Pol, A., Camp, H. J. M. O. D., Harhangi, H. R., and Vogels, G. D. (2000). *Methanosarcina semesiae* sp. nov., a dimethylsulfide-utilizing methanogen from mangrove sediment. *Int. J. Syst. Evol. Microbiol.* 50, 171–178. doi: 10.1099/00207713-50-1-171
- Moran, M. A., and Hodson, R. E. (1990). Contributions of degrading *Spartina alterniflora* lignocellulose to the dissolved organic carbon pool of a salt marsh. *Mar. Ecol. Prog. Ser.* 62, 161–168. doi: 10.3354/meps062161
- Muyzer, G., and Stams, A. J. M. (2008). The ecology and biotechnology of sulphate-reducing bacteria. *Nat. Rev. Microbiol.* 6, 441–454.
- Nie, M., Wang, M., and Li, B. (2009). Effects of salt marsh invasion by *Spartina alterniflora* on sulfate-reducing bacteria in the Yangtze River estuary, China. *Ecol. Eng.* 35, 1804–1808. doi: 10.1016/j.ecoleng.2009.08.002
- Oremland, R. S., Marsh, L. M., and Polcin, S. (1982). Methane production and simultaneous sulfate reduction in anoxic, salt marsh sediments. *Nature* 296, 143–145. doi: 10.1038/296143a0
- Page, H. M., Lastra, M., Rodil, I. F., Briones, M. J. I., and Garrido, J. (2010). Effects of non-native *Spartina patens* on plant and sediment organic matter carbon incorporation into the local invertebrate community. *Biol. Invasions* 12, 3825–3838. doi: 10.1007/s10530-010-9775-y
- Peng, R. H., Fang, C. M., Li, B., and Chen, J. K. (2011). *Spartina alterniflora* invasion increases soil inorganic nitrogen pools through interactions with tidal subsidies in the Yangtze Estuary, China. *Oecologia* 165, 797–807. doi: 10.1007/s00442-010-1887-7
- Purdy, K. J., Nedwell, D. B., and Embley, T. M. (2003). Analysis of the sulfate-reducing bacterial and methanogenic archaeal populations in contrasting antarctic sediments. *Appl. Environ. Microbiol.* 69, 3181–3191. doi: 10.1128/AEM.69.6.3181-3191.2003
- Ravit, B., Ehrenfeld, J. G., and Haggblom, M. M. (2003). A comparison of sediment microbial communities associated with *Phragmites australis* and *Spartina alterniflora* in two brackish wetlands of New Jersey. *Estuaries* 26, 465–474. doi: 10.1007/BF02823723
- Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–7541. doi: 10.1128/AEM.01541-09
- Schubauer, J. P., and Hopkinson, C. S. (1984). Above- and belowground emergent macrophyte production and turnover in a coastal marsh ecosystem, Georgia. *Limnol. Oceanogr.* 29, 1052–1065. doi: 10.4319/lo.1984.29.5.1052
- Senior, E., Borje, E. L., Ibrahim, M. B., and Nedwell, D. B. (1982). Sulfate reduction and methanogenesis in the sediment of a saltmarsh on the east coast of the United Kingdom. *Appl. Environ. Microbiol.* 43, 987–996.
- Steinberg, L. M., and Regan, J. M. (2008). Phylogenetic comparison of the methanogenic communities from an acidic, oligotrophic fen and an anaerobic digester treating municipal wastewater sludge. *Appl. Environ. Microbiol.* 74, 6663–6671. doi: 10.1128/AEM.00553-08
- Steinberg, L. M., and Regan, J. M. (2009). *mcrA*-targeted real-time quantitative PCR method to examine methanogen communities. *Appl. Environ. Microbiol.* 75, 4435–4442. doi: 10.1128/AEM.02858-08
- Tong, C., Wang, W. Q., Huang, J. F., Gauci, V., Zhang, L. H., and Zeng, C. S. (2012). Invasive alien plants increase CH₄ emissions from a subtropical tidal estuarine wetland. *Biogeochemistry* 111, 677–693. doi: 10.1007/s10533-012-9712-5
- Turetsky, M. R., Treat, C. C., Waldrop, M. P., Waddington, J. M., Harden, J. W., and McGuire, A. D. (2008). Short-term response of methane fluxes and methanogen activity to water table and soil warming manipulations in an Alaskan peatland. *J. Geophys. Res.* 113:G00A10. doi: 10.1029/2007JG0004960
- Turner, R. E. (1993). Carbon, nitrogen, and phosphorus leaching rates from *Spartina alterniflora* salt marshes. *Mar. Ecol. Prog. Ser.* 92, 135–140. doi: 10.3354/meps092135
- Wang, D., Chen, Z., and Xu, S. (2009). Methane emission from Yangtze estuarine wetland, China. *J. Geophys. Res.* 114:G02011.
- Watkins, A. J., Roussel, E. G., Webster, G., Parkes, R. J., and Sass, H. (2012). Choline and N,N-Dimethylethanolamine as direct substrates for methanogens. *Appl. Environ. Microbiol.* 78, 8298–8303. doi: 10.1128/AEM.01941-12
- Williams, S. L., and Grosholz, E. D. (2008). The invasive species challenge in estuarine and coastal

- environments: marrying management and science. *Estuar. Coast.* 31, 3–20. doi: 10.1007/s12237-007-9031-6
- Winfrey, M. R., and Ward, D. M. (1983). Substrates for sulfate reduction and methane production in intertidal sediments. *Appl. Environ. Microbiol.* 45, 193–199.
- Zeikus, G., and Winfrey, M. R. (1976). Temperature limitation of methanogenesis in aquatic sediments. *Appl. Environ. Microbiol.* 31, 99–107.
- Zelege, J., Lu, S. L., Wang, J. G., Huang, J. X., Li, B., Ogram, A. V., et al. (2013). Methyl coenzyme M reductase A (*mcrA*) gene-based investigation of methanogens in the mudflat sediments of Yangtze River Estuary, China. *Microb. Ecol.* 66, 257–267. doi: 10.1007/s00248-012-0155-2
- Zhang, G., Tian, J., Jiang, N., Guo, X., Wang, Y., and Dong, X. (2008). Methanogen community in Zoige wetland of Tibetan plateau and phenotypic characterization of a dominant uncultured methanogen cluster ZC-I. *Environ. Microbiol.* 10, 1850–1860. doi: 10.1111/j.1462-2920.2008.01606.x
- Zhang, Y., Ding, W., Cai, Z., Valerie, P., and Han, F. (2010). Response of methane emission to invasion of *Spartina alterniflora* and exogenous N deposition in the coastal salt marsh. *Atmos. Environ.* 44, 4588–4594. doi: 10.1016/j.atmosenv.2010.08.012
- Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
- Received: 15 May 2013; accepted: 04 August 2013; published online: 23 August 2013.
- Citation: Zelege J, Sheng Q, Wang J-G, Huang M-Y, Xia F, Wu J-H and Quan Z (2013) Effects of *Spartina alterniflora* invasion on the communities of methanogens and sulfate-reducing bacteria in estuarine marsh sediments. *Front. Microbiol.* 4:243. doi: 10.3389/fmicb.2013.00243
- This article was submitted to *Terrestrial Microbiology*, a section of the journal *Frontiers in Microbiology*.
- Copyright © 2013 Zelege, Sheng, Wang, Huang, Xia, Wu and Quan. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Rhizosphere priming: a nutrient perspective

Feike A. Dijkstra^{1*}, Yolima Carrillo¹, Elise Pendall² and Jack A. Morgan³

¹ Department of Environmental Sciences, Centre for Carbon, Water, and Food, The University of Sydney, Camden, NSW, Australia

² Department of Botany and Program in Ecology, University of Wyoming, Laramie, WY, USA

³ Rangeland Resources Research Unit, USDA-ARS, Fort Collins, CO, USA

Edited by:

Per Bengtson, Lund University, Sweden

Reviewed by:

Osnat Gillor, Ben Gurion University, Israel

Christopher Blackwood, Kent State University, USA

*Correspondence:

Feike A. Dijkstra, Department of Environmental Sciences, Centre for Carbon, Water, and Food, The University of Sydney, 380 Werombi Road, Camden, NSW 2015, Australia
e-mail: feike.dijkstra@sydney.edu.au

Rhizosphere priming is the change in decomposition of soil organic matter (SOM) caused by root activity. Rhizosphere priming plays a crucial role in soil carbon (C) dynamics and their response to global climate change. Rhizosphere priming may be affected by soil nutrient availability, but rhizosphere priming itself can also affect nutrient supply to plants. These interactive effects may be of particular relevance in understanding the sustained increase in plant growth and nutrient supply in response to a rise in atmospheric CO₂ concentration. We examined how these interactions were affected by elevated CO₂ in two similar semiarid grassland field studies. We found that an increase in rhizosphere priming enhanced the release of nitrogen (N) through decomposition of a larger fraction of SOM in one study, but not in the other. We postulate that rhizosphere priming may enhance N supply to plants in systems that are N limited, but that rhizosphere priming may not occur in systems that are phosphorus (P) limited. Under P limitation, rhizodeposition may be used for mobilization of P, rather than for decomposition of SOM. Therefore, with increasing atmospheric CO₂ concentrations, rhizosphere priming may play a larger role in affecting C sequestration in N poor than in P poor soils.

Keywords: ¹⁵N tracer, microbial mining, N:P stoichiometry, nutrient competition, preferential substrate utilization, progressive nitrogen limitation, root exudates

INTRODUCTION

Rhizosphere priming is the change in soil organic matter (SOM) decomposition caused by plant root activity that is often associated with rhizodeposition (Kuzyakov, 2002). A substantial fraction of net carbon assimilation goes into the soil as rhizodeposition. Estimates of how much C is allocated to rhizodeposition vary widely among plant species, with plant age, soil type, and nutrient availability, and are on average between 11 and 17% of net fixed C (Nguyen, 2003; Jones et al., 2009). Rhizodeposition is an important energy source for the microbial production of extra-cellular enzymes that break down SOM (Schimel and Weintraub, 2003; Blagodatskaya and Kuzyakov, 2008; Averill and Finzi, 2011). The subsequent change in SOM decomposition (i.e., the rhizosphere priming effect) is usually measured by comparing the CO₂ produced from SOM in a soil with and without plants. Often, CO₂ produced from SOM in planted soil is greater than in the unplanted or fallow soil, and is referred to as a positive priming effect (Kuzyakov, 2002). However, smaller CO₂ production in planted compared to unplanted soil, or a negative priming effect, has also been observed (Cheng, 1996; Bader and Cheng, 2007). Although rhizosphere priming effects have frequently been reported in a variety of soil-plant systems, the mechanisms behind these effects remain unclear (Kuzyakov, 2010).

EFFECTS OF SOIL NUTRIENT AVAILABILITY ON RHIZOSPHERE PRIMING

The direction and magnitude of rhizosphere priming have been related to soil nutrient availability. Plants and microbes require C and nutrients within specific boundaries and at the same

time affect the relative availability of C and nutrients in their immediate environment, the rhizosphere. Because of this close interdependence between C and nutrients, the nutrient status of the soil is an important factor for rhizosphere priming. Several hypotheses have been proposed explaining the relationship between rhizosphere priming and soil nutrient availability (Figure 1). First, in soils of low nutrient availability, inputs of energy-rich carbon compounds from roots may be used for the production of extra-cellular enzymes that can release nutrients locked in SOM (Asmar et al., 1994; Brzostek et al., 2012). Under these conditions, microbes may use root exudates to release nutrients thereby meeting their nutrient requirement. This has also been referred to as the microbial mining hypothesis (Craine et al., 2007; Fontaine et al., 2011). Because rhizosphere priming effects are usually measured through changes in CO₂ production, microbial mining for nutrients associated with rhizosphere priming should only relate to nutrients released through oxidation of SOM accompanied by the production CO₂. Much of the N in humified SOM is released through oxidation (biological mineralization). While some organic N compounds (proteins, amino acids, amino sugars) do not need to be oxidized for the N to be utilized, the C skeletons of these compounds are often catabolized by microbes thereby producing CO₂. On the other hand, organic P is mostly released through hydrolysis without CO₂ production (biochemical mineralization, McGill and Cole, 1981). Therefore, the microbial mining hypothesis may be more important for N than for P.

Second, in soils of high nutrient availability, a negative priming effect may occur. Under conditions of high nutrient availability, there is less need for microbes to mine nutrients, but instead,

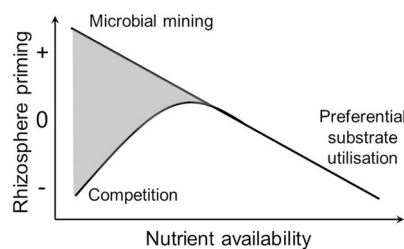


FIGURE 1 | Hypothetical relationship between soil nutrient availability and rhizosphere priming. Three nutrient-centered hypotheses are illustrated: Microbial mining: microbes utilize rhizodeposition to mine for nutrients in SOM thereby causing a positive rhizosphere priming effect when nutrient availability is low; Preferential substrate utilization: microbes switch from decomposing SOM to utilizing rhizodeposition when nutrient availability is high; Competition: microbes compete for nutrients with plants causing a negative rhizosphere priming effect because microbial growth and decomposition are nutrient limited. Both positive and negative rhizosphere priming can occur under low nutrient availability (gray area).

microbes may switch from decomposing recalcitrant SOM to utilizing labile root exudates for their carbon and energy requirements (Blagodatskaya et al., 2007; Guenet et al., 2010). As a result, decomposition of SOM could decrease with inputs of root exudates. This has been referred to as the preferential substrate utilization hypothesis (Cheng, 1999) (Figure 1).

A third nutrient-centered mechanism that has been proposed for explaining negative rhizosphere priming effects is when plants and microbes compete for the same nutrients. When plants remove nutrients from the soil through uptake they may reduce microbial decomposition (Dijkstra et al., 2010b; Pausch et al., 2013). The resulting negative rhizosphere priming effect may be stronger when nutrient availability is already low and limiting both plant and microbial growth (competition hypothesis, Cheng, 1999) (Figure 1). Production of rhizodeposits may also be a strategy for slow-growing plant species to lower soil N availability thereby outcompeting neighboring fast-growing plant species (Meier et al., 2009).

Low soil nutrient conditions can invoke both positive and negative rhizosphere priming (Figure 1). Clearly, there is a need to better understand why rhizodeposition in soils with low nutrient conditions sometimes result in enhanced microbial mining for nutrients (and a positive rhizosphere priming effect) and at other times in enhanced competition for nutrients inducing reduced microbial activity (and a negative rhizosphere priming effect). Several explanations may be involved in observations of positive and negative priming effects, including soil microbial community effects, quality and stoichiometry of the root exudates, and the relative availability of N and P, while none of these explanations are mutually exclusive.

First, dominance of one group of microbes over the other could potentially determine whether rhizodeposition results in negative or positive rhizosphere priming under low nutrient conditions. Microbes vary tremendously in their ability to decompose SOM. While rhizodeposition may primarily increase growth and activity of fast growing microbes (r-strategists), a proportion of the rhizodeposition may be utilized by slow growing microbes

decomposing recalcitrant organic matter (K-strategists), particularly when nutrient availability is low (Fontaine et al., 2003). Fungi, gram-negative and gram-positive bacteria have all been associated with enhanced SOM decomposition with increased rhizodeposition and input of other labile C (Nottingham et al., 2009; Bird et al., 2011; Fontaine et al., 2011; Garcia-Pausas and Paterson, 2011). Bacteria may be more sensitive to competition for nutrients with plants than fungi because fungal hyphae have a greater ability to explore the soil (Otten et al., 2001), and therefore fungi may escape competition for nutrients with plants. Furthermore, mycorrhizae, a special group of fungi that grow in direct association with plants, may supply nutrients directly to plants in return for plant C, thereby reducing nutrient competition between plants and mycorrhizae (Koide, 1991).

If the soil microbial community is dominated by bacteria that are activated close to the root, then the microbial competition hypothesis (or preferential substrate utilization hypothesis under high nutrient availability) may prevail resulting in negative rhizosphere priming. These bacteria would be dominated by r-strategists and utilize fresh exudates (Dorodnikov et al., 2009). On the other hand, if the soil microbial community is dominated by fungi, supply of plant C may stimulate fungi to mine for nutrients further away from the roots resulting in positive rhizosphere priming. These fungal decomposers act more like K-strategists, and may be saprotrophic or mycorrhizal (Talbot et al., 2008).

Second, the direction and magnitude of rhizosphere priming under low nutrient conditions may depend on the type of organic compounds released by plants. Plant roots release a myriad of organic compounds, not only from root exudation, but also from mucilage, as sloughed cells via mechanical abrasion, and from root death (Jones et al., 2004, 2009). Because these compounds have different stoichiometric and energetic properties, rhizodeposition may have variable effects on priming (Mary et al., 1993; Hamer and Marschner, 2005; Kuzyakov and Bol, 2006). Although many of these compounds often showed idiosyncratic priming effects, root exudates that generate more alkalinity during their decomposition or contain more N have been shown to cause greater priming (Rukshana et al., 2012; Drake et al., 2013).

Third, contrasting rhizosphere priming effects under low nutrient availability may occur because priming also depends on soil properties such as total C content and texture (Zhang et al., 2013), mineralogy (Rasmussen et al., 2007), pH (Blagodatskaya and Kuzyakov, 2008; Luo et al., 2011), and heavy metal concentration (Ohm et al., 2011). Here we propose that the contrasting effects of rhizosphere priming under low nutrient availability can also be related to the relative availability of N and P in the soil.

N AND P CYCLING AND THEIR ROLE IN RHIZOSPHERE PRIMING

As discussed above, the supply of N to plants and microbes in soils predominantly occurs through oxidation of organic matter whereby N is mineralized. Phosphorus can also be released from SOM, but in soils with low organic P, inorganic sources are an important source for P supply (Walker and Syers, 1976). For instance, in calcareous soils much of the soil P is contained in calcium phosphates and the supply of P is regulated by precipitation/dissolution equilibria with P in soil solution (Lajtha and

Bloomer, 1988; Tunesi et al., 1999). Similarly, in many acidic soils, P is bound to Al and Fe oxides, and the supply of P to plants is controlled by adsorption/desorption processes (Sanyal and Datta, 1991). Furthermore, much of the organic P is present in soil as monoesters and diesters (Doolette and Smernik, 2011). The P in these bonds can be released by hydrolysis with the help of phosphatase enzymes (without causing CO₂ production, Nannipieri et al., 2011), rather than through oxidation of organic matter (causing CO₂ production). Therefore, the supply of N and P to plants is decoupled in many soil types (McGill and Cole, 1981).

Terrestrial ecosystems are frequently limited by P (Elser et al., 2007; Harpole et al., 2011). Microbes in particular have a high P requirement relative to N, where microbial N:P ratios are often lower than the plant or SOM N:P ratios from the same system (Cleveland and Liptzin, 2007). Microbial activity and growth can be limited by P, which has mostly been observed in highly weathered tropical soils (Cleveland et al., 2002; Ehlers et al., 2010), but also in a calcareous (Raiesi and Ghollarata, 2006), peat (Amador and Jones, 1993), and boreal forest soils (Giesler et al., 2002).

Whether the input of C compounds via rhizodeposition results in altered SOM decomposition may depend on whether microbial activity is N or P limited. In soils where microbes are more limited by N than by P, root exudates could be utilized to mine for N through enhanced SOM decomposition. On the other hand, in soils where microbes are more limited by P than by N, root exudates are not needed by microbes for releasing N from SOM, but instead, could be used to mobilize P from inorganic or organic sources. Root exudates could be utilized by microbes to produce phosphatase extracellular enzymes releasing P through hydrolysis (Dakora and Phillips, 2002). Increased levels of microbial biomass and phosphatase extracellular enzymes have been observed in the rhizosphere (Chen et al., 2002), but it is unclear to what degree phosphatase extracellular enzymes are produced by plants or microbes (George et al., 2011). Root exudates can also directly increase P mobilization by increasing desorption and solubilisation from mineral surfaces through ligand exchange and dissolution (Dakora and Phillips, 2002; George et al., 2011). We hypothesize that microbial N limitation results in rhizosphere priming, while microbial P limitation does not (Figure 2). After discussing the effects of rhizosphere priming on nutrient availability, we will illustrate this hypothesis with examples of rhizosphere priming effects that were observed under elevated atmospheric CO₂ concentration.

RHIZOSPHERE PRIMING EFFECTS ON NUTRIENT AVAILABILITY

While some research has been done on how rhizosphere priming is affected by nutrient availability, particularly N availability, considerably less is known regarding the consequences of rhizosphere priming for nutrient cycling and plant nutrient uptake. From an evolutionary perspective it could be argued that the loss of expensive energy-rich carbon compounds into the soil through root exudation should result in some benefit to the plant. Does the stimulation of SOM decomposition through rhizosphere priming result in increased nutrient availability to plants or does it only result in increased microbial nutrient immobilization? There are only a limited number of studies that have tried to tackle this question.

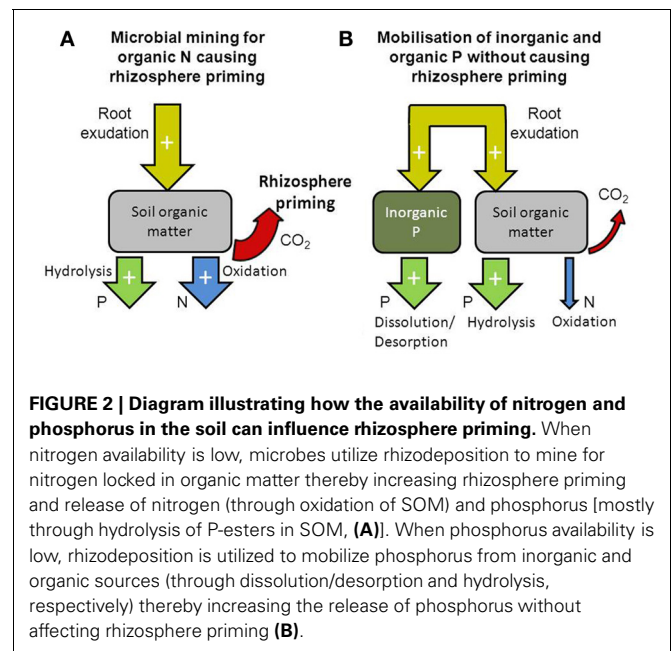


FIGURE 2 | Diagram illustrating how the availability of nitrogen and phosphorus in the soil can influence rhizosphere priming. When nitrogen availability is low, microbes utilize rhizodeposition to mine for nitrogen locked in organic matter thereby increasing rhizosphere priming and release of nitrogen (through oxidation of SOM) and phosphorus [mostly through hydrolysis of P-esters in SOM, (A)]. When phosphorus availability is low, rhizodeposition is utilized to mobilize phosphorus from inorganic and organic sources (through dissolution/desorption and hydrolysis, respectively) thereby increasing the release of phosphorus without affecting rhizosphere priming (B).

Positive priming effects should be associated with net N mineralization, but Cheng (2009) observed that the amount of net mineral N released from the accelerated SOM decomposition associated with the rhizosphere priming effect was much lower than the expected amount based on the C:N ratio of the SOM. This suggests that much of the N associated with the increase in SOM decomposition remained in the microbial biomass or was returned to the organic soil pool. On the other hand, a range of positive rhizosphere priming effects were observed among six different soil type-plant species combinations that also resulted in substantial increases in net N mineralization (Dijkstra et al., 2009). Except for one soil-plant combination, rhizosphere priming effects were positively related to gross N mineralization and plant N uptake, suggesting that rhizosphere priming not only enhanced microbial mining for N, but also enhanced the release of N for plant uptake. An increase in net N mineralization with root exudation could also occur because of microbial grazing by protozoa (Clarholm, 1985) creating a microbial loop. According to this microbial loop hypothesis, increased microbial growth in response to root exudation causes increased microbial immobilization of N, which in return is released for plant uptake after grazing by protozoa or nematodes.

However, there appears to be a fine balance between how rhizosphere priming affects microbial mineralization and immobilization. Microbial immobilization increased more than gross N mineralization with increased root exudation of three species of tree seedlings so that net N mineralization was reduced at high rates of root exudation (Bengtson et al., 2012). In modeling and field experiments Drake et al. (2013), simulated exudates and showed that adding C alone enhanced SOM decomposition, but adding C and N together stimulated SOM decomposition and N mineralization significantly more than C alone. These results suggest that both quantity and quality of root exudation have important consequences for the release of N through rhizosphere priming.

RHIZOSPHERE PRIMING UNDER ELEVATED ATMOSPHERIC CO₂

Atmospheric CO₂ concentrations have increased by more than 35% during the last 150 years and will continue to rise in the future (Forster et al., 2007), causing large impacts on C cycling in terrestrial ecosystems (Heimann and Reichstein, 2008). The immediate plant response to an increase in atmospheric CO₂ is often an increase in photosynthesis and net primary production (Amthor, 1995). Several studies have indicated that elevated CO₂ also increases rhizodeposition (Darragh, 1996; Pendall et al., 2004; Fransson and Johansson, 2010), and could potentially increase rhizosphere priming. Indeed, increased loss of soil C or mineral-associated organic matter under elevated CO₂ has been associated with greater rhizosphere priming (Carney et al., 2007; Hofmockel et al., 2011b).

An increase in SOM decomposition caused by rhizosphere priming under elevated CO₂ may also affect N cycling, and this has important ramifications for long-term responses of terrestrial ecosystems to elevated CO₂. Plant growth in most terrestrial ecosystems is N limited (Vitousek and Howarth, 1991). It has been suggested that without external input of N, elevated CO₂ will reduce N availability to plants in the long-term and that therefore plant growth responses to elevated CO₂ cannot be sustained (Luo et al., 2004). A crucial component of this concept of Progressive N Limitation (PNL) is the expectation that N availability is reduced under elevated CO₂ because of increased plant N uptake and immobilization in long-lived plant biomass, and because of increased microbial N immobilization associated with increased C inputs into the soil. Indeed, elevated CO₂ often reduces N availability in the soil (Diaz et al., 1993; Gill et al., 2002; Reich et al., 2006). Tree growth in a temperate forest increased during the first 6 years in response to elevated CO₂, but this effect disappeared after 11 years (Norby et al., 2010). It was suggested that a decline in soil N availability constrained the plant growth responses to elevated CO₂ in the long-term thereby providing support for the PNL concept.

However, PNL has not always been observed (Luo et al., 2006) and rhizosphere priming may be one of the mechanisms responsible for the lack of PNL. Tree growth in a temperate forest in North Carolina was still higher after 10 years of elevated CO₂ (McCarthy et al., 2010). Moreover, plant N uptake remained higher under elevated CO₂, which appears to have caused the sustained increase in tree growth in response to elevated CO₂ in this study (Drake et al., 2011). While some of this extra N may have been taken up from deeper soil layers (Finzi et al., 2006), it was also shown that elevated CO₂ enhanced root exudation and N release from SOM decomposition through rhizosphere priming (Phillips et al., 2011, 2012). Increased rhizosphere priming and plant N uptake under elevated CO₂ has also been observed in several other studies (Martín-Olmedo et al., 2002; de Graaff et al., 2009; Hofmockel et al., 2011a). These results suggest that enhanced rhizosphere priming could delay or at least alleviate PNL under elevated CO₂.

CONTRASTING ELEVATED CO₂ EFFECTS ON N CYCLING IN SEMIARID GRASSLANDS

Sustained increases in N cycling and plant N uptake under elevated CO₂ were also observed in a semiarid grassland in

Colorado, USA (King et al., 2004; Dijkstra et al., 2008). Enhanced N cycling and microbial mining for N under elevated CO₂ was illustrated with a ¹⁵N tracer study. In this field experiment ¹⁵N (as ¹⁵NH₄ ¹⁵NO₃) was added as a tracer to the soil and followed into aboveground plant biomass collected in plots exposed to ambient and elevated CO₂ using open top chambers, up to 5 years after the pulse addition (OTC experiment). The ¹⁵N label in plant tissue, expressed as a fraction of total aboveground plant tissue N, decreased with time (Dijkstra et al., 2008) (Figure 3A). The decrease of the ¹⁵N label was explained by ongoing mineralization of unlabeled N in the soil that progressively diluted the ¹⁵N label in the available N pool to plants (Dijkstra, 2009). This dilution of the ¹⁵N label in the plant was faster under elevated CO₂, suggesting that mineralization of unlabeled N from SOM was enhanced under elevated CO₂. Rhizodeposition was also greater under elevated CO₂ in this system (Pendall et al., 2004). These combined results suggest that elevated CO₂ may have increased rhizosphere priming through microbial mining for N and subsequent release of N for plant uptake.

We conducted a similar experiment in the Prairie Heating And CO₂ Enrichment (PHACE) experiment in Wyoming, USA (Dijkstra et al., 2010a; Morgan et al., 2011). The northern mixed prairie vegetation of the PHACE experiment is similar to that of the shortgrass steppe of the OTC experiment in Colorado with *Bouteloua gracilis* (a warm season C4 grass), *Pascopyrum smithii* and *Hesperostipa comata* (two cool-season C3 grasses) being the dominant species comprising 80% or more of the aboveground biomass at both sites, although net primary productivity is greater at PHACE than at OTC. Free Air CO₂ Enrichment technology was used in the PHACE experiment to increase the CO₂ concentration to 600 ppm, which is lower than the elevated CO₂ treatment in the OTC experiment (720 ppm). Furthermore, a warming treatment (1.5/3°C above ambient during the day/night) using

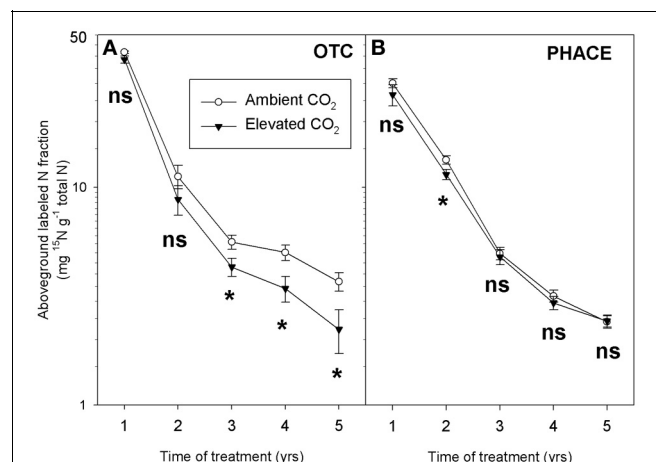


FIGURE 3 | Labeled N fractions (expressed per total amount of N) in aboveground biomass over time in the OTC (A) and PHACE (B) experiment. A ¹⁵N label was added to the soil in the Spring of year 1 and aboveground biomass was sampled at peak biomass in July in the following 5 years. For each year, CO₂ treatment effects were tested with ANOVA (ns: not significant, **P* < 0.05).

infrared heaters was included in the PHACE experiment in a full factorial design.

As in the OTC experiment, we added a ^{15}N tracer to the plots and followed the ^{15}N label into aboveground biomass during the following years. The ^{15}N label was added by spraying a K^{15}NO_3 solution (99 atom% ^{15}N) onto the plots in 2007. As in the OTC experiment the ^{15}N label, expressed as a fraction of the total plant N, decreased with time due to dilution of the label with non-labeled N from mineralization in the soil. However, in contrast to the OTC experiment, the decrease of the ^{15}N label in aboveground biomass was not enhanced under elevated CO_2 (Figure 3B). These results suggest that elevated CO_2 did not enhance microbial mining and release of unlabeled N in the PHACE experiment.

DOES RHIZOSPHERE PRIMING UNDER ELEVATED CO_2 DEPEND ON RELATIVE AVAILABILITY OF N AND P?

Why did these similar semiarid grasslands respond differently to elevated CO_2 in terms of its effect on N cycling? A possible explanation is that in the PHACE experiment elevated CO_2 did not increase rhizosphere priming of SOM. However, elevated CO_2 resulted in larger labile soil C pools, although not in all years (Carrillo et al., 2011). Further, elevated CO_2 increased rates of heterotrophic respiration in the PHACE experiment (Pendall et al., 2013). These results suggest that elevated CO_2 may have enhanced rhizosphere priming of native soil organic C. However, it is also possible that elevated CO_2 enhanced input and cycling of the new and relatively labile soil C only without affecting decomposition of the native soil C pool (e.g., Hungate et al., 1997) (Figure 4). If elevated CO_2 increased the cycling of new C without affecting decomposition of native soil organic C, this would not result in enhanced dilution of the ^{15}N in the plant (Dijkstra, 2009). Only an increase in the decomposition of native soil

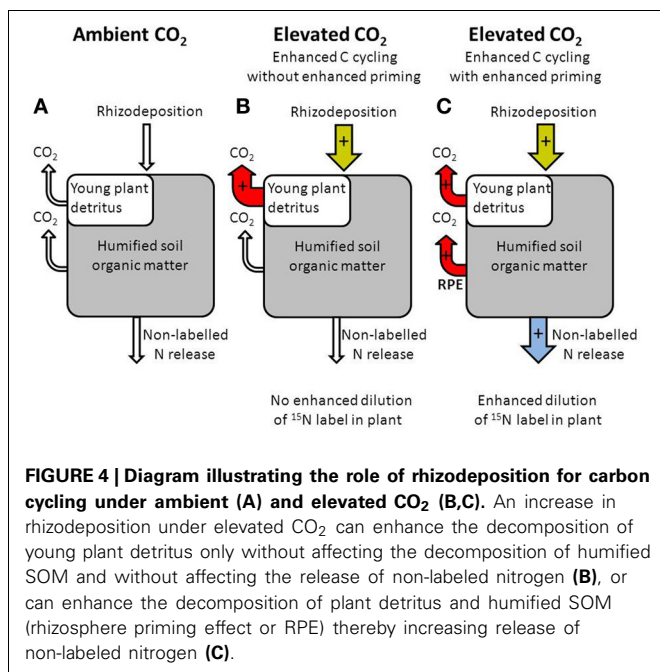
organic C (due to rhizosphere priming) would result in enhanced dilution of the ^{15}N label in the plant. Interestingly, (Allard et al., 2006) also found no enhanced plant N uptake under elevated CO_2 despite enhanced C cycling.

Why then would greater inputs of labile C under elevated CO_2 result in enhanced rhizosphere priming of native soil organic C in the OTC experiment, but only result in enhanced cycling of labile C in the PHACE experiment? We postulate that these contrasting effects of elevated CO_2 on soil C cycling may have occurred because of differences in the availability and cycling of N and P between the two sites.

As explained above, under conditions of low N availability, root exudates could be used by microbes to improve N supply by enhancing the decomposition of recalcitrant SOM that is relatively rich in N (microbial mining hypothesis), possibly with an extra supply of N-degrading enzymes (Drake et al., 2013). However, as described above, root exudates could also be used for the production of phosphatase extracellular enzymes hydrolyzing organic P (by plants and microbes) and to increase mobilization of P (Dakora and Phillips, 2002; George et al., 2011) without affecting SOM decomposition.

In the PHACE experiment we observed that the availability and plant uptake of P compared to that of N increased under elevated CO_2 (Dijkstra et al., 2012). The PHACE experiment was conducted on a calcareous soil high in insoluble calcium phosphates (41% of total soil P was in inorganic form, Dijkstra et al., 2012) that are not directly available to plants. The fixation of P as calcium phosphates may have caused low P availability possibly limiting microbial activity and plant growth. An increase in root exudation under elevated CO_2 in this system may have increased P dissolution and mobilization without affecting net N release from native SOM. We have limited data on the availability of P compared to N in the soil of the OTC experiment. While soil P availability was similarly low at both sites (between 4 and 11 mg P kg^{-1} soil in the OTC experiment and between 4 and 7 mg P kg^{-1} soil in the PHACE experiment using 0.5 M NaHCO_3 extractions), a semiarid grassland similar to the grassland used in the OTC experiment strongly responded to N fertilization (Lauenroth et al., 1978). This suggests that plants and microbes may have been more limited by N than by P in the OTC experiment.

We propose that contrasting effects of elevated CO_2 on ^{15}N dilution in plant biomass in the OTC and PHACE experiments were due to differences in N and P availability to microbial activity and plant growth. In the N limited semiarid grassland, where the OTC experiment was conducted (Lauenroth et al., 1978), increased root exudation under elevated CO_2 resulted in a greater rhizosphere priming thereby enhancing SOM decomposition and mineralization of N, and possibly P. The enhanced N mineralization from native SOM then resulted in enhanced dilution of the ^{15}N label in the plant (Figure 2). On the other hand, in the PHACE experiment where P availability was low (Dijkstra et al., 2012) and that may have limited microbial activity and plant growth more than N, the increase in root exudation under elevated CO_2 may have increased the dissolution/desorption and mobilization of P, more so than enhancing decomposition of native SOM. As a result, the dilution of the ^{15}N label in



aboveground plant biomass with time was unaffected by elevated CO₂. Others have also suggested that enhanced rhizodeposition under elevated CO₂ may be utilized for mobilizing P, rather than for enhancing SOM decomposition (Cardon, 1996; Lloyd et al., 2001).

CONCLUSION

Several studies have suggested that elevated CO₂ can enhance SOM decomposition through increased rhizosphere priming effects (Cheng, 1999; Paterson et al., 2008; Phillips et al., 2011). An increase in rhizosphere priming has important implications for long-term C sequestration in soils under elevated CO₂ and how this feedbacks to the global climate. However, the magnitude and direction of the rhizosphere priming effect may strongly depend on the relative availability of N and P in the soil (Bradford et al., 2008; Milcu et al., 2011; Sullivan and

Hart, 2013). Although rhizosphere priming is influenced by several factors, we argue that the relative availability of N and P has to be considered in understanding how perturbations such as climate change affect rhizosphere priming and soil C sequestration.

ACKNOWLEDGMENTS

This project was supported by the US Department of Agriculture Agricultural Research Service Climate Change, Soils and Emissions Program, USDA-CSREES Soil Processes Program (Grant no. 2008-35107-18655), US Department of Energy's Office of Science (BER), through the Terrestrial Ecosystem Science program and the Western Regional Center of the National Institute for Climatic Change Research at Northern Arizona University, by NSF (DEB# 1021559), and by the Australian Research Council (FT100100779).

REFERENCES

- Allard, V., Robin, C., Newton, P. C. D., Lieffering, M., and Soussana, J. F. (2006). Short and long-term effects of elevated CO₂ on *Lolium perenne* rhizodeposition and its consequences on soil organic matter turnover and plant N yield. *Soil Biol. Biochem.* 38, 1178–1187. doi: 10.1016/j.soilbio.2005.10.002
- Amador, J., and Jones, R. D. (1993). Nutrient limitations on microbial respiration in peat soils with different total phosphorus content. *Soil Biol. Biochem.* 25, 793–801. doi: 10.1016/0038-0717(93)90125-U
- Amthor, J. S. (1995). Terrestrial higher-plant response to increasing atmospheric [CO₂] in relation to the global carbon cycle. *Glob. Change Biol.* 1, 243–274. doi: 10.1111/j.1365-2486.1995.tb00025.x
- Asmar, F., Eiland, F., and Nielsen, N. E. (1994). Effect of extracellular enzyme activities on solubilization rate of soil organic nitrogen. *Biol. Fertil. Soils* 17, 32–38. doi: 10.1007/BF00418669
- Averill, C., and Finzi, A. (2011). Plant regulation of microbial enzyme production *in situ*. *Soil Biol. Biochem.* 43, 2457–2460. doi: 10.1016/j.soilbio.2011.09.002
- Bader, N. E., and Cheng, W. (2007). Rhizosphere priming effect of *Populus fremontii* obscures the temperature sensitivity of soil organic carbon respiration. *Soil Biol. Biochem.* 39, 600–606. doi: 10.1016/j.soilbio.2006.09.009
- Bengtson, P., Barker, J., and Grayston, S. J. (2012). Evidence of a strong coupling between root exudation, C and N availability, and stimulated SOM decomposition caused by rhizosphere priming effects. *Ecol. Evol.* 2, 1843–1852. doi: 10.1002/ecs3.311
- Bird, J. A., Herman, D. J., and Firestone, M. K. (2011). Rhizosphere priming of soil organic matter by bacterial groups in a grassland soil. *Soil Biol. Biochem.* 43, 718–725. doi: 10.1016/j.soilbio.2010.08.010
- Blagodatskaya, E., and Kuzyakov, Y. (2008). Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biol. Fertil. Soils* 45, 115–131. doi: 10.1007/s00374-008-0334-y
- Blagodatskaya, E. V., Blagodatsky, S. A., Anderson, T. H., and Kuzyakov, Y. (2007). Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. *Appl. Soil Ecol.* 37, 95–105. doi: 10.1016/j.apsoil.2007.05.002
- Bradford, M. A., Fierer, N., and Reynolds, J. F. (2008). Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. *Funct. Ecol.* 22, 964–974. doi: 10.1111/j.1365-2435.2008.01404.x
- Brzostek, E. R., Greco, A., Drake, J. E., and Finzi, A. C. (2012). Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in temperate forest soils. *Biogeochemistry* doi: 10.1007/s10533-012-9818-9.
- Cardon, Z. G. (1996). Influence of rhizodeposition under elevated CO₂ on plant nutrition and soil organic matter. *Plant Soil* 187, 277–288. doi: 10.1007/BF00017093
- Carney, K. M., Hungate, B. A., Drake, B. G., and Megonigal, J. P. (2007). Altered soil microbial community at elevated CO₂ leads to loss of soil carbon. *Proc. Natl. Acad. Sci. U.S.A.* 104, 4990–4995. doi: 10.1073/pnas.0610045104
- Carrillo, Y., Pendall, E., Dijkstra, F. A., Morgan, J. A., and Newcomb, J. M. (2011). Response of soil organic matter pools to elevated CO₂ and warming in a semi-arid grassland. *Plant Soil* 347, 339–350. doi: 10.1007/s11104-011-0853-4
- Chen, C. R., Condon, L. M., Davis, M. R., and Sherlock, R. R. (2002). Phosphorus dynamics in the rhizosphere of perennial ryegrass (*Lolium perenne* L.) and radiata pine (*Pinus radiata* D. Don). *Soil Biol. Biochem.* 34, 487–499. doi: 10.1016/S0038-0717(01)00207-3
- Cheng, W. (1996). Measurement of rhizosphere respiration and organic matter decomposition using natural ¹³C. *Plant Soil* 183, 263–268. doi: 10.1007/BF00011441
- Cheng, W. (1999). Rhizosphere feedbacks in elevated CO₂. *Tree Physiol.* 19, 313–320. doi: 10.1093/treephys/19.4.5.313
- Cheng, W. (2009). Rhizosphere priming effect: Its functional relationships with microbial turnover, evapotranspiration, and C-N budgets. *Soil Biol. Biochem.* 41, 1795–1801. doi: 10.1016/j.soilbio.2008.04.018
- Clarholm, M. (1985). Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. *Soil Biol. Biochem.* 17, 181–187. doi: 10.1016/0038-0717(85)90113-0
- Cleveland, C. C., and Liptzin, D. (2007). C:N:P stoichiometry in soil: Is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry* 85, 235–252. doi: 10.1007/s10533-007-9132-0
- Cleveland, C. C., Townsend, A. R., and Schmidt, S. K. (2002). Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies. *Ecosystems* 5, 680–691. doi: 10.1007/s10021-002-0202-9
- Craine, J. M., Morrow, C., and Fierer, N. (2007). Microbial nitrogen limitation increases decomposition. *Ecology* 88, 2105–2113. doi: 10.1890/06-1847.1
- Dakora, F. D., and Phillips, D. A. (2002). Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant Soil* 245, 35–47. doi: 10.1023/A:1020809400075
- Darrah, P. R. (1996). Rhizodeposition under ambient and elevated CO₂ levels. *Plant Soil* 187, 265–275. doi: 10.1007/BF00017092
- de Graaff, M.-A., Van Kessel, C., and Six, J. (2009). Rhizodeposition-induced decomposition increases N availability to wild and cultivated wheat genotypes under elevated CO₂. *Soil Biol. Biochem.* 41, 1094–1103. doi: 10.1016/j.soilbio.2009.02.015
- Díaz, S., Grime, J. P., Harris, J., and McPherson, E. (1993). Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. *Nature* 364, 616–617. doi: 10.1038/364616a0
- Dijkstra, F. A. (2009). Modeling the flow of ¹⁵N after a ¹⁵N pulse to study long-term N dynamics in a semiarid grassland. *Ecology* 90, 2171–2182. doi: 10.1890/08-1172.1
- Dijkstra, F. A., Bader, N. E., Johnson, D. W., and Cheng, W. (2009). Does accelerated soil organic matter decomposition in the presence of plants increase plant N availability? *Soil Biol. Biochem.* 41, 1080–1087. doi: 10.1016/j.soilbio.2009.02.013

- Dijkstra, F. A., Blumenthal, D., Morgan, J. A., Pendall, E., Carrillo, Y., and Follett, R. F. (2010a). Contrasting effects of elevated CO₂ and warming on nitrogen cycling in a semiarid grassland. *New Phytol.* 187, 426–437. doi: 10.1111/j.1469-8137.2010.03293.x
- Dijkstra, F. A., Morgan, J. A., Blumenthal, D., and Follett, R. F. (2010b). Water limitation and plant inter-specific competition reduce rhizosphere-induced C decomposition and plant N uptake. *Soil Biol. Biochem.* 42, 1073–1082. doi: 10.1016/j.soilbio.2010.02.026
- Dijkstra, F. A., Pendall, E., Morgan, J. A., Blumenthal, D. M., Carrillo, Y., LeCain, D. R., et al. (2012). Climate change alters stoichiometry of phosphorus and nitrogen in a semiarid grassland. *New Phytol.* 196, 807–815. doi: 10.1111/j.1469-8137.2012.04349.x
- Dijkstra, F. A., Pendall, E., Mosier, A. R., King, J. Y., Milchunas, D. G., and Morgan, J. A. (2008). Long-term enhancement of N availability and plant growth under elevated CO₂ in a semi-arid grassland. *Funct. Ecol.* 22, 975–982. doi: 10.1111/j.1365-2435.2008.01398.x
- Doolette, A. L., and Smernik, R. J. (2011). “Soil organic phosphorus speciation using spectroscopic techniques,” in *Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling*, eds E. K. Bünemann, A. Oberson, and E. Frossard (Heidelberg: Springer), 3–36. doi: 10.1007/978-3-642-15271-9_1
- Dorodnikov, M., Blagodatskaya, E., Blagodatsky, S., Fangmeier, A., and Kuzyakov, Y. (2009). Stimulation of r- vs. K-selected microorganisms by elevated atmospheric CO₂ depends on soil aggregate size: research article. *FEMS Microbiol. Ecol.* 69, 43–52. doi: 10.1111/j.1574-6941.2009.00697.x
- Drake, J. E., Darby, B. A., Giasson, M. A., Kramer, M. A., Phillips, R. P., and Finzi, A. C. (2013). Stoichiometry constrains microbial response to root exudation- insights from a model and a field experiment in a temperate forest. *Biogeosciences* 10, 821–838. doi: 10.5194/bg-10-821-2013
- Drake, J. E., Gallet-Budynek, A., Hofmockel, K. S., Bernhardt, E. S., Billings, S. A., Jackson, R. B., et al. (2011). Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO₂. *Ecol. Lett.* 14, 349–357. doi: 10.1111/j.1461-0248.2011.01593.x
- Ehlers, K., Bakken, L. R., Frostegård, Å., Frossard, E., and Bünemann, E. K. (2010). Phosphorus limitation in a ferralsol: impact on microbial activity and cell internal P pools. *Soil Biol. Biochem.* 42, 558–566. doi: 10.1016/j.soilbio.2009.11.025
- Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., et al. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.* 10, 1135–1142. doi: 10.1111/j.1461-0248.2007.01113.x
- Finzi, A. C., Moore, D. J. P., DeLucia, E. H., Lichter, J., Hofmockel, K. S., Jackson, R. B., et al. (2006). Progressive nitrogen limitation of ecosystem processes under elevated CO₂ in a warm-temperate forest. *Ecology* 87, 15–25. doi: 10.1890/04-1748
- Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J. M. G., Maire, V., et al. (2011). Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. *Soil Biol. Biochem.* 43, 86–96. doi: 10.1016/j.soilbio.2010.09.017
- Fontaine, S., Mariotti, A., and Abbadie, L. (2003). The priming effect of organic matter: a question of microbial competition? *Soil Biol. Biochem.* 35, 837–843. doi: 10.1016/S0038-0717(03)00123-8
- Forster, P., Ramaswamy, V., Artaxo, P., Bernsten, T., Betts, R., Fahey, D. W., et al. (2007). “Changes in atmospheric constituents and in radiative forcing,” in *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, eds S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller (Cambridge: Cambridge University Press), 129–234.
- Fransson, P. M. A., and Johansson, E. M. (2010). Elevated CO₂ and nitrogen influence exudation of soluble organic compounds by ectomycorrhizal root systems. *FEMS Microbiol. Ecol.* 71, 186–196. doi: 10.1111/j.1574-6941.2009.00795.x
- García-Pausas, J., and Paterson, E. (2011). Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon. *Soil Biol. Biochem.* 43, 1705–1713. doi: 10.1016/j.soilbio.2011.04.016
- George, T. S., Fransson, A. M., Hammond, J. P., and White, P. J. (2011). “Phosphorus nutrition: rhizosphere processes, plant response and adaptation,” in *Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling*, eds E. K. Bünemann, A. Oberson, and E. Frossard (Heidelberg: Springer), 245–271. doi: 10.1007/978-3-642-15271-9_10
- Giesler, R., Petersson, T., and Höglberg, P. (2002). Phosphorus limitation in boreal forests: effects of aluminum and iron accumulation in the humus layer. *Ecosystems* 5, 300–314. doi: 10.1007/s10021-001-0073-5
- Gill, R. A., Polley, H. W., Johnson, H. B., Anderson, L. J., Maherali, H., and Jackson, R. B. (2002). Nonlinear grassland responses to past and future atmospheric CO₂. *Nature* 417, 279–282. doi: 10.1038/417279a
- Guenet, B., Neill, C., Bardoux, G., and Abbadie, L. (2010). Is there a linear relationship between priming effect intensity and the amount of organic matter input? *Appl. Soil Ecol.* 46, 436–442. doi: 10.1016/j.apsoil.2010.09.006
- Hamer, U., and Marschner, B. (2005). Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. *Soil Biol. Biochem.* 37, 445–454. doi: 10.1016/j.soilbio.2004.07.037
- Harpole, W. S., Ngai, J. T., Cleland, E. E., Seabloom, E. W., Borer, E. T., Bracken, M. E. S., et al. (2011). Nutrient co-limitation of primary producer communities. *Ecol. Lett.* 14, 852–862. doi: 10.1111/j.1461-0248.2011.01651.x
- Heimann, M., and Reichstein, M. (2008). Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* 451, 289–292. doi: 10.1038/nature06591
- Hofmockel, K. S., Gallet-Budynek, A., McCarthy, H. R., Currie, W. S., Jackson, R. B., and Finzi, A. (2011a). Sources of increased N uptake in forest trees growing under elevated CO₂: results of a large-scale ¹⁵N study. *Glob. Change Biol.* 17, 3338–3350. doi: 10.1111/j.1365-2486.2011.02465.x
- Hofmockel, K. S., Zak, D. R., Moran, K. K., and Jastrow, J. D. (2011b). Changes in forest soil organic matter pools after a decade of elevated CO₂ and O₃. *Soil Biol. Biochem.* 43, 1518–1527. doi: 10.1016/j.soilbio.2011.03.030
- Hungate, B. A., Holland, E. A., Jackson, R. B., Chapin, F. S. III, Mooney, H. A., and Field, C. B. (1997). The fate of carbon in grassland under carbon dioxide enrichment. *Nature* 388, 576–579. doi: 10.1038/41550
- Jones, D., Nguyen, C., and Finlay, R. (2009). Carbon flow in the rhizosphere: carbon trading at the soil–root interface. *Plant Soil* 321, 5–33. doi: 10.1007/s11104-009-9925-0
- Jones, D. L., Hodge, A., and Kuzyakov, Y. (2004). Plant and mycorrhizal regulation of rhizodeposition. *New Phytol.* 163, 459–480. doi: 10.1111/j.1469-8137.2004.01130.x
- King, J. Y., Mosier, A. R., Morgan, J. A., LeCain, D. R., Milchunas, D. G., and Parton, W. J. (2004). Plant nitrogen dynamics in shortgrass steppe under elevated atmospheric carbon dioxide. *Ecosystems* 7, 147–160. doi: 10.1007/s10021-003-0201-5
- Koide, R. T. (1991). Tansley Review No.29. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytol.* 117, 365–386. doi: 10.1111/j.1469-8137.1991.tb00001.x
- Kuzyakov, Y. (2002). Factors affecting rhizosphere priming effects (review). *J. Plant Nutr. Soil Sci.* 165, 382–396.
- Kuzyakov, Y. (2010). Priming effects: interactions between living and dead organic matter. *Soil Biol. Biochem.* 42, 1363–1371. doi: 10.1016/j.soilbio.2010.04.003
- Kuzyakov, Y., and Bol, R. (2006). Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. *Soil Biol. Biochem.* 38, 747–758. doi: 10.1016/j.soilbio.2005.06.025
- Lajtha, K., and Bloomer, S. H. (1988). Factors affecting phosphate sorption and phosphate retention in a desert ecosystem. *Soil Sci.* 146, 160–167. doi: 10.1097/00010694-198809000-00003
- Lauenroth, W. K., Dodd, J. L., and Sims, P. L. (1978). The effects of water- and nitrogen-induced stresses on plant community structure in a semiarid grassland. *Oecologia* 36, 211–222. doi: 10.1007/BF00349810
- Lloyd, J., Bird, M. I., Veenendaal, E. M., and Kruijt, B. (2001). “Should phosphorus availability be constraining moist tropical forest responses to increasing CO₂ concentrations?” in *Global Biogeochemical Cycles in the Climate System* eds E. D. Schulze, M. Heimann, S. Harrison, E. Holland, J. Lloyd, I. C. Prentice, and D. Schimel (San Diego, CA: Academic Press), 95–114.
- Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., and Brookes, P. C. (2011). Short term soil priming effects and the mineralisation of

- biochar following its incorporation to soils of different pH. *Soil Biol. Biochem.* 43, 2304–2314. doi: 10.1016/j.soilbio.2011.07.020
- Luo, Y., Field, C. B., and Jackson, R. B. (2006). Does nitrogen constrain carbon cycling, or does carbon input stimulate nitrogen cycling? *Ecology* 87, 3–4. doi: 10.1890/05-0923
- Luo, Y., Su, B., Currie, W. S., Dukes, J. S., Finzi, A., Hartwig, U., et al. (2004). Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *Bioscience* 54, 731–739. doi: 10.1641/0006-3568(2004)054[0731:PNLOER]2.0.CO;2
- Martin-Olmedo, P., Rees, R. M., and Grace, J. (2002). The influence of plants grown under elevated CO₂ and N fertilization on soil nitrogen dynamics. *Glob. Change Biol.* 8, 643–657. doi: 10.1046/j.1365-2486.2002.00499.x
- Mary, B., Fresneau, C., Morel, J. L., and Mariotti, A. (1993). C and N cycling during decomposition of root mucilage, roots and glucose in soil. *Soil Biol. Biochem.* 25, 1005–1014. doi: 10.1016/0038-0717(93)90147-4
- McCarthy, H. R., Oren, R., Johnsen, K. H., Gallet-Budynek, A., Pritchard, S. G., Cook, C. W., et al. (2010). Re-assessment of plant carbon dynamics at the Duke free-air CO₂ enrichment site: interactions of atmospheric [CO₂] with nitrogen and water availability over stand development. *New Phytol.* 185, 514–528. doi: 10.1111/j.1469-8137.2009.03078.x
- McGill, W. B., and Cole, C. V. (1981). Comparative aspects of cycling of organic C, N, S and P through soil organic matter. *Geoderma* 26, 267–286. doi: 10.1016/0016-7061(81)90024-0
- Meier, C. L., Keyserling, K., and Bowman, W. D. (2009). Fine root inputs to soil reduce growth of a neighbouring plant via distinct mechanisms dependent on root carbon chemistry. *J. Ecol.* 97, 941–949. doi: 10.1111/j.1365-2745.2009.01537.x
- Milcu, A., Heim, A., Ellis, R., Scheu, S., and Manning, P. (2011). Identification of general patterns of nutrient and labile carbon control on soil carbon dynamics across a successional gradient. *Ecosystems* 14, 710–719. doi: 10.1007/s10021-011-9440-z
- Morgan, J. A., Lécain, D. R., Pendall, E., Blumenthal, D. M., Kimball, B. A., Carrillo, Y., et al. (2011). C₄ grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. *Nature* 476, 202–205. doi: 10.1038/nature10274
- Nannipieri, P., Giagnoni, L., Landi, L., and Renella, G. (2011). “Role of phosphatase enzymes in soil,” in *Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling*, eds E. K. Bünnemann, A. Oberson, and E. Frossard (Heidelberg: Springer), 215–243. doi: 10.1007/978-3-642-15271-9_9
- Nguyen, C. (2003). Rhizodeposition of organic C by plants: mechanisms and controls. *Agronomie* 23, 375–396. doi: 10.1051/agro:2003011
- Norby, R. J., Warren, J. M., Iversen, C. M., Medlyn, B. E., and McMurtrie, R. E. (2010). CO₂ enhancement of forest productivity constrained by limited nitrogen availability. *Proc. Natl. Acad. Sci. U.S.A.* 107, 19368–19373. doi: 10.1073/pnas.1006463107
- Nottingham, A. T., Griffiths, H., Chamberlain, P. M., Stott, A. W., and Tanner, E. V. J. (2009). Soil priming by sugar and leaf-litter substrates: a link to microbial groups. *Appl. Soil Ecol.* 42, 183–190. doi: 10.1016/j.apsoil.2009.03.003
- Ohm, H., Marschner, B., and Broos, K. (2011). Respiration and priming effects after fructose and alanine additions in two copper- and zinc-contaminated Australian soils. *Biol. Fertil. Soils* 47, 523–532. doi: 10.1007/s00374-011-0566-0
- Otten, W., Hall, D., Harris, K., Ritz, K., Young, I. M., and Gilligan, C. A. (2001). Soil physics, fungal epidemiology and the spread of *Rhizoctonia solani*. *New Phytol.* 151, 459–468. doi: 10.1046/j.0028-646x.2001.00190.x
- Paterson, E., Thornton, B., Midwood, A. J., Osborne, S. M., Sim, A., and Millard, P. (2008). Atmospheric CO₂ enrichment and nutrient additions to planted soil increase mineralisation of soil organic matter, but do not alter microbial utilisation of plant- and soil C-sources. *Soil Biol. Biochem.* 40, 2434–2440. doi: 10.1016/j.soilbio.2008.06.005
- Pausch, J., Zhu, B., Kuzyakov, Y., and Cheng, W. (2013). Plant inter-species effects on rhizosphere priming of soil organic matter decomposition. *Soil Biol. Biochem.* 57, 91–99. doi: 10.1016/j.soilbio.2012.08.029
- Pendall, E., Heisler-White, J. L., Williams, D. G., Dijkstra, F. A., Carrillo, Y., Morgan, J. A., et al. (2013). Warming reduces carbon losses from grassland exposed to elevated atmospheric carbon dioxide. *PLoS ONE* (in press).
- Pendall, E., Mosier, A. R., and Morgan, J. A. (2004). Rhizodeposition stimulated by elevated CO₂ in a semiarid grassland. *New Phytol.* 162, 447–458. doi: 10.1111/j.1469-8137.2004.01054.x
- Phillips, R. P., Finzi, A. C., and Bernhardt, E. S. (2011). Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO₂ fumigation. *Ecol. Lett.* 14, 187–194. doi: 10.1111/j.1461-0248.2010.01570.x
- Phillips, R. P., Meier, I. C., Bernhardt, E. S., Grandy, A. S., Wickings, K., and Finzi, A. C. (2012). Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO₂. *Ecol. Lett.* 15, 1042–1049. doi: 10.1111/j.1461-0248.2012.01827.x
- Raiesi, F., and Ghollarata, M. (2006). Interactions between phosphorus availability and an AM fungus (*Glomus intraradices*) and their effects on soil microbial respiration, biomass and enzyme activities in a calcareous soil. *Pedobiologia* 50, 413–425. doi: 10.1016/j.pedobi.2006.08.001
- Rasmussen, C., Southard, R. J., and Horwath, W. R. (2007). Soil mineralogy affects conifer forest soil carbon source utilization and microbial priming. *Soil Sci. Soc. Am. J.* 71, 1141–1150. doi: 10.2136/sssaj2006.0375
- Reich, P. B., Hobbie, S. E., Lee, T., Ellsworth, D. S., West, J. B., Tilman, D., et al. (2006). Nitrogen limitation constrains sustainability of ecosystem response to CO₂. *Nature* 440, 922–925. doi: 10.1038/nature04486
- Rukshana, F., Butterly, C. R., Baldock, J. A., Xu, J. M., and Tang, C. (2012). Model organic compounds differ in priming effects on alkalinity release in soils through carbon and nitrogen mineralisation. *Soil Biol. Biochem.* 51, 35–43. doi: 10.1016/j.soilbio.2012.03.022
- Sanyal, S. K., and Datta, S. K. (1991). “Chemistry of phosphorus transformations in soil,” in *Advances in Soil Science*, ed B. A. Stewart (New York, NY: Springer), 1–120.
- Schimel, J. P., and Weintraub, M. N. (2003). The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biol. Biochem.* 35, 549–563. doi: 10.1016/S0038-0717(03)00015-4
- Sullivan, B. W., and Hart, S. C. (2013). Evaluation of mechanisms controlling the priming of soil carbon along a substrate age gradient. *Soil Biol. Biochem.* 58, 293–301. doi: 10.1016/j.soilbio.2012.12.007
- Talbot, J. M., Allison, S. D., and Treseder, K. K. (2008). Decomposers in disguise: Mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. *Funct. Ecol.* 22, 955–963. doi: 10.1111/j.1365-2435.2008.01402.x
- Tunesi, S., Poggi, V., and Gessa, C. (1999). Phosphate adsorption and precipitation in calcareous soils: the role of calcium ions in solution and carbonate minerals. *Nutr. Cycl. Agroecosys.* 53, 219–227. doi: 10.1023/A:1009709005147
- Vitousek, P. M., and Howarth, R. W. (1991). Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13, 87–115. doi: 10.1007/BF00002772
- Walker, T. W., and Syers, J. K. (1976). The fate of phosphorus during pedogenesis. *Geoderma* 15, 1–19. doi: 10.1016/0016-7061(76)90066-5
- Zhang, W., Wang, X., and Wang, S. (2013). Addition of external organic carbon and native soil organic carbon decomposition: a meta-analysis. *PLoS ONE* 8:e54779. doi: 10.1371/journal.pone.0054779

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 29 April 2013; accepted: 10 July 2013; published online: 29 July 2013.

Citation: Dijkstra FA, Carrillo Y, Pendall E and Morgan JA (2013) Rhizosphere priming: a nutrient perspective. *Front. Microbiol.* 4:216. doi: 10.3389/fmicb.2013.00216

This article was submitted to *Frontiers in Terrestrial Microbiology*, a specialty of *Frontiers in Microbiology*.

Copyright © 2013 Dijkstra, Carrillo, Pendall and Morgan. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.



Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources

Maria Mooshammer¹, Wolfgang Wanek^{1*}, Sophie Zechmeister-Boltenstern² and Andreas Richter¹

¹ Terrestrial Ecosystem Research, Department of Microbiology and Ecosystem Science, University of Vienna, Vienna, Austria

² Institute of Soil Research, Department of Forest and Soil Sciences, University of Natural Resources and Life Sciences Vienna, Vienna, Austria

Edited by:

Johannes Rousk, Lund University, Sweden

Reviewed by:

Feike Auke Dijkstra, The University of Sydney, Australia

Mark Gessner, Institute of Freshwater Ecology and Inland Fisheries, Germany

*Correspondence:

Wolfgang Wanek, Terrestrial Ecosystem Research, Department of Microbiology and Ecosystem Science, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria
e-mail: wolfgang.wanek@univie.ac.at

Terrestrial microbial decomposer communities thrive on a wide range of organic matter types that rarely ever meet their elemental demands. In this review we synthesize the current state-of-the-art of microbial adaptations to resource stoichiometry, in order to gain a deeper understanding of the interactions between heterotrophic microbial communities and their chemical environment. The stoichiometric imbalance between microbial communities and their organic substrates generally decreases from wood to leaf litter and further to topsoil and subsoil organic matter. Microbial communities can respond to these imbalances in four ways: first, they adapt their biomass composition toward their resource in a non-homeostatic behavior. Such changes are, however, only moderate, and occur mainly because of changes in microbial community structure and less so due to cellular storage of elements in excess. Second, microbial communities can mobilize resources that meet their elemental demand by producing specific extracellular enzymes, which, in turn, is restricted by the C and N requirement for enzyme production itself. Third, microbes can regulate their element use efficiencies (ratio of element invested in growth over total element uptake), such that they release elements in excess depending on their demand (e.g., respiration and N mineralization). Fourth, diazotrophic bacteria and saprotrophic fungi may trigger the input of external N and P to decomposer communities. Theoretical considerations show that adjustments in element use efficiencies may be the most important mechanism by which microbes regulate their biomass stoichiometry. This review summarizes different views on how microbes cope with imbalanced supply of C, N and P, thereby providing a framework for integrating and linking microbial adaptation to resource imbalances to ecosystem scale fluxes across scales and ecosystems.

Keywords: Ecological stoichiometry, homeostasis, carbon/nutrient use efficiency, elemental imbalance, soil microbial communities, extracellular enzymes, mineralization, organic matter decomposition

INTRODUCTION

Soil microbial communities are key players in global biogeochemical cycles, regulating core ecosystem processes such as organic matter decomposition, soil C sequestration and nutrient recycling. Microbial decomposers release extracellular enzymes (EEs), which deconstruct plant macromolecules and ultimately liberate soluble substrates for microbial uptake (Schimel and Bennett, 2004). In turn, microbes use these substrates to fuel biomass production and EE synthesis (Moorhead et al., 2012). The amount of inorganic nutrients released into the ecosystem by mineralization depends on the relative C to the nutrient demand of the microorganisms, as well as the nutrient content of organic matter.

Microorganisms can be linked to these ecosystem processes through the theory of ecological stoichiometry, which has emerged as a powerful tool for studying the functioning of both aquatic and terrestrial ecosystems (Sterner and Elser, 2002). Soil is spatially and temporally heterogeneous, comprising chemically diverse compounds as well as harboring a vast diversity of microbes. Ecological stoichiometry uses elemental ratios and is thus a

simplification of natural complexity, which can explain ecological dynamics simply by acknowledging chemical constraints on the metabolic and physiologic functions of organisms. Stoichiometric invariance (homeostasis) of organisms is a central concept in ecological stoichiometry to predict nutrient retention and recycling, as well as biomass production, from subcellular to ecosystem scales (Sterner and Elser, 2002). Stoichiometric homeostasis is defined as the degree to which organisms maintain a constant chemical composition despite variations in the chemical composition and availability of their resources (Sterner and Elser, 2002). Strictly homeostatic organisms have invariable C:N:P ratios, where changes in resource stoichiometry have no influence on their stoichiometry, whereas non-homeostatic organisms vary their elemental composition in response to changes in resource composition.

On a global scale, the stoichiometry of soil microbial biomass has been shown to be more constrained in range and variance compared to its resource, which implies that microbes are largely homeostatic in terms of their biomass C:N:P (Cleveland

and Liptzin, 2007). Homeostatic regulation of microbial biomass composition constitutes the basis for the consumer-driven nutrient recycling theory (CNR; Sterner, 1990; Elser and Urabe, 1999; Sterner and Elser, 2002), according to which the elemental ratios of consumers and their resources determine the ratio of C:nutrient released through differential recycling of C and nutrients (N or P). This is of special interest in terrestrial ecosystems because microbial decomposers recycle C and N mainly as carbon dioxide and ammonium, respectively, contributing to soil respiration and soil N mineralization. Thus, the constraints on microbial growth and activity by the stoichiometric imbalance between microbial communities and their resource play a pivotal role in shaping ecosystem processes (Manzoni et al., 2010; Mooshammer et al., 2012), with the regulation of microbial homeostasis being an underlying, determining factor.

The key questions regarding stoichiometric imbalances, i.e., how soil microbes regulate their C:N:P homeostasis and how this in turn affects the processing of organic matter, are still insufficiently understood. The aim of this review is to synthesize the current knowledge on the mechanisms that allow terrestrial microbial communities to thrive in a stoichiometrically imbalanced world. First, we review the spatial and temporal variability of

resource stoichiometry and its effect on microbial biomass stoichiometry. Second, we present a mechanistic framework of how soil microbial communities cope with resource imbalances (**Figure 1**), including (i) plasticity of microbial biomass C:N:P, (ii) production of EEs as C and nutrient acquisition strategy, (iii) adjustments in microbial element use efficiencies, and (iv) input of external nutrients by N-fixing prokaryotes or by saprotrophic fungi.

STOICHIOMETRIC IMBALANCES BETWEEN RESOURCES AND SOIL MICROBIAL COMMUNITIES

For soil microbes the most important resources are plant necromass (wood, plant, and root litter), exudates and soil organic matter (SOM). Decomposable plant products (detritus, necromass, root exudates) exhibit much wider and more variable C:N:P ratios than microbes [see data compiled in **Table 1** and (Sistla and Schimel, 2012); all C:N:P ratios in this publication refer to molar ratios]. For instance, leaf litter C:N:P is globally constrained with a molar ratio of 3,007:45:1 (McGroddy et al., 2004), or 3,055:43:1, based on a larger dataset (Yuan and Chen, 2009). Leaf litter C:N:P, however, varies between ecosystems and biomes (McGroddy et al., 2004), and even more between plant species, life forms (e.g.,

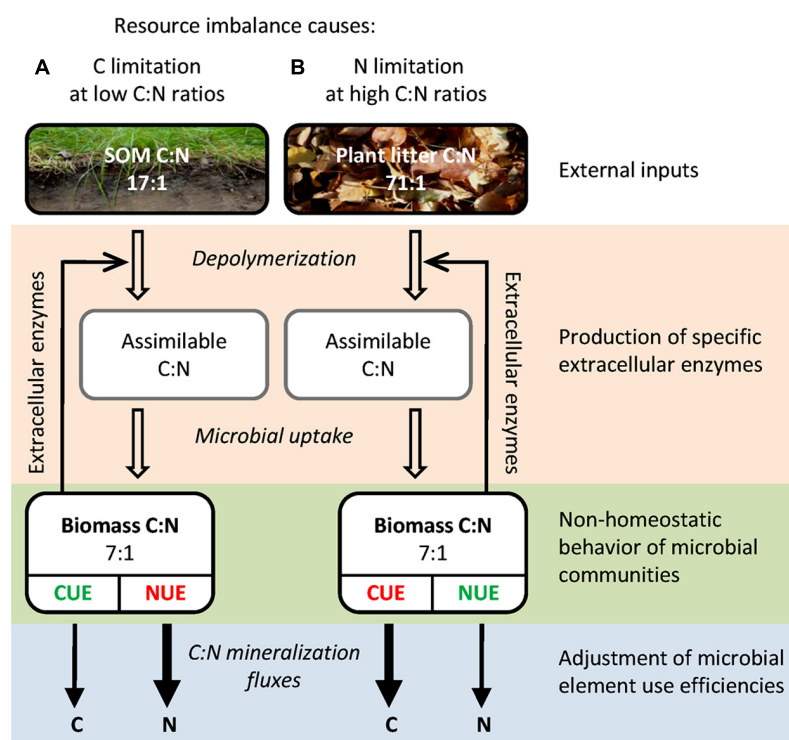


FIGURE 1 | Simplified schematic representation of important C:N components and fluxes during organic matter breakdown by litter or soil microbial communities. The differences in C:N:P stoichiometry between (A) soil organic matter and (B) plant litter result in distinct elemental limitations for the respective microbial communities, with different implications for element mineralization fluxes. Mechanisms for microbial adaptation to these resource imbalances are indicated: input of external nutrients by N-fixing prokaryotes or by saprotrophic fungi,

adjustment in the production of extracellular enzymes as C and nutrient acquisition strategy, non-homeostatic behavior of microbial communities and adjustment of microbial element use efficiencies. Given are global average molar C:N ratios (Yuan and Chen, 2009; Xu et al., 2013). The scheme is also applicable for P, by substitution of C:N ratios by C:P (plant litter, 3,055:1; SOM, 287:1; microbial biomass, 42:1; **Table 1**) as well as NUE by phosphorus use efficiency (PUE). Abbreviations: SOM, soil organic matter; CUE, carbon use efficiency; NUE, nitrogen use efficiency.

Table 1 | Globally averaged element ratios in potential resources and in soil microbial biomass, and stoichiometric imbalances between resources and microbes calculated as the ratio of C:N_{resource} (or C:P, N:P) over C:N_{microbes} (or C:P, N:P).

Organic material	Molar C:N:P	C:N imbalance	C:P imbalance	N:P imbalance	Reference
Wood	14,103 (±2,898):40 (±13):1	50	336	7	Harmon et al. (1986), Martinelli et al. (2000), Weedon et al. (2009)
Dead roots	4,184 (±991):43 (±4):1	14	100	7	Yuan et al. (2011)
Leaf litter	3,055 (±181):43 (±1):1	10	73	7	Yuan and Chen (2009)
Soil organic matter	287 (±25):17 (±1):1	2	7	3	Xu et al. (2013)
Soil microbes	42 (±4):6 (±0.4):1				Xu et al. (2013)

grasses, forbs, shrubs, deciduous or evergreen trees) and broader phylogenetic groups (i.e., angiosperms, gymnosperms; Yuan and Chen, 2009). Therefore, in diverse multispecies plant communities, one can expect a large variability of C:N:P of leaf litter inputs. Root litter also has wide C:N:P ratios, with 4,184:43:1, although it remains unclear if systematic differences between species, ecosystems and biomes exist due to limited available data (Yuan et al., 2011). Nevertheless, molar C:N:P ratios of living fine root biomass clearly differ between biomes and decline exponentially with latitude, similar to those of leaf litter (Yuan et al., 2011). Woody debris has even wider C:N:P ratios than root litter due to very low N and P concentrations, with C:N:P ratios of boles and other coarse woody debris of 14,103:40:1 (Table 1; Harmon et al., 1986; Martinelli et al., 2000; Weedon et al., 2009). Wood of gymnosperms typically has wider C:N:P ratios (15,980:28:1) than that of angiosperms (6,122:28:1; Weedon et al., 2009), and tropical wood has a higher N:P ratio (93:1) than wood from temperate and boreal plants (27:1).

Adding to this complexity, the availability and type of plant necromass varies seasonally (e.g., Bardgett et al., 2005). For instance, in temperate forests leaf litter fall occurs as a short pulse of a few weeks in autumn. In temperate ecosystems root turnover increases in late autumn and consequently root necromass becomes available for microbial decomposition in topsoil and subsoil (Drake et al., 2011). Root exudation also affects the availability of organic C relative to N and P. Root exudation peaks during the plant growing season (Phillips et al., 2011), changing the stoichiometry of available resources in the rhizosphere. Root exudates are thought to consist mainly of sugars and organic acids, with a minor contribution of amino acids (Marschner, 1995). Consequently, the C:N ratio of root exudates has been assumed to range between 50 and 100 (Drake et al., 2013). Although little empirical evidence exists, it is safe to suggest that C:N ratios of root exudates are much wider than those of SOM and microbial biomass. No data are currently available for root exudate P fluxes, but P concentration in exudates is presumably low. Our current knowledge of root exudate composition is notably limited and empirical data on exudate C:N:P fluxes are urgently needed to better define its effect on decomposition processes in soil.

Soil organic matter, which is the result of microbial decomposition of plant necromass, is another major resource available for heterotrophic microorganisms. Microbial residues play an important role in the formation of SOM, as plant resources are converted

to microbial biomass during decomposition, comprising a large proportion of SOM (e.g., Simpson et al., 2007; Miltner et al., 2009). Consequently, resource C:N:P ratios decline and the stoichiometric variability decreases with SOM processing. For instance, the C:N ratios of deeper soil horizons, which have been processed for longer, converge toward the C:N ratios of microbial biomass. Therefore, soils have narrower molar C:N:P ratios than plant litter, with a global soil average of 287:17:1 (Xu et al., 2013). Similar to other resources, SOM shows significant differences in C:N:P between ecosystem types and biomes (Xu et al., 2013). A meta-analysis of ~2400 soil profiles collected in China further showed that soil C:N, C:P, and N:P ratios decrease with soil depth (Tian et al., 2010a). Soil C:N ratios are significantly higher in organic soils than in mineral soils, increasing linearly with soil organic carbon (SOC) content (Table 2, dataset of Xu et al., 2013). Based on this relationship, a C:N ratio of 13.6 is predicted for soils with a SOC content of 1%, whereas a C:N ratio of 39.2 is predicted for high SOC soil (i.e., 50% SOC). Along the same line, soil C:P ratios are predicted to be 156 and 1,610 for low and high SOC soils, and soil N:P ratios to be 10.3 and 55.4, respectively (Table 2). Significant differences in soil C:N:P along the SOM decomposition continuum therefore become evident, despite soils being stoichiometrically more constrained than plant detritus. As a result of the spatio-temporal variability in the availability and type of plant detritus and SOM, terrestrial microbial communities must cope with large spatio-temporal variability in resource C:N:P ratios.

The range of biomass C, N, and P concentrations in soil microbial communities spans several orders of magnitude, although the linear and isometric relationships between these elements suggest a fixed, or at least a highly constrained, microbial C:N:P ratio (Cleveland and Liptzin, 2007; Hartman and Richardson, 2013). A meta-analysis of microbial biomass C:N:P showed remarkably constant molar ratios around 60:7:1 (Cleveland and Liptzin, 2007), and more recently, an area-weighted global soil microbial biomass C:N:P of 42:6:1 was reported based on a fourfold larger dataset (Xu et al., 2013). Although similarities in microbial elemental ratios among sites across large scales have been stressed by several authors, there is also evidence for some stoichiometric flexibility of microbial communities (Li et al., 2012; Fanin et al., 2013; Hartman and Richardson, 2013). Significant differences in microbial biomass elemental ratios were found between different ecosystems, e.g., forests and grasslands (Cleveland and Liptzin, 2007), and also between major biomes, as shown by wide microbial

Table 2 | Relationships between soil organic carbon (SOC) content, soil C:N:P and microbial biomass C:N:P.

Relationship	Equation	n	R	R ²	P	Estimate	
						1% SOC	50% SOC
Soil C:N vs. SOC (%)	Soil C:N = 13.1 + 0.523 × SOC	2135	0.577	0.333	<0.001	13.6	39.2
Soil C:P vs. SOC (%)	Soil C:P = 126 + 29.7 × SOC	528	0.650	0.423	<0.001	156	1611
Soil N:P vs. SOC (%)	Soil N:P = 9.42 + 0.920 × SOC	506	0.505	0.255	<0.001	10.3	55.4
Mic C:N vs. SOC (%)	Mic C:N = 8.13 + 0.041 × SOC	1108	0.076	0.005	0.012	8.2	10.2
Mic C:P vs. SOC (%)	Mic C:P = 64.2 + 0.730 × SOC	561	0.088	0.008	0.037	64.9	100.7
Mic N:P vs. SOC (%)	Mic N:P = 7.16 + 0.067 × SOC	440	0.084	0.007	0.079	7.2	10.5
Mic C:N vs. soil C:N	Mic C:N = 7.81 + 0.031 × soil C:N	1023	0.063	0.004	0.044	7.8	9.4
Mic C:N vs. soil C:N (log-log)			0.121	0.014	<0.001		
Mic C:P vs. soil C:P	Mic C:P = 66.5 + 0.015 × soil C:P	405	0.078	0.006	0.118	66.5	67.3
Mic C:P vs. soil C:P (log-log)			0.000	0.000	0.992		
Mic N:P vs. soil N:P	Mic N:P = 6.81 + 0.046 × soil N:P	294	0.102	0.010	0.081	6.9	9.1
Mic N:P vs. soil N:P (log-log)			−0.097	0.009	0.091		

The respective equations are used to estimate element ratios for low-C soils (1% soil organic carbon, SOC) and high-C soils (50% SOC). Data from Xu et al. (2013).

biomass C:N:P ratios in natural wetlands and tundra, in contrast to the narrow C:N:P ratios in boreal forests, croplands, pastures and deserts (Xu et al., 2013). Microbial C:N ranged between 4.5 and 12.5 (95% confidence boundaries for major biomes), microbial C:P between 24 and 275, and microbial N:P between 3.5 and 10.6 (excluding wetlands, Xu et al., 2013). In another meta-analysis, Hartman and Richardson (2013) also reported significant variations in microbial N:P ratios among sites with different vegetation and land use types, across soils and litter layers, and suggested that these differences might be linked to variation in size-dependent scaling relationships of biomass C:N and C:P (i.e., slight increases in the proportions of N and P with increasing soil microbial biomass C pools).

Biome- and ecosystem-level differences in microbial C:N:P ratios may be explained by co-variation with resource stoichiometry, or by soil type, soil pH, soil C content and/or latitude (mean annual temperature). Xu et al. (2013) found significant negative correlations between latitude and microbial C:N, N:P, and C:P, although low correlation coefficients indicate a weak effect of mean annual temperature on microbial stoichiometry. Soil pH was not found to affect microbial C:N:P ratios (Cleveland and Liptzin, 2007; Hartman and Richardson, 2013; Xu et al., 2013). Interestingly a re-analysis of the existing global dataset of Xu et al. (2013), presented in **Table 2**, shows that microbial biomass C:N and C:P, but not N:P, are positively correlated with SOC content. This points to indirect control of SOC content on microbial stoichiometry, i.e., soil C:N and C:P ratios increase with SOC content, and microbial C:N and C:P as well. Moreover, this pattern indicates that in soil with high SOC content and high C:N (C:P), N or P may become limiting while C availability is high, thereby increasing microbial biomass C:N:P. On a global scale, microbial C:N:P ratios were not (Cleveland and Liptzin, 2007; Hartman and Richardson, 2013), or only weakly, positively related to the respective soil

element ratios (Xu et al., 2013; **Table 2**), indicating a large degree of stoichiometric homeostasis of soil microbial communities. Xu et al. (2013) reported a positive relationship between resource C:N and microbial biomass C:N (**Table 2**), though the relationship between resource C:N and microbial C:N is strongly dampened in relation to the variability in resource elemental ratios. For example, a resource C:N ratio of 10 would predict a microbial biomass C:N of 8.1, but a 10-fold higher resource C:N of 100 would only lead to a 1.3-fold increase in microbial biomass C:N to 10.9.

As a consequence of the relatively low variation in microbial biomass C:N:P, large C:N:P imbalances arise between different types of decomposable organic matter and decomposer communities (**Table 1**). We estimated the average global C:N imbalance – calculated as resource C:N divided by microbial biomass C:N – ranging from 2 (SOM) to values between 10 and 14 (leaf litter, dead roots), and up to 50 (wood). The C:P imbalances are higher than the C:N imbalances, ranging from 7 (SOM), to 73 (litter), 100 (root litter) and 336 (wood). Smallest imbalances are found for N:P, ranging from 3 in SOM to 7 in plant detritus (leaf litter, dead roots, and wood). These patterns in resource stoichiometry raise the question of how soil microbial communities cope with such large elemental imbalances.

ADAPTATIVE MECHANISMS OF SOIL MICROBIAL COMMUNITIES TO STOICHIOMETRIC IMBALANCES

There are four main mechanisms for microbial decomposers to cope with elemental imbalances and thrive on substrates that do not meet their elemental demand for growth (**Figure 1**): First, microbes can adjust their biomass C:N:P ratios to meet the elemental composition of their substrates; second, they change the elemental composition of their immediate substrates by producing EEs that preferentially deconstruct polymers that meet their demand for C and nutrients; third, after substrate uptake, they

mineralize and excrete the elements in excess of their demand by regulating their element use efficiencies; and fourth, diazotrophic bacteria and saprotrophic fungi may trigger the input of external N and P to decomposer communities. Below, we review these four mechanisms in more detail:

(1) *Non-homeostatic behavior of microbes reduces the stoichiometric imbalance to their resource.* As demonstrated above, small adjustments of microbial biomass C:N:P ratios to resource C:N:P do indeed occur, and may help as an adaptive mechanism of microbial communities to stoichiometric imbalances. Adjustment of microbial biomass C:N:P to resource C:N:P ratios can occur due to two main mechanisms: first, microbial storage of elements in excess, leading to a convergence between the biomass and resource stoichiometries, and second, shifts in microbial community structure and concomitant shifts in biomass stoichiometry. Whereas the first mechanism would be a physiological adjustment of the stoichiometry of microorganisms, requiring that microbes are non-homeostatic, the latter is not a true adjustment, as a community change may have very different reasons.

Despite the lack of information regarding changes in microbial C, N, or P storage, analyses of cultured organisms have shown that P storage in polyphosphates by bacteria and fungi is related to increased P availability (Kornberg, 1995; Achbergerova and Nahalka, 2011), and C storage, for example, in lipids (triacylglycerols, TAG, and poly- β -hydroxyalkanoates, PHA) and glucans (such as glycogen), can increase with C availability (Wilkinson, 1963; Lee, 1996; Wilson et al., 2010). However, glucan and PHA storage usually does not exceed a few percent (up to a maximum of 20%) of biomass. Unlike C and P, N has no specific storage pool beside small, intermittent accumulation of essential amino acids, and no significant N storage in microbes has been reported to date (e.g., Banham and Whatley, 1991). It rather seems that N is either directly incorporated into the biomass, or mineralized and excreted. Therefore, C:N ratios are not expected to change markedly due to storage of excess C or N. Under P-limited conditions, microbes are thought to be relatively homeostatic in regard to C:P and N:P ratios (Makino et al., 2003). However, it has recently been shown in decomposition experiments with tropical leaf litter (at low P availability) that microbial N:P ratios followed that of the soluble litter fraction (Fanin et al., 2013). When P is available in excess, microbes can become strongly non-homeostatic (Scott et al., 2012). In such case, luxury P consumption and cellular P storage in the form of polyphosphates has been reported (Kornberg, 1995). For example, accumulated polyphosphate can comprise up to 10–20% of yeast cells' dry mass, thereby strongly affecting their C:N:P stoichiometry. However, whether P supply in excess of microbial demand really exists in natural terrestrial ecosystems remains unclear.

On the other hand, the stoichiometric plasticity of microbial communities may be generated by shifts in the dominance of strains of distinct stoichiometry. Bacteria in general exhibit lower C:N ratios than fungi (Strickland and Rousk, 2010), and fast-growing microbes (copiotrophs, r-strategists) have been suggested to exhibit lower biomass C:N:P ratios (higher nutrient requirements) than slow-growing ones (oligotrophs, K-strategists; Elser et al., 2003; Fierer et al., 2007). Therefore, changes in fungal: bacterial ratios and shifts in the dominance of r- or K-strategists

are expected to result in concomitant shifts in microbial biomass C:N:P ratios. Moreover, fast- and slow-growing microorganisms do not only exhibit distinct biomass stoichiometries, but also the requirement for stoichiometric homeostasis might vary with their growth rates, with tight requirements for fast-growing and relaxed requirements for slow-growing microorganisms (Egli, 1995; Fierer et al., 2007). Recently, Fanin et al. (2013) showed that a non-homeostatic behavior of microbial biomass was due to shifts in the community composition rather than due to stoichiometric flexibility of the same community. It is well known that fungal: bacterial ratios decrease from litter and organic soils toward mineral soils (Maassen et al., 2006; Moore et al., 2010; Lee et al., 2013). For example, since both fungal: bacterial ratios and the C:N ratio of SOM decrease with soil depth, a positive correlation of microbial biomass C:N with resource C:N is expected. However, it remains unclear whether such associations between microbial biomass stoichiometry and community composition reflect a causal effect, and to what extent, or are merely coincidental due to adaptation to resource chemistry. Assessing the effect of microbial community composition on overall microbial biomass stoichiometry will therefore require more direct approaches, such as *in situ* measurements of single-cell C:N:P ratios in soils by techniques such as X-ray microanalysis or NanoSIMS coupled with phylogenetic classification at broad scale (bacteria, fungi) or fine scale (fluorescence *in situ* hybridization; Hall et al., 2011).

(2) *Microbes adjust their EE production, to maximize the mobilization of substrates rich in the limiting element.* Microbial regulation of EE production is complex, including constitutive secretion of low levels of EE (most likely to detect suitable substrates), induction of EE synthesis in response to increasing availability of complex substrates, and feedback inhibition of EE activity by their products (Wallenstein and Weintraub, 2008; Burns et al., 2013). Many EEs in soils become stabilized through association with clay minerals, humic acids and particulate organic matter, often leading to lower activities and greater residence times in soils, but such immobilized enzymes may also serve as a first sensor communicating changes in substrate availability to microbes (Burns et al., 2013). Another factor that complicates the EE response of microbes lies in the fact that enzyme production itself requires an investment of N and C, which can further increase elemental limitation (Schimel and Weintraub, 2003). This may be especially true for N, as the C:N ratio of proteins is usually much lower than microbial biomass C:N. Thus, under strong N limitation, excretion of EE to mobilize N-containing substrates may not be an adequate strategy for microbes to regulate their N homeostasis, unless the benefit of enzyme production (i.e., N released from litter or SOM through EE) outweighs the costs involved (Schimel and Weintraub, 2003). Given that EE production involves no direct P investment we do not expect similar patterns for P acquiring enzymes.

On a global scale, the stoichiometry of EE was shown to be strongly constrained, with a mean C:N:P ratio near 1:1:1 using log-transformed potential activities of hydrolytic C, N, and P acquiring enzymes (Sinsabaugh et al., 2008, 2009). However, significant variations in the stoichiometry of soil EE were found at the ecosystem scale (Sinsabaugh et al., 2008). In terms of microbial C:N:P homeostasis, we would indeed expect that EE activities are not stoichiometrically invariant to resource elemental ratios,

because according to resource allocation theory microbes invest abundant elements into EE production mining for scarce elements (Allison et al., 2011). There is evidence that increasing availability of N stimulates C mobilizing enzymes (Allison and Vitousek, 2005; Geisseler and Horwath, 2009) and causes enhanced mobilization of P by enhancing phosphatase activity, i.e., an effective investment of N to mine for P in order to keep elemental balance (Olander and Vitousek, 2000; Marklein and Houlton, 2012; Mooshammer et al., 2012). In contrast, P availability did not affect N acquiring enzymes (Olander and Vitousek, 2000). In some cases, the input of labile C, which can induce N limitation in microbes, did enhance the activity of N acquiring enzymes although in other cases it did not (Allison and Vitousek, 2005; Geisseler and Horwath, 2009; Hernandez and Hobbie, 2010), which possibly depends on the overall N limitation of the microbial community.

Input of labile C was also shown to make N accessible for microbes by increased production of oxidative enzymes, resulting in enhanced SOM decomposition (“rhizosphere priming effect” or “nitrogen mining”) (Craine et al., 2007; Blagodatskaya and Kuzyakov, 2008; Sinsabaugh, 2010). In contrast to this release of N through oxidation of SOM, much of the organic P in soils is present in monoesters and diesters, which is released by hydrolysis through the activity of phosphatases (McGill and Cole, 1981; Vitousek and Howarth, 1991). Therefore, rhizosphere priming may not occur in systems that are P limited, as rhizodeposition may be utilized to mobilize P from organic and inorganic sources (through dissolution/desorption and hydrolysis, respectively), rather than for decomposition of SOM (Dijkstra et al., 2013). Overall, the available data indicate that the regulation of hydrolytic and oxidative EEs can bring nutrient and C supply closer to microbial element demand, although the general validity and relative role of this mechanism must yet be demonstrated.

If microbes indeed reduce the elemental imbalance between bulk substrate (soil or litter) and their biomass composition through the release of EE, we would expect that the C:N:P ratio of dissolved organic matter (DOM) is closer to the stoichiometry of microbial biomass than the bulk resource. However, C:N ratios of DOM (DOC:DON) in soil water and leachates are often higher than C:N ratios of SOM and are only weakly, or not significantly, related across sites (Neff et al., 2000; Wu et al., 2010; Haney et al., 2012). Soil microbial activity was nonetheless more strongly related to water-soluble organic C:N than to soil C:N in arable fields (Haney et al., 2012), which highlights that water-soluble C:N represents a more sensitive measure of the soil substrate driving microbial activity. Moreover, the bioavailability of DOM, or extractable organic matter, can be reduced (i.e., the interaction between microorganisms and DOM is restricted) by physical (e.g., inaccessibility of DOM) and chemical (e.g., DOM sorption to solid surfaces) restrictions (Marschner and Kalbitz, 2003), thus causing great differences in DOM availability between soils and soil microhabitats. For example, DOM bioavailability was found to range between 10 and 20% in organic soils (Kiikkilä et al., 2005), and reached 80 to 90% in agricultural (mineral) soils (Tian et al., 2010b). Bulk measurements of C:N:P in DOM therefore also do not represent the immediate source of elements for microbes.

The composition of DOM is not only a result of the input, but is also affected by uptake through the soil microbial community, which is a virtually unexplored field given the methodological difficulties to assess the immediate substrates for microbial uptake. An inverse approach that may hold promise to dissect the elemental composition of the resource used *in situ* by soil microbes is to infer microbial C:N:P uptake from measured C:N:P ratios of mineralization fluxes, microbial biomass and bulk soil. However, this approach relies strongly on knowledge about microbial element use efficiencies (Murphy et al., 2003; Herrmann and Witter, 2008), and these are hardly ever measured for N and P (see below). An alternative approach is to determine the pools, and gross production and consumption rates of major low-molecular weight compounds that serve as the immediate substrates for heterotrophic soil microbes, including sugars, organic acids, amino acids, amino sugars, and organic phosphates, and thereby estimate the C:N:P ratios of substrates taken up by soil microbial communities.

(3) *Microbes excrete elements that are present in excess in their resource compared to their biomass composition by adjusting their element use efficiencies.* If microbes cannot change the elemental composition of their immediate substrates or adjust their biomass composition accordingly, they could take up whatever substrate is available and release elements in excess of their requirement, while keeping those in short supply for growth. Microbes are able to achieve this balance by regulating their element use efficiencies, such as the carbon use efficiency (CUE, sometimes also called growth yield or gross growth efficiency). Microbial CUE is defined as the ratio of C invested in growth (new biomass production) over total C taken up (Del Giorgio and Cole, 1998; Manzoni et al., 2012).

The considerable differences in C:N:P stoichiometry between plant detritus (litter) and SOM result in distinct elemental limitations for the respective microbial communities (**Table 1, Figure 1**). Progressively lower C:N and C:P ratios from litter to topsoil, and further to subsoil, correspond also to a decreasing C availability in relation to N and P (increasing C limitation). In order to cope with such differences in resource elemental composition, microbes are expected to adjust their element use efficiencies accordingly. For example, assuming strict homeostasis and negligible adjustment of EE, when microbes with a biomass C:N of 7 (global average, **Table 1**) decompose plant litter with a relatively high C:N ratio of 70, they must release 63 units of C as CO₂ per unit of N invested in growth, yielding a low CUE of about 0.1. By contrast, if SOM with a C:N close to that of the microbial biomass would be decomposed (e.g., SOM C:N of 12 and biomass C:N of 7.2), CUE would converge toward the theoretical maximum of about 0.6 (Sinsabaugh et al., 2013). CUE cannot reach the maximum of 1, given that a significant amount of C taken up is required to produce energy for growth, maintenance and enzyme production (Schimel and Weintraub, 2003; Manzoni et al., 2012; Sinsabaugh et al., 2013). In this situation, when C becomes limiting and CUE cannot be further increased, microbes need to lower their nitrogen use efficiency (NUE), i.e., excrete N in excess (Manzoni and Porporato, 2009). This transition from net nutrient immobilization to net nutrient mineralization (critical C:N ratio) corresponds to the threshold elemental ratio (TER), which defines the transition of an ecological

system from being controlled by a limiting nutrient (N or P) to being controlled by energy (C; e.g., Urabe and Watanabe, 1992; Anderson and Hessen, 1995; Frost et al., 2006). Thus, a certain nutrient becomes limiting for growth, when resource C:nutrient ratios are greater than TER.

Whereas the stoichiometric regulation of CUE has been recently reviewed in detail (Manzoni et al., 2012; Sinsabaugh et al., 2013), very little is known about regulation of microbial NUE, and even less about microbial phosphorus use efficiency (PUE) in soils. From theoretical considerations, we can postulate that, in contrast to CUE, NUE can approach the theoretical maximum of 1 if all organic N taken up is used for growth. In addition, NUE can be regulated independently from CUE, allowing microbial communities to adjust to resources with low C:N, which leads to C limitation and consequently N excess. It remains to be seen, however, if microbial NUE is also regulated at high substrate C:N ratios where microbial N limitation is expected. In such a case, microorganisms could respond to stoichiometrically unbalanced substrates by concurrent fine-tuning of CUE, NUE, and PUE, depending on the limiting element. In conclusion, the regulation of element use efficiencies is likely an important microbial strategy to cope with variations in resource stoichiometry.

Adaptations in microbial element use efficiencies have also a potentially great impact on the elemental ratios of major biogeochemical fluxes (e.g., the ratio of heterotrophic respiration to microbial N mineralization), with broad implications for soil C sequestration and N losses from terrestrial ecosystems. This means that microbial homeostasis achieved through adaptations in microbial element use efficiencies is expected to cause a strong positive relationship between resource C:N:P and mineralization flux C:N:P (CNR theory; Sterner and Elser, 2002), as the limiting elements are retained and incorporated into microbial biomass, and those in excess are excreted. In contrast, both non-homeostatic regulation of microbial biomass stoichiometry and compensatory EE regulation are expected to cause reductions in microbial element imbalances. Thus, if such a mechanism dominates, the ratios of element mineralization fluxes should not, or only slightly, increase with resource C:N:P, except when those mechanisms cannot compensate for the resource imbalance, especially with non-homeostatic regulation of microbial biomass stoichiometry at wide resource C:N:P ratios. However, the relationship between the stoichiometry of resource and mineralization fluxes as predicted by CNR theory has not yet been explicitly tested for terrestrial microbial communities.

There are few reports showing that C:N:P ratios of mineralization fluxes are strongly positively related to resource C:N:P. For instance, in decomposing litter, resource C:N and C:P were strongly negatively correlated with the respective gross N mineralization and gross P mineralization fluxes (but less so with respiration), suggestive of increasing microbial NUE and PUE at high resource C:N and C:P ratios while microbial communities were homeostatic with respect to these element ratios (Mooshammer et al., 2012). Moreover, in forest soils, Achat et al. (2010) demonstrated a similar relationship, with relatively constant microbial biomass C:P, while C:P mineralization fluxes were highly variable and strongly positively related to resource C:P [data recalculated

from Achat et al. (2010)]. Both studies therefore point to the importance of regulation of microbial NUE and PUE as an adaptation to stoichiometric imbalances. Concurrent measurements of the C:N:P ratios of resources, microbial biomass, EE activities and mineralization fluxes would therefore allow deeper insights into the mechanisms used by soil microorganisms to adapt to elemental imbalances in their resources, and to constrain the most important mechanisms and their environmental controls. Addressing these questions will require advances in the measurement of CUE, NUE, and PUE in soil microbial communities.

(4) *Nitrogen-fixing prokaryotes and saprotrophic fungi increase the N and P availability by inputs from external sources.* There are two major pathways for input of N or P to the decomposing material from external sources, (i) N fixation by prokaryotes and (ii) fungal transfer of N or P from nutrient-rich patches. Prokaryotic N fixers (diazotrophs) convert atmospheric N₂ to ammonia, a highly energy demanding process that is under strict physiological control (Raymond et al., 2004). Amongst other controls, biological N fixation is stimulated by elevated concentrations of labile organic C and P and feedback inhibited by high concentrations of ammonium and amino acids (Reed et al., 2011). Accordingly N fixation by free-living microbes is distributed heterogeneously, with hotspots of N fixation in habitats with high C and low N availability, i.e., in woody debris, leaf litter and the forest floor, and lowest rates in mineral soils (Hope and Li, 1997; Wei and Kimmins, 1998; Reed et al., 2007; Cusack et al., 2009). Moreover, release of C-rich exudates by roots also causes increased diazotroph abundances in the plant rhizosphere compared to bulk soils (Hamelin et al., 2002; Bürgmann et al., 2005). Through the release of ammonium and amino acids and upon death and lysis of diazotrophs, fixed N becomes available to other decomposers. However, though globally important amounts of N are fixed by free-living diazotrophs (Wang and Houlton, 2009), their importance to lower the C:N imbalance between resources and decomposer communities in litter has yet to be demonstrated. Diazotrophs represent only a small fraction of the decomposer community (Reed et al., 2010; Jung et al., 2012; Ducey et al., 2013) and N fixation rates are therefore orders of magnitude lower than microbial respiration rates in woody debris, leaf litter, forest floor, and mineral soils (Hope and Li, 1997; Hicks et al., 2003). Therefore the community level impact of N subsidy by diazotrophs to meet substrate C:N imbalances can be expected to be small.

Though for P there is no analogous process to N fixation, fungal mycelia may relocate P in addition to N from other sources to supplement bacterial decomposer communities at sites where these elements are scarce. Hyphae of fungal saprophytes have been shown to often extend well beyond the resource that they decompose (Strickland and Rousk, 2010) and have been demonstrated to mediate nutrient import from nutrient-rich patches into nutrient-poor habitats, e.g., from soil into decomposing litter (Osono et al., 2003; Chigineva et al., 2011) or from nutrient-rich to nutrient-poor litter (Schimel and Hättenschwiler, 2007). In addition, they may mediate reciprocal transfer of C and N between soil and litter, relocating C from litter to soil and N from soil to litter (Frey et al., 2003). Fungi thereby can significantly contribute to close the stoichiometric imbalance between resources and decomposer communities.

CONCLUSION

In this review, we demonstrate that terrestrial microbial communities have to cope with a large spatio-temporal variability in resource stoichiometry, which can result in strong imbalances between resource composition and elemental demands by soil microbes. We highlight four major mechanisms that allow microbial communities to adapt to these environmental constraints (**Figure 1**): (i) plasticity of microbial biomass C:N:P, (ii) compensatory regulation of EE production as C and nutrient acquisition strategy, (iii) adjustments in microbial element use efficiencies and (iv) input of external nutrients by diazotrophic bacteria or saprotrophic fungi. Although the four described mechanisms clearly operate in parallel, their contributions on spatial, temporal and ecosystem scales remain underexplored. The wealth of measurements of microbial biomass C:N:P ratios sets limits to the non-homeostatic behavior of soil microbial communities. Growth of terrestrial decomposer communities on many types of resources with either very wide C:nutrient ratios (e.g., wood) or very low C:nutrient ratios (e.g., deep SOM) cannot be achieved solely through non-homeostatic behavior. Therefore, adaptation of microbial element use efficiencies and compensatory regulation of EE production are expected to contribute significantly to the adaptation of microorganisms to chemically diverse environments, together with external inputs of nutrients mediated by subgroups of the microbial community.

ACKNOWLEDGMENTS

We thank Xiaofeng Xu for providing his database on soil and microbial biomass stoichiometry, Ricardo J. E. Alves for helpful comments on the manuscript and Jörg Schneckner for providing the picture of a soil profile. This manuscript is an outcome of the MICDIF integrated project (Linking microbial diversity and function across scales and ecosystems) funded by the Austrian Science Fund FWF (S 10006-B01, S 10006-B06, S 10006-B07).

REFERENCES

- Achat, D. L., Bakker, M. R., Zeller, B., Pellerin, S., Benaïme, S., and Morel, C. (2010). Long-term organic phosphorus mineralization in Spodosols under forests and its relation to carbon and nitrogen mineralization. *Soil Biol. Biochem.* 42, 1479–1490. doi: 10.1016/j.soilbio.2010.05.020
- Achbergerova, L., and Nahalka, J. (2011). Polyphosphate – an ancient energy source and active metabolic regulator. *Microb. Cell Fact.* 10, 63. doi: 10.1186/1475-2859-10-63
- Allison, S. D., and Vitousek, P. M. (2005). Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biol. Biochem.* 37, 937–944. doi: 10.1016/j.soilbio.2004.09.014
- Allison, S. D., Weintraub, M. N., Gartner, T. B., and Waldrop, M. P. (2011). “Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function,” in *Soil Enzymology*, eds G. Shukla and A. Varma (Berlin Heidelberg: Springer-Verlag), 229–243.
- Anderson, T. R., and Hessen, D. O. (1995). Carbon or nitrogen limitation in marine copepods? *J. Plankton Res.* 17, 317–331. doi: 10.1093/plankt/17.2.317
- Banham, A. H., and Whatley, F. R. (1991). Lack of nitrogen storage by *Paracoccus denitrificans*. *Proc. R. Soc. B Biol. Sci.* 245, 211–214. doi: 10.1098/rspb.1991.0111
- Bardgett, R. D., Bowman, W. D., Kaufmann, R., and Schmidt, S. K. (2005). A temporal approach to linking aboveground and belowground ecology. *Trends Ecol. Evol.* 20, 634–641. doi: 10.1016/j.tree.2005.08.005
- Blagodatskaya, E., and Kuzyakov, Y. (2008). Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biol. Fertil. Soils* 45, 115–131. doi: 10.1007/s00374-008-0334-y
- Bürgmann, H., Meier, S., Bunge, M., Widmer, F., and Zeyer, J. (2005). Effects of model root exudates on structure and activity of a soil diazotroph community. *Environ. Microbiol.* 7, 1711–1724. doi: 10.1111/j.1462-2920.2005.00818.x
- Burns, R. G., Deforest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallenstein, M. D., et al. (2013). Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biol. Biochem.* 58, 216–234. doi: 10.1016/j.soilbio.2012.11.009
- Chigineva, N. I., Aleksandrova, A. V., Marhan, S., Kandeler, E., and Tiunov, A. V. (2011). The importance of mycelial connection at the soil-litter interface for nutrient translocation, enzyme activity and litter decomposition. *Appl. Soil Ecol.* 51, 35–41. doi: 10.1016/j.apsoil.2011.08.009
- Cleveland, C. C., and Liptzin, D. (2007). C:N:P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry* 85, 235–252. doi: 10.1007/s10533-007-9132-0
- Craine, J. M., Morrow, C., and Fierer, N. (2007). Microbial nitrogen limitation increases decomposition. *Ecology* 88, 2105–2113. doi: 10.1890/06-1847.1
- Cusack, D. F., Silver, W., and McDowell, W. H. (2009). Biological nitrogen fixation in two tropical forests: ecosystem-level patterns and effects of nitrogen fertilization. *Ecosystems* 12, 1299–1315. doi: 10.1007/s10021-009-9290-0
- Del Giorgio, P. A., and Cole, J. J. (1998). Bacterial growth efficiency in natural aquatic systems. *Annu. Rev. Ecol. Syst.* 29, 503–541. doi: 10.1146/annurev.ecolsys.29.1.503
- Dijkstra, F. A., Carrillo, Y., Pendall, E., and Morgan, J. A. (2013). Rhizosphere priming: a nutrient perspective. *Front. Microbiol.* 4:216. doi: 10.3389/fmicb.2013.00216
- Drake, J. E., Darby, B. A., Giasson, M. A., Kramer, M. A., Phillips, R. P., and Finzi, A. C. (2013). Stoichiometry constrains microbial response to root exudation: insights from a model and a field experiment in a temperate forest. *Biogeosciences* 10, 821–838. doi: 10.5194/bg-10-821-2013
- Drake, J. E., Gallet-Budynek, A., Hofmockel, K. S., Bernhardt, E. S., Billings, S. A., Jackson, R. B., et al. (2011). Increases in the flux of carbon below-ground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO₂. *Ecol. Lett.* 14, 349–357. doi: 10.1111/j.1461-0248.2011.01593.x
- Ducey, T. F., Ippolito, J. A., Cantrell, K. B., Novak, J. M., and Lentz, R. D. (2013). Addition of activated switchgrass biochar to an arid subsoil increases microbial nitrogen cycling gene abundances. *Appl. Soil Ecol.* 65, 65–72. doi: 10.1016/j.apsoil.2013.01.006
- Egli, T. (1995). The ecological and physiological significance of the growth of heterotrophic microorganisms with mixtures of substrates. *Adv. Microb. Ecol.* 14, 305.
- Elser, J. J., Acharya, K., Kyle, M., Cotner, J., Makino, W., Markow, T., et al. (2003). Growth rate-stoichiometry couplings in diverse biota. *Ecol. Lett.* 6, 936–943. doi: 10.1046/j.1461-0248.2003.00518.x
- Elser, J. J., and Urabe, J. (1999). The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. *Ecology* 80, 735–751. doi: 10.1890/0012-9658(1999)080[0735:TSOCDN]2.0.CO;2
- Fanin, N., Fromin, N., Buatois, B., and Häntenschwiler, S. (2013). An experimental test of the hypothesis of non-homeostatic consumer stoichiometry in a plant litter-microbe system. *Ecol. Lett.* 16, 764–772. doi: 10.1111/ele.12108
- Fierer, N., Bradford, M. A., and Jackson, R. B. (2007). Toward an ecological classification of soil bacteria. *Ecology* 88, 1354–1364. doi: 10.1890/05-1839
- Frey, S. D., Six, J., and Elliott, E. T. (2003). Reciprocal transfer of carbon and nitrogen by decomposer fungi at the soil-litter interface. *Soil Biol. Biochem.* 35, 1001–1004. doi: 10.1016/S0038-0717(03)00155-X
- Frost, P. C., Benstead, J. P., Cross, W. F., Hillebrand, H., Larson, J. H., Xenopoulos, M. A., et al. (2006). Threshold elemental ratios of carbon and phosphorus in aquatic consumers. *Ecol. Lett.* 9, 774–779. doi: 10.1111/j.1461-0248.2006.00919.x
- Geisseler, D., and Horwath, W. R. (2009). Relationship between carbon and nitrogen availability and extracellular enzyme activities in soil. *Pedobiologia* 53, 87–98. doi: 10.1016/j.pedobi.2009.06.002
- Hall, E. K., Maixner, F., Franklin, O., Daims, H., Richter, A., and Battin, T. (2011). Linking microbial and ecosystem ecology using ecological stoichiometry: a synthesis of conceptual and empirical approaches. *Ecosystems* 14, 261–273. doi: 10.1007/s10021-010-9408-4
- Hamelin, J., Fromin, N., Tarnawski, S., Teyssier-Cuvellé, S., and Aragno, M. (2002). nifH gene diversity in the bacterial community associated with the rhizosphere

- of *Molinia coerulea*, an oligonitrophilic perennial grass. *Environ. Microbiol.* 4, 477–481. doi: 10.1046/j.1462-2920.2002.00319.x
- Haney, R. L., Franzluebbbers, A. J., Jin, V. L., Johnson, M.-V., Haney, E. B., White, M. J., et al. (2012). Soil organic C:N vs. water-extractable organic C:N. *Open J. Sci.* 2, 269–274. doi: 10.4236/ojss.2012.23032
- Harmon, M. E., Franklin, J. F., Swanson, F. J., Sollins, P., Gregory, S. V., Lattin, J. D., et al. (1986). Ecology of coarse woody debris in temperate ecosystems. *Adv. Ecol. Res.* 15, 133–302. doi: 10.1016/S0065-2504(08)60121-X
- Hartman, W. H., and Richardson, C. J. (2013). Differential nutrient limitation of soil microbial biomass and metabolic quotients ($q\text{CO}_2$): is there a biological stoichiometry of soil microbes? *PLoS ONE* 8:e57127. doi: 10.1371/journal.pone.0057127
- Hernandez, D. L., and Hobbie, S. E. (2010). The effects of substrate composition, quantity, and diversity on microbial activity. *Plant Soil* 335, 397–411. doi: 10.1007/s11104-010-0428-9
- Herrmann, A. M., and Witter, E. (2008). Predictors of gross N mineralization and immobilization during decomposition of stabilized organic matter in agricultural soil. *Eur. J. Soil Sci.* 59, 653–664. doi: 10.1111/j.1365-2389.2008.01023.x
- Hicks, W. T., Harmon, M. E., and Myrold, D. D. (2003). Substrate controls on nitrogen fixation and respiration in woody debris from the Pacific Northwest, USA. *For. Ecol. Manage.* 176, 25–35. doi: 10.1016/S0378-1127(02)00229-3
- Hope, S. M., and Li, C. Y. (1997). Respiration, nitrogen fixation, and mineralizable nitrogen spatial and temporal patterns within two Oregon Douglas-fir stands. *Can. J. For. Res.* 27, 501–509.
- Jung, J., Yeom, J., Han, J., Kim, J., and Park, W. (2012). Seasonal changes in nitrogen-cycle gene abundances and in bacterial communities in acidic forest soils. *J. Microbiol.* 50, 365–373. doi: 10.1007/s12275-012-1465-2
- Kiikkilä, O., Kitunen, V., and Smolander, A. (2005). Degradability of dissolved soil organic carbon and nitrogen in relation to tree species. *FEMS Microbiol. Ecol.* 53, 33–40. doi: 10.1016/j.femsec.2004.08.011
- Kornberg, A. (1995). Inorganic polyphosphate – toward making a forgotten polymer unforgettable. *J. Bacteriol.* 177, 491–496.
- Lee, S. H., Jang, L., Chae, N., Choi, T., and Kang, H. (2013). Organic layer serves as a hotspot of microbial activity and abundance in arctic tundra soils. *Microb. Ecol.* 65, 405–414. doi: 10.1007/s00248-012-0125-8
- Lee, S. Y. (1996). Bacterial polyhydroxyalkanoates. *Biotechnol. Bioeng.* 49, 1–14. doi: 10.1002/(SICI)1097-0290(19960105)49:1<1::AID-BIT1>3.3.CO;2-1
- Li, Y., Wu, J., Liu, S., Shen, J., Huang, D., Su, Y., et al. (2012). Is the C:N:P stoichiometry in soil and soil microbial biomass related to the landscape and land use in southern subtropical China? *Global Biogeochemical Cycles* 26. doi: 10.1029/2012GB004399
- Maassen, S., Fritze, H., and Wirth, S. (2006). Response of soil microbial biomass, activities, and community structure at a pine stand in northeastern Germany 5 years after thinning. *Can. J. For. Res.* 36, 1427–1434. doi: 10.1139/x06-039
- Makino, W., Cotner, J. B., Sterner, R. W., and Elser, J. J. (2003). Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C : N : P stoichiometry. *Funct. Ecol.* 17, 121–130. doi: 10.1046/j.1365-2435.2003.00712.x
- Manzoni, S., and Porporato, A. (2009). Soil carbon and nitrogen mineralization: theory and models across scales. *Soil Biol. Biochem.* 41, 1355–1379. doi: 10.1016/j.soilbio.2009.02.031
- Manzoni, S., Taylor, P., Richter, A., Porporato, A., and Ågren, G. I. (2012). Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytol.* 196, 79–91. doi: 10.1111/j.1469-8137.2012.04225.x
- Manzoni, S., Trofymow, J. A., Jackson, R. B., and Porporato, A. (2010). Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. *Ecol. Monogr.* 80, 89–106. doi: 10.1890/09-0179.1
- Marklein, A. R., and Houlton, B. Z. (2012). Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytol.* 193, 696–704. doi: 10.1111/j.1469-8137.2011.03967.x
- Marschner, B., and Kalbitz, K. (2003). Controls of bioavailability and biodegradability of dissolved organic matter in soils. *Geoderma* 113, 211–235. doi: 10.1016/S0016-7061(02)00362-2
- Marschner, H. (1995). *Mineral Nutrition of Higher Plants*. London: Academic Press.
- Martinelli, L. A., Almeida, S., Brown, I. F., Moreira, M. Z., Victoria, R. L., Filoso, S., et al. (2000). Variation in nutrient distribution and potential nutrient losses by selective logging in a humid tropical forest of Rondonia, Brazil. *Biotropica* 32, 597–613. doi: 10.1646/0006-3606(2000)032[0597:VINDAP]2.0.CO;2
- McGill, W. B., and Cole, C. V. (1981). Comparative aspects of cycling of organic C, N, S and P through soil organic matter. *Geoderma* 26, 267–286. doi: 10.1016/0016-7061(81)90024-0
- McGroddy, M. E., Daufresne, T., and Hedin, L. O. (2004). Scaling of C:N:P stoichiometry in forests worldwide: implications of terrestrial redfield-type ratios. *Ecology* 85, 2390–2401. doi: 10.1890/03-0351
- Miltner, A., Kindler, R., Knicker, H., Richnow, H.-H., and Kaestner, M. (2009). Fate of microbial biomass-derived amino acids in soil and their contribution to soil organic matter. *Org. Geochem.* 40, 978–985. doi: 10.1016/j.orggeochem.2009.06.008
- Moore, J., Macalady, J. L., Schulz, M. S., White, A. E., and Brantley, S. L. (2010). Shifting microbial community structure across a marine terrace grassland chronosequence, Santa Cruz, California. *Soil Biol. Biochem.* 42, 21–31. doi: 10.1016/j.soilbio.2009.09.015
- Moorhead, D. L., Lashermes, G., and Sinsabaugh, R. L. (2012). A theoretical model of C- and N-acquiring exoenzyme activities, which balances microbial demands during decomposition. *Soil Biol. Biochem.* 53, 133–141. doi: 10.1016/j.soilbio.2012.05.011
- Mooshammer, M., Wanek, W., Schneckner, J., Wild, B., Leitner, S., Hofhansl, F., et al. (2012). Stoichiometric controls of nitrogen and phosphorus cycling in decomposing beech leaf litter. *Ecology* 93, 770–782. doi: 10.1890/11-0721.1
- Murphy, D. V., Recous, S., Stockdale, E. A., Fillery, I. R. P., Jensen, L. S., Hatch, D. J., et al. (2003). Gross nitrogen fluxes in soil: theory, measurement and application of ^{15}N pool dilution techniques. *Adv. Agron.* 79, 69–118. doi: 10.1016/S0065-2113(02)79002-0
- Neff, J. C., Hobbie, S. E., and Vitousek, P. M. (2000). Nutrient and mineralogical control on dissolved organic C, N and P fluxes and stoichiometry in Hawaiian soils. *Biogeochemistry* 51, 283–302. doi: 10.1023/A:1006414517212
- Olander, L. P., and Vitousek, P. M. (2000). Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry* 49, 175–190. doi: 10.1023/A:1006316117817
- Osono, T., Ono, Y., and Takeda, H. (2003). Fungal ingrowth on forest floor and decomposing needle litter of *Chamaecyparis obtusa* in relation to resource availability and moisture condition. *Soil Biol. Biochem.* 35, 1423–1431. doi: 10.1016/S0038-0717(03)00236-0
- Phillips, R. P., Finzi, A. C., and Bernhardt, E. S. (2011). Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. *Ecol. Lett.* 14, 187–194. doi: 10.1111/j.1461-0248.2010.01570.x
- Raymond, J., Siefert, J. L., Staples, C. R., and Blankenship, R. E. (2004). The natural history of nitrogen fixation. *Mol. Biol. Evol.* 21, 541–554. doi: 10.1093/molbev/msh047
- Reed, S. C., Cleveland, C. C., and Townsend, A. R. (2007). Controls over leaf litter and soil nitrogen fixation in two lowland tropical rain forests. *Biotropica* 39, 585–592. doi: 10.1111/j.1744-7429.2007.00310.x
- Reed, S. C., Cleveland, C. C., and Townsend, A. R. (2011). Functional ecology of free-living nitrogen fixation: a contemporary perspective. *Annu. Rev. Ecol. Evol. Syst.* 42, 489–512. doi: 10.1146/annurev-ecolsys-102710-145034
- Reed, S. C., Townsend, A. R., Cleveland, C. C., and Nemergut, D. R. (2010). Microbial community shifts influence patterns in tropical forest nitrogen fixation. *Oecologia* 164, 521–531. doi: 10.1007/s00442-010-1649-6
- Schimel, J. P., and Bennett, J. (2004). Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85, 591–602. doi: 10.1890/03-8002
- Schimel, J. P., and Hättenschwiler, S. (2007). Nitrogen transfer between decomposing leaves of different N status. *Soil Biol. Biochem.* 39, 1428–1436. doi: 10.1016/j.soilbio.2006.12.037
- Schimel, J. P., and Weintraub, M. N. (2003). The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biol. Biochem.* 35, 549–563. doi: 10.1016/S0038-0717(03)00015-4
- Scott, J. T., Cotner, J. B., and Lapara, T. M. (2012). Variable stoichiometry and homeostatic regulation of bacterial biomass elemental composition. *Front. Microbiol.* 3:42. doi: 10.3389/fmicb.2012.00042
- Simpson, A. J., Simpson, M. J., Smith, E., and Kelleher, B. P. (2007). Microbially derived inputs to soil organic matter: are current estimates too low? *Environ. Sci. Technol.* 41, 8070–8076. doi: 10.1021/es071217x
- Sinsabaugh, R. L. (2010). Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biol. Biochem.* 42, 391–404. doi: 10.1016/j.soilbio.2009.10.014

- Sinsabaugh, R. L., Hill, B. H., and Shah, J. J. F. (2009). Eoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature* 462, 795–798. doi: 10.1038/nature08632
- Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., Allison, S. D., Crenshaw, C., et al. (2008). Stoichiometry of soil enzyme activity at global scale. *Ecol. Lett.* 11, 1252–1264. doi: 10.1111/j.1461-0248.2008.01245.x
- Sinsabaugh, R. L., Manzoni, S., Moorhead, D. L., and Richter, A. (2013). Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. *Ecol. Lett.* 16, 930–939. doi: 10.1111/ele.12113
- Sistla, S. A., and Schimel, J. P. (2012). Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change. *New Phytol.* 196, 68–78. doi: 10.1111/j.1469-8137.2012.04234.x
- Sterner, R. W. (1990). The ratio of nitrogen to phosphorus resupplied by herbivores: zooplankton and the algal competitive arena. *Am. Nat.* 136, 209–229. doi: 10.1086/285092
- Sterner, R. W., and Elser, J. J. (2002). *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton: Princeton University Press.
- Strickland, M. S., and Rousk, J. (2010). Considering fungal:bacterial dominance in soils – Methods, controls, and ecosystem implications. *Soil Biol. Biochem.* 42, 1385–1395. doi: 10.1016/j.soilbio.2010.05.007
- Tian, H. Q., Chen, G. S., Zhang, C., Melillo, J. M., and Hall, C. A. S. (2010a). Pattern and variation of C:N:P ratios in China's soils: a synthesis of observational data. *Biogeochemistry* 98, 139–151. doi: 10.1007/s10533-009-9382-0
- Tian, L., Dell, E., and Shi, W. (2010b). Chemical composition of dissolved organic matter in agroecosystems: correlations with soil enzyme activity and carbon and nitrogen mineralization. *Appl. Soil Ecol.* 46, 426–435. doi: 10.1016/j.apsoil.2010.09.007
- Urabe, J., and Watanabe, Y. (1992). Possibility of N or P limitation for planktonic cladocerans: an experimental test. *Limnol. Oceanogr.* 37, 244–251. doi: 10.4319/lo.1992.37.2.0244
- Vitousek, P. M., and Howarth, R. W. (1991). Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13, 87–115. doi: 10.1007/BF00002772
- Wallenstein, M. D., and Weintraub, M. N. (2008). Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes. *Soil Biol. Biochem.* 40, 2098–2106. doi: 10.1016/j.soilbio.2008.01.024
- Wang, Y. P., and Houlton, B. Z. (2009). Nitrogen constraints on terrestrial carbon uptake: implications for the global carbon-climate feedback. *Geophys. Res. Lett.* 36, 24. doi: 10.1029/2009GL041009
- Weedon, J. T., Cornwell, W. K., Cornelissen, J. H. C., Zanne, A. E., Wirth, C., and Coomes, D. A. (2009). Global meta-analysis of wood decomposition rates: a role for trait variation among tree species? *Ecol. Lett.* 12, 45–56. doi: 10.1111/j.1461-0248.2008.01259.x
- Wei, X., and Kimmins, J. P. (1998). Asymbiotic nitrogen fixation in harvested and wildfire-killed lodgepole pine forests in the central interior of British Columbia. *For. Ecol. Manag.* 109, 343–353. doi: 10.1016/S0378-1127(98)00288-6
- Wilkinson, J. F. (1963). Carbon and energy storage in bacteria. *J. Gen. Microbiol.* 32, 171–176. doi: 10.1099/00221287-32-2-171
- Wilson, W. A., Roach, P. J., Montero, M., Baroja-Fernandez, E., Munoz, F. J., Eydollin, G., et al. (2010). Regulation of glycogen metabolism in yeast and bacteria. *FEMS Microbiol. Rev.* 34, 952–985. doi: 10.1111/j.1574-6976.2010.00220.x
- Wu, Y. J., Clarke, N., and Mulder, J. (2010). Dissolved organic nitrogen concentrations and ratios of dissolved organic carbon to dissolved organic nitrogen in throughfall and soil waters in Norway spruce and Scots pine forest stands throughout Norway. *Water Air Soil Pollut.* 210, 171–186. doi: 10.1007/s11270-009-0239-x
- Xu, X., Thornton, P. E., and Post, W. M. (2013). A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Glob. Ecol. Biogeogr.* 22, 737–749. doi: 10.1111/geb.12029
- Yuan, Z. Y., Chen, H. Y. H., and Reich, P. B. (2011). Global-scale latitudinal patterns of plant fine-root nitrogen and phosphorus. *Nat. Commun.* 2, 344. doi: 10.1038/ncomms1346
- Yuan, Z. Y. Y., and Chen, H. Y. H. (2009). Global trends in senesced-leaf nitrogen and phosphorus. *Glob. Ecol. Biogeogr.* 18, 532–542. doi: 10.1111/j.1466-8238.2009.00474.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 03 June 2013; accepted: 14 January 2014; published online: 03 February 2014.

Citation: Mooshammer M, Wanek W, Zechmeister-Boltenstern S and Richter A (2014) Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. *Front. Microbiol.* 5:22. doi: 10.3389/fmicb.2014.00022

This article was submitted to *Terrestrial Microbiology*, a section of the journal *Frontiers in Microbiology*.

Copyright © 2014 Mooshammer, Wanek, Zechmeister-Boltenstern and Richter. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Moss-cyanobacteria associations as biogenic sources of nitrogen in boreal forest ecosystems

Kathrin Rousk^{1*}, Davey L. Jones¹ and Thomas H. DeLuca²

¹ School of Environment, Natural Resources and Geography, Bangor University, Bangor, Gwynedd, UK

² School of Environment and Forest Sciences, University of Washington, Seattle, WA, USA

Edited by:

Per Bengtson, Lund University, Sweden

Reviewed by:

Marja A. Tirola, University of Jyväskylä, Finland

Richard S. Winder, Natural Resources Canada, Canada

*Correspondence:

Kathrin Rousk, School of Environment, Natural Resources and Geography, Bangor University, Deiniol Road, Bangor, Gwynedd LL 57 2UW, UK
e-mail: kathrin.rousik@gmx.net

The biological fixation of atmospheric nitrogen (N) is a major pathway for available N entering ecosystems. In N-limited boreal forests, a significant amount of N₂ is fixed by cyanobacteria living in association with mosses, contributing up to 50% to the total N input. In this review, we synthesize reports on the drivers of N₂ fixation in feather moss-cyanobacteria associations to gain a deeper understanding of their role for ecosystem-N-cycling. Nitrogen fixation in moss-cyanobacteria associations is inhibited by N inputs and therefore, significant fixation occurs only in low N-deposition areas. While it has been shown that artificial N additions in the laboratory as well as in the field inhibit N₂ fixation in moss-cyanobacteria associations, the type, as well as the amounts of N that enters the system, affect N₂ fixation differently. Another major driver of N₂ fixation is the moisture status of the cyanobacteria-hosting moss, wherein moist conditions promote N₂ fixation. Mosses experience large fluctuations in their hydrological status, undergoing significant natural drying and rewetting cycles over the course of only a few hours, especially in summer, which likely compromises the N input to the system via N₂ fixation. Perhaps the most central question, however, that remains unanswered is the fate of the fixed N₂ in mosses. The cyanobacteria are likely to leak N, but whether this N is transferred to the soil and if so, at which rates and timescales, is unknown. Despite our increasing understanding of the drivers of N₂ fixation, the role moss-cyanobacteria associations play in ecosystem-N-cycling remains unresolved. Further, the relationship mosses and cyanobacteria share is unknown to date and warrants further investigation.

Keywords: acetylene reduction, boreal biome, bryophytes, global change, N-cycle, nitrogenase, symbioses

THE N-CYCLE IN BOREAL FORESTS

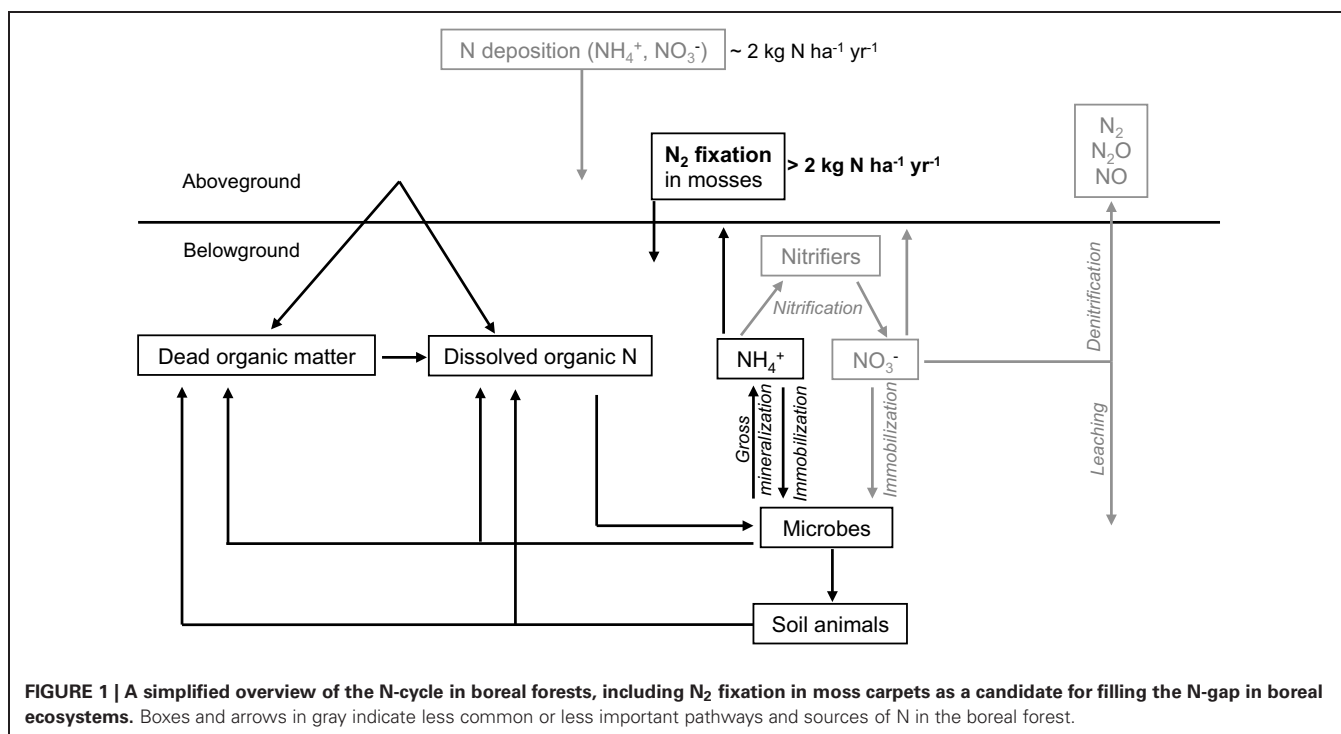
Nitrogen (N) is the limiting nutrient for productivity in boreal forests (Tamm, 1991) due to limited N introduction and the accumulation of carbon (C)-rich recalcitrant litter and plant material, which leads to rapid immobilization of inorganic N and decreased net N mineralization rates (Keeney, 1980; Scott and Binkley, 1997). Therefore, boreal forest soils are characterized by a tight internal N-cycle where immobilization processes dominate (Giesler et al., 1998; Schimel and Bennett, 2004). Considering that the boreal biome accounts for 17% of the Earth's land surface (DeLuca and Boisvenue, 2012), the ability of this ecosystem to sustain productivity is important to consider for global biogeochemical budgets.

One main source of biological available N is the fixation of atmospheric N₂ performed by free-living and symbiotic bacteria (Vitousek et al., 1997; Reed et al., 2011). This conversion of N₂ to ammonia (NH₃) is the initial step in the N-cycle. Nitrogen-fixing cyanobacteria have been found to colonize a range of moss species in pristine, unpolluted environments (Basilier and Granhall, 1978; DeLuca et al., 2002; Sorensen et al., 2006; Ininbergs et al., 2011), where the N₂ fixation of moss-cyanobacteria associations contribute >2 kg N ha⁻¹ yr⁻¹ to the total N input in these systems (DeLuca et al., 2002; Gundale et al., 2011; Sorensen and Michelsen, 2011). These moss-cyanobacteria

associations contribute significantly to the N-input in boreal forests (**Figure 1**) by accumulating N in the moss tissue, which becomes available upon disturbances like drying-rewetting -and fire events (Carleton and Read, 1991; Wilson and Coxson, 1999) as well as via slow mineralization (Hobbie, 1996) and mycorrhizal associations (Kausrud et al., 2008; Davey et al., 2009). Thus, moss-cyanobacteria associations represent a vital feature for maintaining productivity in boreal ecosystems.

BOREAL FORESTS, MOSSES, AND CYANOBACTERIA

Boreal forests receive low amounts of background N-deposition (Phil-Karlsson et al., 2009). In addition, boreal forest soils are characterized by low concentrations of inorganic N, low pH and low temperatures (Read, 1991), contributing to the N-limitation in these systems. Mosses likely play a crucial role in boreal forest ecosystems due to their contribution to habitat heterogeneity (Longton, 1988), their influence on hydrology, temperature, and chemistry of boreal forest soils (Cornelissen et al., 2007). For instance, summer soil temperatures below moss carpets are lower compared to sites without moss cover (Bonan, 1991; Startsev et al., 2007), leading to slower decomposition rates below mosses (Prescott et al., 1993). However, mosses release substantial amounts of nutrients [C, N, phosphorus (P)] upon rewetting of dried tissue, funneling plant and



microbial-available nutrients into the soil (Carleton and Read, 1991; Wilson and Coxson, 1999). Further, mosses contribute fundamentally to the biomass and productivity in boreal forests, and may exceed tree biomass [e.g., 120 g m⁻² yr⁻¹ for feather mosses vs. 102 g m⁻² yr⁻¹ for black spruce, (Van Cleve et al., 1983)] (see also Martin and Adamson, 2001; Turetsky, 2003; Lindo and Gonzalez, 2010). For instance, the ubiquitous feather moss *Pleurozium schreberi* (Brid.) Mitt. accounts for 70–100% of the ground cover in boreal forests (Oechel and Van Cleve, 1986; DeLuca et al., 2002; Zackrisson et al., 2004; Street et al., 2013) (**Figure 2A**).

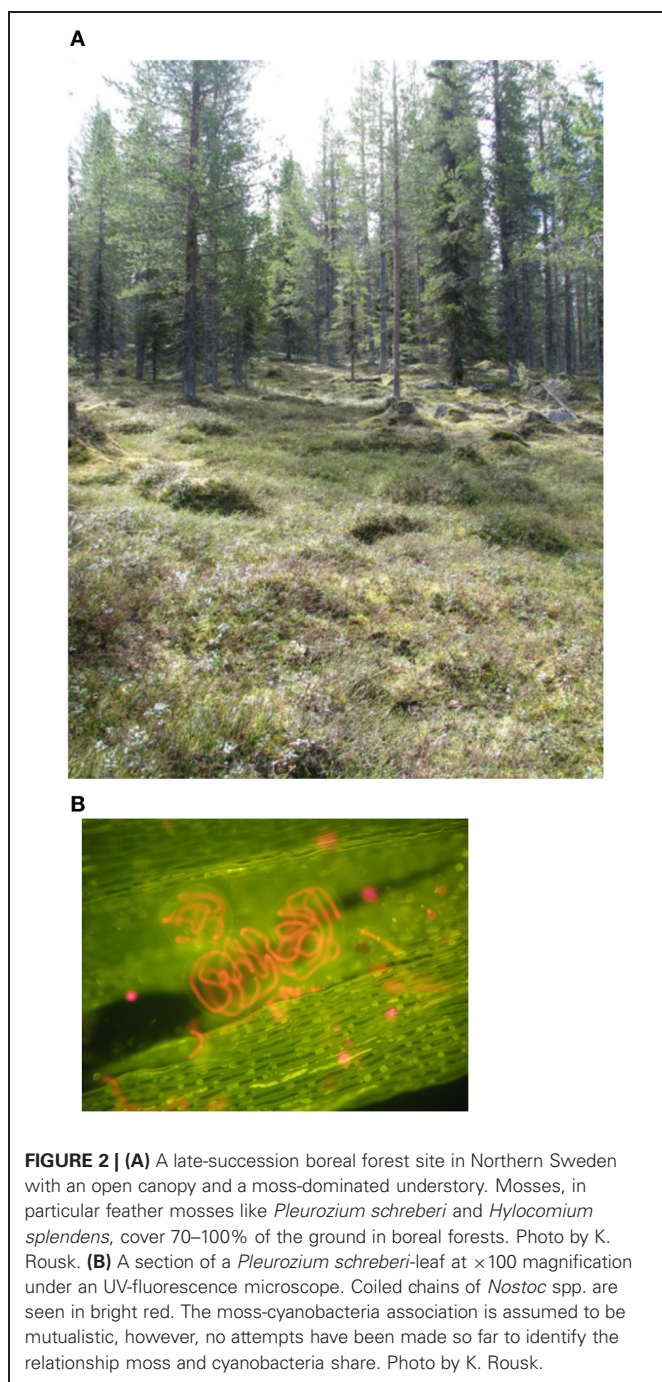
By buffering abiotic factors (e.g., temperature, wind) and exhibiting a high water retention capacity (Dickson, 2000), mosses can provide a stable and favorable habitat for cyanobacterial colonizers, promoting N₂ fixation in N-limited ecosystems (DeLuca et al., 2002). The association between mosses and cyanobacteria (**Figure 2B**) could play a fundamental role for the N-cycle in N-limited boreal forests by contributing >2 kg N ha⁻¹ yr⁻¹ via N₂ fixation to the N-pool in mature forest ecosystems (DeLuca et al., 2002). This value is on par with the magnitude of atmospheric N-deposition in the boreal biome, which ranges between 1 and 2 kg N ha⁻¹ yr⁻¹ (e.g., Gundale et al., 2011).

To date, several genera of cyanobacteria (*Nostoc*, *Stigonema*, *Calothrix*, *Cylindrospermum*) have been identified living epiphytically on feather mosses like *P. schreberi* and *Hylocomium splendens* (Hedw.) (Gentili et al., 2005; Ininbergs et al., 2011). Numbers of cyanobacterial cells and N₂ fixation rates in feather mosses follow a linear relationship (DeLuca et al., 2007), indicating that cyanobacteria are responsible for N₂ fixation, whereas the contribution of methanotrophs to N₂ fixation in feather mosses might be negligible (Leppänen et al., 2013).

ABIOTIC CONTROLS OF N₂ FIXATION IN MOSS-CYANOBACTERIA ASSOCIATIONS

NITROGEN

Moss biomass (Solga et al., 2005; Nordin et al., 2006) and biomass and activity (nitrogenase enzyme) of cyanobacteria (DeLuca et al., 2007, 2008; Sorensen et al., 2012) are sensitive to N inputs (**Table 1**), leading to drastic reductions in the abundance of dominant moss species and to significant reductions or total exclusion of N₂ fixation in moss-cyanobacteria associations (Zackrisson et al., 2004; DeLuca et al., 2008; Gundale et al., 2011; Ackermann et al., 2012). The amount of N input dictates in which form N enters the ecosystem: either as organic N via the moss layer when N deposition is low (<3 kg N ha⁻¹ yr⁻¹) and N₂ fixation is high or as inorganic N when N deposition is higher and bypasses the moss layer. Mosses effectively absorb nutrients and water from atmospheric deposition, making them extremely sensitive to increased nutrient inputs (e.g., Bengtsson et al., 1982). For instance, Ackermann et al. (2012) showed that N₂ fixation in moss-cyanobacteria associations along road-derived N-deposition gradients in Northern Sweden was significantly inhibited close to busy roads. While other measured factors (soil-N, -C concentrations, microbial PLFAs, heavy metals in moss tissue) did not change along the road-gradients, N₂ fixation increased with increasing distance to the busy roads, suggesting that N₂ fixation in feather mosses is a sensitive indicator for N-deposition (Ackermann et al., 2012). Artificial N additions *in-situ* have been shown to significantly decrease numbers of cyanobacterial cells on moss leaves at levels of only 3 kg N ha⁻¹ yr⁻¹ coinciding with a significant reduction in N₂ fixation in moss-cyanobacteria associations (Gundale et al., 2011). However, additions of 10 kg N ha⁻¹ in laboratory experiments did



not inhibit N_2 fixation in mosses (Ackermann, 2013), suggesting that the mosses likely experience higher N loads in the field than expected. When combining values of atmospheric N-deposition ($1\text{--}2\text{ kg N ha}^{-1}\text{ yr}^{-1}$) (Phil-Karlsson et al., 2009; Gundale et al., 2011) with values of N throughfall ($>8\text{ kg N ha}^{-1}\text{ yr}^{-1}$) (Rousk et al., 2013a) in boreal forests, N input will easily reach values higher than 10 kg N ha^{-1} . Further, mosses collected from a high N-deposition area in Wales ($12\text{--}15\text{ kg N ha}^{-1}\text{ yr}^{-1}$) were shown to start fixing N_2 after a period of N deprivation (Ackermann, 2013).

PHOSPHORUS

In contrast to N additions that would likely only decrease N_2 fixation rates, nutrient additions (other than N) to moss carpets have the potential to increase N_2 fixation rates. The addition of soluble P to Arctic mosses has been reported to increase N_2 fixation rates (Chapin et al., 1991). Studies in boreal forest ecosystems have been conducted both on late succession forest stands that had high rates of N_2 fixation and on early succession stands that had low rates of N_2 fixation (Zackrisson et al., 2004). Phosphorus additions (0 and 5 kg P ha^{-1} as NaH_2PO_4) to field plots resulted in a slight increase in N_2 fixation rates in late succession plots just 8 weeks after the original application of P (Zackrisson et al., 2004), but a more prominent effect was recorded 1 year after the original treatment in the late and a significant effect in the early succession site (see Figure 3). Five years after the P additions, the positive effects were less pronounced and again, only significant in the early succession site (Figure 3). Phosphorus additions with and without N additions to plots of *H. splendens* showed little direct response to P additions of 5 kg P ha^{-1} , but also demonstrated this species to be more tolerant to N deposition and to respond somewhat to P additions in the presence of added N (Zackrisson et al., 2009).

There have also been reports of P suppression of N_2 fixation in Subantarctic epiphytic cyanobacteria (Smith, 1984), however, the findings by Chapin et al. (1991) and Zackrisson et al. (2009) suggest a positive effect of P additions on N_2 fixation in mosses. Nevertheless, reported results on the effects of P additions are ambiguous and the outcome of P-fertilizations seems to be dependent on the availability of P and other nutrients. Studies on symbiotic N_2 fixation in tropical rainforests suggest that N_2 fixation can be limited by P (Vitousek and Hobbie, 2000; Reed et al., 2013), molybdenum (Mo) (Barron et al., 2009), or by P and Mo in combination (Reed et al., 2013), depending on the availability of P and Mo in the sites studied (Wurzburger et al., 2012).

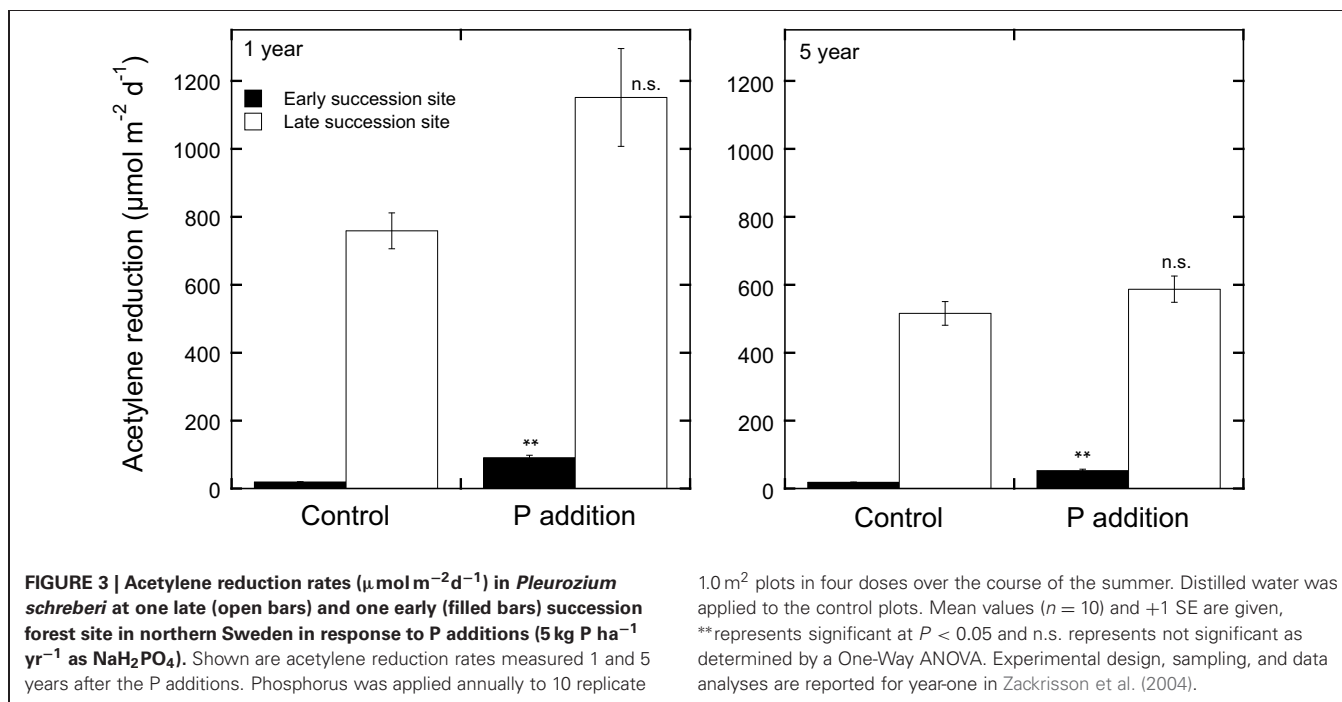
MOISTURE, TEMPERATURE, AND LIGHT

Besides the availability of N and P, other abiotic factors dramatically affect N_2 fixation in moss-cyanobacteria associations. For instance, the hydrological status of the moss seems to be a crucial factor driving N_2 fixation. Moisture, as well as frequent rainfall promotes N_2 fixation rates in mosses (Gundale et al., 2009, 2012a,b; Jackson et al., 2011; Jean et al., 2012). This is not surprising, given the fact that mosses absorb water over their entire surface from the atmosphere and do not take up water from the soil (Tyler, 1990). Therefore, mosses readily lose water under dry conditions, which could in turn affect the activity of cyanobacterial associates. Moss growth, as well as cyanobacterial activity, peak in early spring after snowmelt (May–June) and in late summer (September), and drop in between (July–August) (Basilier and Granhall, 1978; Zackrisson et al., 2004). This reduction in moss growth and activity in summer could correspond to a period of dormancy in mosses as a result of dry conditions or photoinhibition (Sveinbjörnsson and Oechel, 1992; Zackrisson et al., 2004). In the boreal forest, mosses can be exposed to extreme daily fluctuations in moisture and temperature conditions. Frequently, dry episodes are followed by heavy rainfall over the course of only few hours. Thus, mosses experience natural and

Table 1 | Effects of selected abiotic factors on N₂ fixation in different moss species from boreal and arctic environments.

Moss species	Abiotic factor	Effect on N ₂ fixation	References
<i>Sphagnum riparium</i>	Temperature	+ up to 15°C; T _{opt} 16°C	Basilier and Granhall, 1978
<i>Brachythecium subplicatum</i>	Temperature	+ up to 25°C; T _{opt} 25–27°C	Smith, 1984
<i>Pleurozium schreberi</i> -Nostoc –associate	Temperature	T _{max} 13°C	Gentili et al., 2005
<i>Pleurozium schreberi</i> -Calothrix –associate	Temperature	T _{max} 30°C	Gentili et al., 2005
<i>Pleurozium schreberi</i>	Temperature	+ T _{opt} 25°C	Gundale et al., 2012a
<i>Hylocomium splendens</i>	Temperature	–	Gundale et al., 2012a
<i>Hylocomium splendens</i>	Temperature	No effect	Sorensen et al., 2012
<i>Aulacomnium turgidum</i>	Temperature	–	Sorensen et al., 2012
<i>Pleurozium schreberi</i>	N-addition	– (4.25; 25.5 kg N ha ⁻¹ yr ⁻¹)	Zackrisson et al., 2004
<i>Pleurozium schreberi</i>	N-addition	– (3; 6; 12; 50 kg N ha ⁻¹ yr ⁻¹)	Gundale et al., 2011
<i>Hylocomium splendens</i>	N-addition	– (100 kg N ha ⁻¹ yr ⁻¹)	Sorensen et al., 2012
<i>Aulacomnium turgidum</i>	N-addition	– (100 kg N ha ⁻¹ yr ⁻¹)	Sorensen et al., 2012
<i>Brachythecium subplicatum</i>	Moisture	+	Smith, 1984
<i>Pleurozium schreberi</i>	Water addition	+	Gundale et al., 2009
<i>Pleurozium schreberi</i>	Water addition	+	Gundale et al., 2012b
<i>Pleurozium schreberi</i>	P-addition	+	Zackrisson et al., 2004
<i>Hylocomium splendens</i>	P-addition	No effect	Zackrisson et al., 2009
<i>Sphagnum riparium</i>	P-addition	+	Basilier and Granhall, 1978
<i>Brachythecium subplicatum</i>	P-addition	–	Smith, 1984

P, Phosphorus; N, Nitrogen; T_{opt}, optimum temperature; T_{max}, maximum temperature; positive, +; negative, –; or no effects are given.



intensive recurrent drying and rewetting events. Significant leaching of nutrients from mosses has been found upon rewetting of dried moss (Carleton and Read, 1991; Wilson and Coxson, 1999), resulting in nutrient-rich leachates available for soil biota. Mosses are relatively desiccation-tolerant; they are able to withstand drying until no free water remains in the cells and quickly return to normal metabolism and growth upon rewetting (Proctor, 2001).

Also, many fundamental processes like photosynthesis resume quickly after rewetting, with some moss species starting to fix CO₂ within minutes upon rewetting (Proctor et al., 2007). However, the moisture condition of the moss could change the nutrient supply and exchange between moss and associated cyanobacteria. Scott (1960) suggested that not only nutrients, but also light and moisture could affect the balance and rates of nutrients exchanged

between the symbiotic partners in lichen symbioses, upsetting the relationship between them.

The recovery of nitrogenase activity in free-living cyanobacteria after desiccation is supposed to be slower than the recovery of photosynthesis because *de novo* protein synthesis is required for N₂ fixation (>24 h vs. 4 h for N₂ fixation and photosynthesis, respectively) (Hawes et al., 1992). Cyanobacteria can form dormant cells during dry conditions and resuscitate upon rewetting to resume fixing N₂ (Kaplan-Levy et al., 2010). However, recovery of N₂ fixation in the moss-cyanobacteria association after rewetting of dried moss has been shown to be very slow. It took 5 days for N₂ fixation to reach values comparable to moist moss after rewetting of air-dried moss (Ackermann, 2013). In contrast, other processes like photosynthesis have been reported to recover much faster than N₂ fixation upon rewetting in free-living cyanobacteria (Hawes et al., 1992; Belnap, 2001). This lag-time between rewetting and N₂ fixation activity in cyanobacteria is likely the result of *de novo* synthesis of proteins for N₂ fixation, and the need for differentiation of vegetative cells to heterocysts, in which the reduction of N₂ takes place (Belnap, 2001). Thus, N input via N₂ fixation could be compromised in summer months when the moss is desiccated. In addition, predicted increases in temperatures and more extreme weather events in the next century (IPCC, 2007) could fundamentally affect the N₂ fixation capacity in moss-cyanobacteria associations.

Reports on the temperature relationship of N₂ fixation in moss-cyanobacteria associations are varied (Table 1), ranging from temperature optima at 16°C (Basilier and Granhall, 1978) to 22–27°C (Smith, 1984; Gundale et al., 2012a) and depending on light conditions, moss species and the associated species of cyanobacteria (Smith, 1984; Gentili et al., 2005; Gundale et al., 2012a; Jean et al., 2012; Sorensen et al., 2012). The varying reports on the temperature effects on N₂ fixation call for further studies.

Nitrogen fixation is a metabolically costly process (Turetsky, 2003; Houlton et al., 2008; Reed et al., 2011). In autotrophic N₂-fixers, this high energy demand can be met via the products of photosynthesis (Belnap, 2001), which is dependent on light conditions. The effects of light intensities on N₂ fixation in moss-cyanobacteria associations have rarely been studied. There are indications that N₂ fixation in mosses decreases at high light intensities (500–900 μmol m⁻²s⁻¹) (Smith, 1984; Gundale et al., 2012a). However, the effects of light, moisture and temperature on processes like N₂ fixation are tightly coupled (Gundale et al., 2012a,b), making the identification of the most influencing driver difficult.

THE ECOLOGY OF MOSS-CYANOBACTERIA ASSOCIATIONS—WHAT RELATION DO THEY SHARE?

The term symbiosis (*Symbiotismus*) was first introduced in 1877 by Frank, who described it as a case in which two different species (symbionts) live in or on one another, irrespective of the role of the individuals. Cyanobacteria are an ancient, diverse and widespread group found as free-living cells and colonies as well as living in symbiosis and associations with higher plants, lichens and bryophytes (Rai et al., 2000; Adams and Duggan, 2008; Meeks, 2009). Cyanobacteria are facultative autotrophs, they

possess the ability to fix C as well as N, which allows the establishment of the cyanobacteria-plant symbioses in ecosystems where these essential nutrients are limiting. In their free-living state, cyanobacteria retain the ability to fix both essential nutrients (C, N). However, when living in association with a plant partner, cyanobacteria commonly discontinue photosynthesis and instead obtain C from their symbiotic partner in exchange for fixed N₂ (Meeks and Elhai, 2002; Adams and Duggan, 2008; Meeks, 2009). The plant partner receives N as NH₄⁺ or amino acids from the cyanobacteria and in return provides carbohydrates, shelter and protection (Steinberg and Meeks, 1991). Given that N₂ fixation is a highly energy demanding process (Scherer and Zhong, 1991; Turetsky, 2003; Houlton et al., 2008; Reed et al., 2011), living in association with a symbiotic partner could compensate for energy needs. Although direct evidence is lacking, similar mechanisms and principles are assumed to take place in moss-cyanobacteria associations (Rai et al., 2000; Turetsky, 2003): the moss offers protection and carbohydrates while receiving fixed N₂ in return. However, in the lichen symbiosis for instance, the balance between the exchange of nutrients seems to be not entirely mutually beneficial, but rather depends on the nutrient demands of the partners (Johansson et al., 2011). Over 50 years ago, Scott (1960) reported that variations in the supply of nutrients, light, and moisture could upset the symbiotic balance between the mycobiont and photobiont in lichen symbioses. The growth of both symbionts is controlled by moisture levels and availability of N and C, resulting in a delicate balance between the partners (Scott, 1960).

In addition to nutrient exchange, mutual protection between the partners could play a role in the moss-cyanobacteria relationship. Although mosses are a characteristic and dominant feature of boreal forests, they are consumed by very few herbivores (Prins, 1982; Eskelinen, 2002) and decomposition of moss litter is very slow [>150 vs. 30 days for mosses vs. vascular plants, respectively; (Hobbie, 1996)]. Mosses produce inhibitory compounds like phenols and moss-specific secondary metabolites (oxylipins) (Matsui, 2006; Croisier et al., 2010). These inhibitory compounds could be related to the recalcitrant nature of moss litter and the resistance of mosses to decomposition and can repress enzyme activity involved in the breakdown process (Triebwasser et al., 2012). Given the low density of easily decomposable plants in boreal ecosystems, the low litter quality of mosses (Prins, 1982; Hobbie, 1996; Lang et al., 2009) seems to be an insufficient explanation for the lack of decomposition of this plentiful plant material. Cyanobacteria are known to produce toxins (e.g., microcystins) (Cox et al., 2005; Adams and Duggan, 2008; Kaasalainen et al., 2012). Microcystins are highly toxic, small, cyclic peptides produced by cyanobacteria in freshwater systems (predominantly strains of the genus *Nostoc*) that are reported to be responsible for animal poisoning (Sivonen, 2009). *Nostoc* has been found to also produce the toxin when living in symbioses with lichens (Kaasalainen et al., 2009, 2012). Reindeer thus avoid eating cyanolichens, even during periods of starvation (Rai et al., 2002; Storeheier et al., 2002). Given that *Nostoc* colonizes mosses as well, it is possible that toxic substances produced by the cyanobacterial colonizer provide protection and would explain the moss' resistance toward decomposition, which would

add to the proposed mutualistic relationship between mosses and cyanobacteria. However, the inhibition of soil bacterial growth by mosses colonized by cyanobacteria is reported to be negatively correlated with the numbers of colonizing cyanobacteria (Rousk et al., 2013b). The moss had a higher inhibitory effect on soil bacterial growth when colonized by fewer cyanobacteria. This suggests that the cyanobacteria do not contribute to the moss' resistance toward decomposition. Nevertheless, the N and C-exchange between mosses and cyanobacteria requires further study in order to identify and characterize the relationship they share.

SOIL-N-CYCLING AND N-UTILIZATION PATHWAYS BY MOSSES

Nitrogen is an essential nutrient for plants, animals, and microbes; however, the boreal forest is typically considered to be N-limited in terms of primary productivity (Tamm, 1991). Whilst it has been assumed that this is due to the slow rate of turnover of soil organic matter and therefore the production of NH_4^+ and NO_3^- (Read, 1991), recent evidence suggests that this is only part of the story. There is no doubt that tree needles can decompose relatively slowly in some environments after shedding (e.g., anaerobic soils); however, in mature forests, there is often only a small net accumulation of needle litter at the soil surface considering the high rate of needle shedding, suggesting that turnover is actually relatively rapid (Muukkonen, 2005). Further, much of the N entering soil occurs via fine root turnover (Yuan and Chen, 2012). However, rarely are masses of dead roots observed in the soil profile, suggesting rapid turnover possibly related to intrinsically high N and labile C content (Chertov et al., 2003). Part of the reason for initially thinking that slow rates of organic matter turnover were responsible for N-limitation was the finding that concentrations of NH_4^+ and NO_3^- were often very low in soil solution. This could be partially due to blockage of protease enzymes by high concentrations of polyphenolics in soil solution or a low pH-induced block in nitrification (Butler and Ladd, 1971; Pajuste and Frey, 2003; Triebwasser et al., 2012). However, recent evidence suggests that it may largely reflect rapid rates of removal rather than slow rates of production (Jones and Kielland, 2002, 2012). As soil microorganisms prefer taking up the primary products of protein degradation (peptides, amino acids) this essentially prevents the direct release of NH_4^+ and thus NO_3^- during mineralization (Farrell et al., 2013).

Due to low rates of input and rapid microbial immobilization, in most high latitude or high altitude ecosystems, inorganic N fluxes are found to be insufficient to cover the N demands of plants (e.g., Kielland, 1994). Additionally, soil solution concentrations of organic N concentrations are often found to be higher than inorganic N, especially in soils with low pH and low inorganic N availability (Kielland, 1994, 1995; Nordin et al., 2001; Finzi and Berthrong, 2005). Thus, N demand of plants has to be satisfied by a combination of sources and pathways (Jones et al., 2005). Besides the uptake of mineralized, inorganic N, plants possess the ability to take up organic N in the form of amino acids, urea, polyamines, and small polypeptides (Kielland, 1994; Schimel and Bennett, 2004; Krab et al., 2008; Persson and Näsholm, 2008; Hill et al., 2011) (see **Figure 1**). For instance,

Näsholm et al. (1998) and Persson and Näsholm (2008) showed that many boreal forest and taiga plant species have the ability to take up amino acids from soil pools. This uptake can occur indirectly via mycorrhizae or directly by the roots themselves. However, other studies showed that organic N represents only a minor source for plants to cover their N needs (Hodge et al., 2000), suggesting that the importance of this process is dependent on a range of factors including: plant, soil type, chemical form and concentration of the organic N source, the availability of inorganic N, the activity of the competing microbial biomass or other plants and the time of year. Further, amino acids are removed rapidly from the soil-N pool via microbial activity, resulting in fast turnover rates of amino acids in soils (Jones and Kielland, 2002; Rousk and Jones, 2010), indicating that gross rates of N production are much greater than the typically measured net rates of N mineralization (Inselsbacher and Näsholm, 2012). It should be remembered, however, that almost all studies have investigated the unidirectional uptake of organic N into plants (using ^{13}C - ^{15}N tracers) and have largely ignored the counter efflux of amino acids and other N containing solutes (i.e., rhizodeposition; Jones et al., 2009). Therefore, most measured rates of uptake are therefore probably overestimates (Jones et al., 2005).

In addition to vascular plants, mosses have been found to take up amino acids from solution and directly from soil (Ayres et al., 2006; Krab et al., 2008; Hill et al., 2011). Further, it has been suggested that mosses are associated with fungi (Kausrud et al., 2008; Davey et al., 2009), which could enhance the uptake of N from the soil. Mosses, however, are thought to receive most of their N via absorption of N originating from atmospheric deposition, leaching and throughfall (Li and Vitt, 1997; Kotanen, 2002). While bulk atmospheric deposition is dominated by NO_3^- and NH_4^+ , it should be noted that canopy throughfall is often dominated by organic forms of N (Pelster et al., 2009). Another source of N for mosses is the relocation and recycling of nutrients along the moss-profile, from dead moss tissue to the growing parts at the apex (Aldous, 2002). Additionally, mosses possess an endogenous N supply due to their association with N_2 fixing cyanobacteria (DeLuca et al., 2002; Berg et al., 2013). Thus, mosses are able to gain N via various sources and pathways. However, only few attempts have been made to qualitatively relate N-acquisition processes with N-utilization pathways in mosses. When linking N_2 fixation in the feather moss *Pleurozium schreberi* with uptake of organic and inorganic N from soil by the moss, one study found no correlations (Rousk et al., 2013a). Further, the uptake of N from soil was very low. Thus, the moss seems to be independent of soil-N resources and features an internal N-cycle that acquires N via absorption of atmospheric N and epiphytic N_2 fixation, and recycling of N within the moss.

WHERE DOES THE N GO? THE FATE OF THE FIXED N_2

Since total N input in boreal forests is low (Tamm, 1991; Gundale et al., 2011), moss-cyanobacteria associations likely represent a major N source in N-limited ecosystems (DeLuca et al., 2002; Gundale et al., 2011). Given the great abundance of moss biomass in boreal forests (Oechel and Van Cleve, 1986) (see also **Figure 2A**), their N input could be crucial for the overall N-cycle. However, since mosses capture and retain significant amounts

of N from throughfall and deposition as well as hosting N₂-fixing cyanobacteria, it has been suggested that forest ecosystems are dependent on the release of N from moss carpets (Weber and Van Cleve, 1984; Oechel and Van Cleve, 1986; Carleton and Read, 1991), especially in low-N deposition areas where N₂ fixation rates in mosses are high. Thus, the biological N₂ fixation in mosses is the main N input into the boreal forest as long as atmospheric N deposition is low ($\sim 3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) (Li and Vitt, 1997; Gundale et al., 2011). However, little is known about the fate of the N₂ that is fixed by cyanobacteria associated with mosses. To date, there are no published reports that directly describe the transfer of biologically fixed N₂ into plants (via mycorrhizae) or into the soil or to what extent that N is available for microorganisms and plants. Most likely, the transfer of fixed N₂ to higher plants has to follow a tortuous pathway that involves decomposition of recalcitrant moss tissue (see also Hyodo et al., 2013). Assuming the fixed N₂ is transferred to the soil, questions about the amount, extent and rates of the transferred N emerge. Several studies have shown that mosses represent a short-term (<1 year) N-sink due to efficient capturing and retaining of N from the atmosphere (Startsev and Loeffers, 2006; Startsev et al., 2008; Friedrich et al., 2011). However, mosses can turn into a long-term (>1 year) N source after disturbances like drying-rewetting and fire events (Carleton and Read, 1991; Wilson and Coxson, 1999), upon which N is released from cyanobacterial-N-enriched moss tissue and made available for N-cycling in soils.

CONCLUDING REMARKS

Mosses colonized by diazotrophic cyanobacteria contribute significantly to the N pool in pristine, N-limited forests (Figure 1). Besides that, mosses represent an iconic and important feature

in boreal forests due to their ability to influence soil hydrology and chemistry; they form extensive carpets and contribute to biomass and productivity in these forests. Further, the associated N₂ fixing cyanobacteria could alleviate the pronounced N-limitation in boreal forest ecosystems. It has been shown that N-deposition can inhibit N₂ fixation, but also, this fundamental process can recover from increased N loads and resume. Nitrogen fixation is strongly inhibited in dry moss, and recovery is slow, compromising the N input to the system, especially in dry summers. Considering future scenarios predicting changes in these factors (increasing N input and temperatures), the effects on the N-cycle in boreal forest could be dramatic. Mosses use several pathways to acquire N, however, the most prominent is the absorption of N from throughfall and deposition, which could limit the N input to forests that are characterized by a moss-dominated ground cover. Further, transfer of N from moss to the soil is slow and is only promoted after disturbances, indicating that the moss represents a N sink in the short-term (<1 year). However, mosses colonized by N₂-fixing cyanobacteria likely act as a N source in the long term, releasing N upon disturbances like drying-rewetting and fire events. Given the moss' abundance in the boreal biome, models of ecosystem N and C-budgets should incorporate the nutrient fluxes within the moss layer, between the moss and its environment (atmosphere and soil) as well as the factors driving N₂ fixation in moss-cyanobacteria associations. Further, more research is needed to explore and identify the relationship mosses and cyanobacteria share (see also Figure 2B).

ACKNOWLEDGMENT

This work was supported by the UK Natural Environment Research Council (Grant number: NE/I027150/1).

REFERENCES

- Ackermann, K. (2013). *Nitrogen Dynamics in Boreal Forests: a Feather Moss' Perspective*. Ph.D. thesis, Bangor: Bangor University, 1–162.
- Ackermann, K., Zackrisson, O., Rousk, J., Jones, D. L., and DeLuca, T. H. (2012). N₂ fixation in feather mosses is a sensitive indicator of N deposition in boreal forests. *Ecosystems* 15, 986–998. doi: 10.1007/s10021-012-9562-y
- Adams, D. G., and Duggan, P. S. (2008). Cyanobacteria-bryophyte symbioses. *J. Exp. Bot.* 59, 1047–1058. doi: 10.1093/jxb/ern005
- Aldous, A. R. (2002). Nitrogen retention by Sphagnum mosses: response to atmospheric nitrogen deposition. *Can. J. Bot.* 80, 721–731. doi: 10.1139/b02-054
- Ayres, E., Van der Wal, R., Sommerkorn, M., and Bardgett, R. D. (2006). Direct uptake of soil nitrogen by mosses. *Biol. Lett.* 2, 286–288. doi: 10.1098/rsbl.2006.0455
- Barron, A. R., Wurzbarger, N., Bellenger, J. P., Wright, S. J., Kraepiel, A. M. L., and Hedin, L. O. (2009). Molybdenum limitation of symbiotic nitrogen fixation in tropical forest soils. *Nat. Geosci.* 2, 42–45. doi: 10.1038/ngeo366
- Basilier, K., and Granhall, U. (1978). Nitrogen fixation in wet minerotrophic moss communities of a subarctic mire. *Oikos* 31, 236–246. doi: 10.2307/3543568
- Belnap, J. (2001). "Factors influencing nitrogen fixation and nitrogen release in biological soil crusts," in *Biological Soil Crusts: Structure, Function, and Management*, eds J. Belnap and O. L. Lange (Berlin-Heidelberg: Springer), 241–261. doi: 10.1007/978-3-642-56475-8_19
- Bengtsson, C., Folkesson, L., and Göransson, A. (1982). Growth reduction and branching frequency in *Hylocomium splendens* near a foundry emitting copper and zinc. *Lindbergia* 8, 129–138.
- Berg, A., Danielsson, Å., and Svensson, B. H. (2013). Transfer of fixed-N from N₂-fixing cyanobacteria associated with the moss *Sphagnum riparium* results in enhanced growth of the moss. *Plant Soil* 362, 271–278. doi: 10.1007/s11104-012-1278-4
- Bonan, G. B. (1991). A biophysical surface energy budget analysis of soil temperature in the boreal forests of interior Alaska. *Water Res.* 27, 767–781. doi: 10.1029/91WR00143
- Butler, J. H. A., and Ladd, J. N. (1971). Importance of the molecular weight of humic and fulvic acids in determining their effects on protease activity. *Soil Biol. Biochem.* 3, 249–257. doi: 10.1016/0038-0717(71)90021-6
- Chapin, D. M., Bliss, L. C., and Bledsoe, L. J. (1991). Environmental regulation of Nitrogen-fixation in a high arctic lowland ecosystem. *Can. J. Bot.* 69, 2744–2755. doi: 10.1139/b91-345
- Carleton, T. J., and Read, D. J. (1991). Ectomycorrhizas and nutrient transfer in conifer-feather moss ecosystems. *Can. J. Bot.* 69, 778–785. doi: 10.1139/b91-101
- Chertov, O., Komarov, A., Kolstrom, M., Pitkanen, S., Strandman, H., Zudin, S., et al. (2003). Modelling the long-term dynamics of populations and communities of trees in boreal forests based on competition for light and nitrogen. *For. Ecol. Manage.* 176, 355–369.
- Cornelissen, J. H. C., Lang, S. I., Soudzilovskaia, N. A., and During, H. J. (2007). Comparative cryptogam ecology: a review of bryophyte and lichen traits that drive biogeochemistry. *Annu. Rev. Bot.* 99, 987–1001. doi: 10.1093/aob/mcm030
- Cox, P. A., Banack, S. A., Murch, S. J., Rasmussen, U., Tien, G., and Bidigare, R. R. (2005). Diverse taxa of cyanobacteria produce β -N-methylamino-l-alanine, a neurotoxic amino acid. *Proc. Natl. Acad. Sci. U.S.A.* 102, 5074–5078. doi: 10.1073/pnas.0501526102
- Croisier, E., Rempt, M., and Pohnert, G. (2010). Survey of volatile

- oxylipins and their biosynthetic precursors in bryophytes. *Phytochemistry* 71, 574–580. doi: 10.1016/j.phytochem.2009.12.004
- Davey, M. L., Nybakken, L., Kausrud, H., and Ohlson, M. (2009). Fungal biomass associated with the phyllosphere of bryophytes and vascular plants. *Mycol. Res.* 113, 1254–1260. doi: 10.1016/j.mycres.2009.08.001
- DeLuca, T. H., and Boissvenue, C. (2012). Boreal forest soil carbon: distribution, function and modeling. *Forestry* 85, 161–184. doi: 10.1093/forestry/cps003
- DeLuca, T. H., Zackrisson, O., Gentili, F., Sellstedt, A., and Nilsson, M. C. (2007). Ecosystem controls on nitrogen fixation in boreal feather moss communities. *Oecologia* 152, 121–130. doi: 10.1007/s00442-006-0626-6
- DeLuca, T. H., Zackrisson, O., Gundale, M. J., and Nilsson, M. C. (2008). Ecosystem feedbacks and nitrogen fixation in boreal forests. *Science* 320, 1181. doi: 10.1126/science.1154836
- DeLuca, T. H., Zackrisson, O., Nilsson, M. C., and Sellstedt, A. (2002). Quantifying nitrogen-fixation in feather moss carpets of boreal forests. *Nature* 419, 917–920. doi: 10.1038/nature01051
- Dickson, L. G. (2000). Constraints to nitrogen fixation by cryptogamic crusts in a polar desert ecosystem, Devon Island, NWT, Canada. *Arct. Antarct. Alp. Res.* 32, 40–45. doi: 10.2307/1552408
- Eskelinen, O. (2002). Diet of the wood lemming *Myopus schisticolor*. *Ann. Zool. Fenn.* 39, 49–57.
- Farrell, M., Hill, P. W., Farrar, J., DeLuca, T. H., Roberts, P., Kielland, K., et al. (2013). Oligopeptides represent a preferred source of organic N uptake: a global phenomenon? *Ecosystems* 16, 133–145. doi: 10.1007/s10021-012-9601-8
- Finzi, A. C., and Berthrong, S. T. (2005). The uptake of amino acids by microbes and trees in three cold-temperate forests. *Ecology* 86, 3345–3353. doi: 10.1890/04-1460
- Frank, A. B. (1877). Über die biologischen verhältnisse des thallus einiger krustenflechten. *Beitr. Biol. Pflanz.* 2, 123–200.
- Friedrich, U., Falk, K., Bahlmann, E., Marquardt, T., Meyer, H., Niemeyer, T., et al. (2011). Fate of airborne nitrogen in heathland ecosystems: a ^{15}N tracer study. *Glob. Change Biol.* 17, 1549–1559. doi: 10.1111/j.1365-2486.2010.02322.x
- Gentili, F., Nilsson, M. C., Zackrisson, O., DeLuca, T. H., and Sellstedt, A. (2005). Physiological and molecular diversity of feather moss associative N_2 -fixing cyanobacteria. *J. Exp. Bot.* 56, 3121–3127. doi: 10.1093/jxb/eri309
- Giesler, R., Högberg, M., and Högberg, P. (1998). Soil chemistry and plants in Fennoscandian Boreal Forest as exemplified by a local gradient. *Ecology* 79, 119–137. doi: 10.1890/0012-9658(1998)079[0119:SCAPIF]2.0.CO;2
- Gundale, M. J., DeLuca, T. H., and Nordin, A. (2011). Bryophytes attenuate anthropogenic nitrogen inputs in boreal forests. *Glob. Change Biol.* 17, 2743–2753. doi: 10.1111/j.1365-2486.2011.02407.x
- Gundale, M. J., Gustafsson, H., and Nilsson, M. C. (2009). The sensitivity of nitrogen fixation by a feathermoss–cyanobacteria association to litter and moisture variability in young and old boreal forest. *Can. J. For. Res.* 39, 2542–2549. doi: 10.1139/X09-160
- Gundale, M. J., Nilsson, M., Bansal, S., and Jäderlund, A. (2012a). The interactive effects of temperature and light on biological nitrogen fixation in boreal forests. *New Phytol.* 194, 453–463. doi: 10.1111/j.1469-8137.2012.04071.x
- Gundale, M. J., Wardle, D. A., and Nilsson, M. C. (2012b). The effect of altered macroclimate on N-fixation by boreal feather mosses. *Biol. Lett.* 8, 805–808. doi: 10.1098/rsbl.2012.0429
- Hawes, I., Howard-Williams, C., and Vincent, W. F. (1992). Desiccation and recovery of Antarctic cyanobacterial mats. *Polar Biol.* 12, 587–594. doi: 10.1007/BF00236981
- Hill, P. W., Farrar, J., Roberts, P., Farrell, M., Grant, H., Newsham, K. K., et al. (2011). Vascular plant success in a warming Arctic may be due to efficient nitrogen acquisition. *Nat. Clim. Change* 1, 50–53. doi: 10.1038/nclimate1060
- Hobbie, S. E. (1996). Temperature and plant species control over litter decomposition in alaskan tundra. *Ecol. Monogr.* 66, 503–522. doi: 10.2307/2963492
- Hodge, A., Stewart, J., Robinson, D., Griffiths, B. S., and Fitter, A. H. (2000). Spatial and physical heterogeneity of N supply from soil does not influence N capture by two grass species. *Funct. Ecol.* 14, 575–584. doi: 10.1046/j.1365-2435.2000.t011-1-00470.x
- Houlton, B. Z., Wang, Y. P., Vitousek, P. M., and Field, C. B. (2008). A unifying framework for denitrification in the terrestrial biosphere. *Nature* 454, 327–330. doi: 10.1038/nature07028
- Hyodo, F., Kusaka, S., Wardle, D. A., and Nilsson, M. C. (2013). Changes in stable nitrogen and carbon isotope ratios of plants and soil across a boreal forest fire chronosequence. *Plant Soil* 364, 315–323. doi: 10.1007/s11104-012-1339-8
- Ininbergs, K., Bay, G., Rasmussen, U., Wardle, D. A., and Nilsson, M. C. (2011). Composition and diversity of nifH genes of nitrogen-fixing cyanobacteria associated with boreal forest feather mosses. *New Phytol.* 192, 507–517. doi: 10.1111/j.1469-8137.2011.03809.x
- Inselsbacher, E., and Näsholm, T. (2012). The below-ground perspective of forest plants: soil provides mainly organic nitrogen for plants and mycorrhizal fungi. *New Phytol.* 195, 329–334. doi: 10.1111/j.1469-8137.2012.04169.x
- IPCC. (2007). “In climate change 2007: the physical science basis,” in *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, eds S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt et al. (Cambridge, UK; New York, NY: Cambridge University Press).
- Jackson, B. G., Martin, P., Nilsson, M. C., and Wardle, D. A. (2011). Response of feather moss associated N_2 fixation and litter decomposition to variations in simulated rainfall intensity and frequency. *Oikos* 120, 570–581. doi: 10.1111/j.1600-0706.2010.18641.x
- Jean, M. E., Cassar, N., Setzer, C., and Bellenger, J. P. (2012). Short-term N_2 fixation kinetics in a moss-associated cyanobacteria. *Environ. Sci. Technol.* 46, 8667–8671. doi: 10.1021/es3018539
- Johansson, O., Olofsson, J., Giesler, R., and Plamqvist, K. (2011). Lichen responses to nitrogen and phosphorus additions can be explained by the different symbiont responses. *New Phytol.* 191, 795–805. doi: 10.1111/j.1469-8137.2011.03739.x
- Jones, D. L., Healey, J. R., Willett, V. B., Farrar, J. F., and Hodge, A. (2005). Dissolved organic nitrogen uptake by plants—an important N uptake pathway? *Soil Biol. Biochem.* 37, 413–423. doi: 10.1016/j.soilbio.2004.08.008
- Jones, D. L., and Kielland, K. (2002). Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soils. *Soil Biol. Biochem.* 34, 209–219. doi: 10.1016/S0038-0717(01)00175-4
- Jones, D. L., and Kielland, K. (2012). Amino acid, peptide and protein mineralization dynamics in a taiga forest soil. *Soil Biol. Biochem.* 55, 60–69. doi: 10.1016/j.soilbio.2012.06.005
- Jones, D. L., Nguyen, C., and Finlay, R. D. (2009). Carbon flow in the rhizosphere: carbon trading at the soil-root interface. *Plant Soil* 321, 5–33. doi: 10.1007/s11104-009-9925-0
- Kaasalainen, U., Fewer, D. P., Jokela, J., Wahlsten, M., Sivonen, K., and Rikkinen, J. (2012). Cyanobacteria produce a high variety of hepatotoxic peptide in lichen symbiosis. *Proc. Natl. Acad. Sci. U.S.A.* 109, 5886–5891. doi: 10.1073/pnas.1200279109
- Kaasalainen, U., Jokela, J., Fewer, D. P., Sivonen, K., and Rikkinen, J. (2009). Microcystin production in the tripartite cyanolichen *Peltigera leucophlebia*. *Mol. Plant Microbe Interact.* 22, 695–702. doi: 10.1094/MPMI-22-6-0695
- Kaplan-Levy, R. N., Hadas, O., Summers, M. L., Rücker, J., and Sukenik, A. (2010). “Akinetes: dormant cells of cyanobacteria,” in *Dormancy and Resistance in Harsh Environments, Topics in Current Genetics*. Vol. 21, eds E. Lubzens, J. Cerda, and M. Clark (Berlin Heidelberg: Springer), 5–27.
- Kausrud, H., Mathiesen, C., and Ohlson, M. (2008). High diversity of fungi associated with living parts of boreal forest bryophytes. *Botany* 86, 1326–1333. doi: 10.1139/B08-102
- Keeney, D. R. (1980). Prediction of soil nitrogen availability in forest ecosystems: a literature review. *For. Sci.* 26, 159–171.
- Kielland, K. (1994). Amino acid absorption by arctic plants: implications for plant nutrition and nitrogen cycling. *Ecology* 75, 2373–2383. doi: 10.2307/1940891
- Kielland, K. (1995). Landscape patterns of free amino acids in arctic tundra soils. *Biogeochem* 31, 85–98. doi: 10.1007/BF00000940
- Kotanen, P. M. (2002). Fates of added nitrogen in freshwater arctic wetlands grazed by snow geese: the role of mosses. *Arct. Ant. Alp. Res.* 34, 219–225. doi: 10.2307/1552474
- Krab, E. J., Cornelissen, J. H. C., Lang, S. I., and van Logtestijn, R. S. P. (2008). Amino acid uptake among wide-ranging moss species may contribute to their strong position in higher-latitude ecosystems. *Plant Soil* 304, 199–208. doi: 10.1007/s11104-008-9540-5
- Lang, S. I., Cornelissen, J. H. C., Klahn, T., Van Logtestijn, R. S. P.,

- Broekman, R., Schweikert, W., et al. (2009). An experimental comparison of chemical traits and litter decomposition rates in a diverse range of subarctic bryophyte, lichen and vascular plant species. *J. Ecol.* 97, 886–900. doi: 10.1111/j.1365-2745.2009.01538.x
- Leppänen, S. M., Salemaa, M., Smolander, A., Mäkipää, R., and Tirola, M. (2013). Nitrogen fixation and methanotrophy in forest mosses along a N deposition gradient. *Environ. Exp. Bot.* doi: 10.1016/j.envexpbot.2012.12.006
- Li, Y., and Vitt, D. H. (1997). Patterns of retention and utilization of aerially deposited nitrogen in boreal peatlands. *Écoscience* 4, 106–116.
- Lindo, Z., and Gonzalez, A. (2010). The bryosphere: an integral and influential component of the earth's biosphere. *Ecosystems* 13, 612–627. doi: 10.1007/s10021-010-9336-3
- Longton, R. E. (1988). *Biology of Polar Bryophytes and Lichens*. Cambridge: Cambridge University Press. doi: 10.1017/CBO9780511565212
- Martin, C. E., and Adamson, V. J. (2001). Photosynthetic capacity of mosses relative to vascular plants. *J. Bryol.* 23, 319–323.
- Matsui, K. (2006). Green leaf volatiles: hydroperoxide lyase pathway of oxylipin metabolism. *Curr. Opin. Plant Biol.* 9, 274–280. doi: 10.1016/j.pbi.2006.03.002
- Meeks, J. C. (2009). Physiological adaptations in nitrogen-fixing Nostoc-plant symbiotic associations. *Microbiol. Monogr.* 8, 181–205. doi: 10.1007/7171_2007_101
- Meeks, J. C., and Elhai, J. (2002). Regulation of cellular differentiation in filamentous cyanobacteria in free-living and plant associated symbiotic growth states. *Microbiol. Mol. Biol. Rev.* 65, 94–121. doi: 10.1128/MMBR.66.1.94-121.2002
- Muukkonen, P. (2005). Needle biomass turnover rates of Scots pine (*Pinus sylvestris* L.) derived from the needle-shed dynamics. *Trees* 19, 273–279. doi: 10.1007/s00468-004-0381-4
- Näsholm, T., Ekblad, A., Nordin, A., Giesler, R., Högborg, M., and Högborg, P. (1998). Boreal forest plants take up organic nitrogen. *Nature* 392, 914–916. doi: 10.1038/31921
- Nordin, A., Högborg, P., and Näsholm, T. (2001). Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. *Oecologia* 129, 125–132. doi: 10.1007/s004420100698
- Nordin, A., Strengbom, J., and Ericson, L. (2006). Responses to ammonium and nitrate additions by boreal plants and their natural enemies. *Environ. Pollut.* 141, 167–174. doi: 10.1016/j.envpol.2005.08.017
- Oechel, W. C., and Van Cleve, K. (1986). “The role of bryophytes in nutrient cycling in the taiga,” in *Forest Ecosystems in the Alaskan Taiga: A Synthesis of Structure and Function*, eds K. Van Cleve, F. S. Chapin, III, P. W. Flanagan, L. A. Viereck, and C. T. Dyrness (New York, NY: Springer), 121–137.
- Pajuste, K., and Frey, J. (2003). Nitrogen mineralisation in podzol soils under boreal Scots pine and Norway spruce stands. *Plant Soil* 257, 237–247. doi: 10.1023/A:1026222831694
- Pelster, D. E., Kolka, R. K., and Prepas, E. E. (2009). Overstory vegetation influence nitrogen and dissolved organic carbon flux from the atmosphere to the forest floor: Boreal Plain, Canada. *For. Ecol. Manage.* 259, 210–219. doi: 10.1016/j.foreco.2009.10.017
- Persson, J., and Näsholm, T. (2008). Amino acid uptake: a widespread ability among boreal forest plants. *Ecol. Lett.* 4, 434–438. doi: 10.1046/j.1461-0248.2001.00260.x
- Phil-Karlsson, G., Akselsson, C., Hellsten, S., Karlsson, P. E., and Malm, G. (2009). Vol. IVL rapport B 1851. IVL. Svenska Miljöinstitutet. Göteborg.
- Prescott, C. E., Taylor, B. R., Parsons, W. F. J., Durall, D. M., and Parkinson, D. (1993). Nutrient release from decomposing litter in Rocky Mountain coniferous forests: influence of nutrient availability. *Can. J. For. Res.* 23, 1576–1586. doi: 10.1139/x93-198
- Prins, H. H. T. (1982). Why are mosses eaten in cold environments only? *Oikos* 38, 374–380. doi: 10.2307/3544680
- Proctor, M. C. F. (2001). Patterns of desiccation tolerance and recovery in bryophytes. *Plant Growth Regul.* 35, 147–156. doi: 10.1023/A:1014429720821
- Proctor, M. C. F., Oliver, M. J., Wood, A. J., Alpert, P., Stark, L. R., Cleavitt, N. L., et al. (2007). Desiccation tolerance in bryophytes: a review. *Bryologist* 110, 595–621. doi: 10.1639/0007-2745(2007)110[595:DIBAR]2.0.CO;2
- Rai, A. N., Bergmann, B., and Rasmussen, U. (2002). *Cyanobacteria in Symbiosis*. Dordrecht: Kluwer Academic.
- Rai, A. N., Söderbäck, E., and Bergman, B. (2000). Cyanobacterium-plant symbioses. *New Phytol.* 147, 449–481. doi: 10.1046/j.1469-8137.2000.00720.x
- Read, D. J. (1991). Mycorrhizas in ecosystems. *Experientia* 47, 376–391. doi: 10.1007/BF01972080
- Reed, S. C., Cleveland, C. C., and Townsend, A. R. (2011). Functional ecology of free-living nitrogen fixation: a contemporary perspective. *Annu. Rev. Ecol. Evol. Syst.* 42, 489–512. doi: 10.1146/annurev-ecolsys-102710-145034
- Reed, S. C., Cleveland, C. C., and Townsend, A. R. (2013). Relationships among phosphorus, molybdenum and free-living nitrogen fixation in tropical rain forests: results from observational and experimental analyses. *Biogeochem* doi: 10.1007/s10533-013-9835-9833
- Rousk, J., and Jones, D. L. (2010). Loss of low molecular weight dissolved organic carbon (DOC) and nitrogen (DON) in H₂O and 0.5 M K₂SO₄ soil extracts. *Soil Biol. Biochem.* 42, 2331–2335. doi: 10.1016/j.soilbio.2010.08.017
- Rousk, K., Rousk, J., Jones, D. L., Zackrisson, O., and DeLuca, T. H. (2013a). Feather moss nitrogen acquisition across natural fertility gradients in boreal forests. *Soil Biol. Biochem.* 61, 86–95.
- Rousk, K., DeLuca, T. H., and Rousk, J. (2013b). The cyanobacterial role in the resistance of feather mosses to decomposition - toward a new hypothesis. *PLoS ONE* 8:e62058. doi: 10.1371/journal.pone.0062058
- Scherer, S., and Zhong, Z. P. (1991). Desiccation independence of terrestrial Nostoc commune ecotypes (cyanobacteria). *Microbiol. Ecol.* 22, 271–283. doi: 10.1007/BF02540229
- Schimel, J. P., and Bennett, J. (2004). Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85, 591–602. doi: 10.1890/03-8002
- Scott, G. D. (1960). Studies of the lichen symbiosis. I. The relationship between nutrition and moisture content in the maintenance of the symbiotic state. *New Phytol.* 59, 374–381. doi: 10.1111/j.1469-8137.1960.tb06232.x
- Scott, N. A., and Binkley, D. (1997). Foliage litter quality and annual net N mineralization: comparison across North American forest sites. *Oecologia* 111, 151–159. doi: 10.1007/s004420050219
- Sivonen, K. (2009). “Cyanobacterial toxins,” in *The Encyclopedia of Microbiology*, 3rd Edn., eds M. Schaechter (Oxford: Academic), 290–307.
- Smith, V. R. (1984). Effects of abiotic factors on acetylene reduction by cyanobacteria epiphytic on moss at a subantarctic island. *Appl. Environ. Microbiol.* 48, 594–600.
- Solga, A., Burkhardt, J., Zechmeister, H. G., and Frahm, J. P. (2005). Nitrogen content, 15N natural abundance and biomass of the two pleurocarpous mosses *Pleurozium schreberi* (Brid.) Mitt. and *Scleropodium purum* (Hedw.) Limpr. in relation to atmospheric nitrogen deposition. *Environ. Pollut.* 134, 465–473. doi: 10.1016/j.envpol.2004.09.008
- Sorensen, P. L., Jonasson, S. E., and Michelsen, A. (2006). Nitrogen fixation, denitrification, and ecosystem nitrogen pools in relation to vegetation development in the Subarctic. *Arct. Ant. Alp. Res.* 38, 263–272. doi: 10.1657/1523-0430(2006)38[263:NFAEN]2.0.CO;2
- Sorensen, P. L., Lett, S., and Michelsen, A. (2012). Moss-specific changes in nitrogen fixation following two decades of warming, shading, and fertilizer addition. *Plant Ecol.* 213, 695–706. doi: 10.1007/s11258-012-0034-4
- Sorensen, P. L., and Michelsen, A. (2011). Long-term warming and litter addition affects nitrogen fixation in a subarctic heath. *Glob. Change Biol.* 17, 528–537. doi: 10.1111/j.1365-2486.2010.02234.x
- Startsev, N. A., and Lieffers, V. J. (2006). Dynamic of mineral nitrogen release from feather mosses after dehydration or handling stress. *Bryologist* 109, 551–559. doi: 10.1639/0007-2745(2006)109[551:DOMNRF]2.0.CO;2
- Startsev, N. A., Lieffers, V. J., Landhäuser, S. M., and Velazquez-Martinez, A. (2008). N-transfer through aspen litter and feather moss layers after fertilization with ammonium nitrate and urea. *Plant Soil* 311, 51–59. doi: 10.1007/s11104-008-9657-6
- Startsev, N. A., Lieffers, V. J., and McNabb, D. H. (2007). Effects of feather moss removal, thinning and fertilization in lodgepole pine growth, soil microclimate and stand nitrogen dynamics. *For. Ecol. Manage.* 240, 79–86. doi: 10.1016/j.foreco.2006.12.010
- Steinberg, N. A., and Meeks, J. C. (1991). Photosynthetic CO₂ fixation and ribulose biphosphate carboxylase/oxygenase activity of Nostoc sp. strain UCD (7801).

- in symbiotic association with *Anthroceros punctatus*. *J. Bacteriol.* 171, 6227–6233.
- Storeheier, P. V., Mathiesen, S., Tyler, N., and Olsen, M. (2002). Nutritive value of terricolous lichens for reindeer in winter. *Lichenologist* 34, 247–257. doi: 10.1006/lich.2002.0394
- Street, L. E., Subke, J. A., Sommerkorn, M., Sloan, V., Ducrot, H., Phoenix, G. K., et al. (2013). The role of mosses in carbon uptake and partitioning in arctic vegetation. *New Phyt.* doi: 10.1111/nph.12285
- Sveinbjörnsson, B., and Oechel, W. C. (1992). “Controls of growth and productivity of bryophytes: environmental limitations under current and anticipated condition,” in *Bryophytes and Lichens In A Changing Environment* eds J. W. Bates and A. M. Farmer (Oxford, UK: Clarendon Press), 77–102.
- Tamm, C. O. (1991). *Nitrogen in Terrestrial Ecosystems*. Berlin: Springer. doi: 10.1007/978-3-642-75168-4
- Triebwasser, D. J., Tharayil, N., Preston, C. M., and Gerard, P. D. (2012). The susceptibility of soil enzymes to inhibition by leaf litter tannins is dependent on the tannin chemistry, enzyme class and vegetation history. *New Phytol.* 196, 1122–1132. doi: 10.1111/j.1469-8137.2012.04346.x
- Turetsky, M. R. (2003). The role of bryophytes in carbon and nitrogen cycling. *Bryologist* 106, 395–109. doi: 10.1639/05
- Tyler, G. (1990). Bryophytes and heavy metals: a literature review. *Bot. J. Linn. Soc.* 104, 231–253. doi: 10.1111/j.1095-8339.1990.tb02220.x
- Van Cleve, K., Oliver, L. K., Schlentner, P., Viereck, L. A., and Dyrness, C. T. (1983). Productivity and nutrient cycling in taiga forest ecosystems. *Can. J. For. Res.* 13, 747–766. doi: 10.1139/x83-105
- Vitousek, P., and Hobbie, S. (2000). Heterotrophic nitrogen fixation in decomposing litter: patterns, mechanisms, and models. *Ecology* 75, 418–429. doi: 10.2307/1939545
- Vitousek, P. M., Mooney, H. A., Lubchenco, J., and Melilo, J. M. (1997). Human domination of earth's ecosystems. *Science* 277, 494–499. doi: 10.1126/science.277.5325.494
- Weber, M. G., and Van Cleve, K. (1984). Nitrogen transformation in feather moss and forest floor layers of interior Alaska black spruce ecosystems. *Can. J. For. Res.* 14, 278–290. doi: 10.1139/x84-053
- Wilson, J. A., and Coxson, D. S. (1999). Carbon flux in a subalpine spruce-fir forest: pulse release from *Hylocomium splendens* feather-moss mats. *Can. J. Bot.* 77, 564–569. doi: 10.1139/cjb-77-4-564
- Wurzburger, N., Bellenger, J. P., Kraepiel, A. M. L., and Hedin, L. O. (2012). Molybdenum and phosphorus interact to constrain asymbiotic nitrogen fixation in tropical forests. *PLoS ONE* 7:e33710. doi: 10.1371/journal.pone.0033710
- Yuan, Z. Y., and Chen, H. Y. H. (2012). Fine root dynamics with stand development in the boreal forest. *Func. Ecol.* 26, 991–998. doi: 10.1111/j.1365-2435.2012.02007.x
- Zackrisson, O., DeLuca, T. H., Gentili, F., Sellstedt, A., and Jäderlund, A. (2009). Nitrogen fixation in mixed *Hylocomium splendens* moss communities. *Oecologia* 160, 309–319. doi: 10.1007/s00442-009-1299-8
- Zackrisson, O., DeLuca, T. H., Nilsson, M. C., Sellstedt, A., and Berglund, L. M. (2004). Nitrogen fixation increases with successional age in boreal forests. *Ecology* 85, 3327–3334. doi: 10.1890/04-0461

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 12 April 2013; accepted: 28 May 2013; published online: 17 June 2013.

Citation: Rousk K, Jones DL and DeLuca TH (2013) Moss-cyanobacteria associations as biogenic sources of nitrogen in boreal forest ecosystems. *Front. Microbiol.* 4:150. doi: 10.3389/fmicb.2013.00150

This article was submitted to *Frontiers in Terrestrial Microbiology*, a specialty of *Frontiers in Microbiology*.

Copyright © 2013 Rousk, Jones and DeLuca. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.



Microbes in nature are limited by carbon and energy: the starving-survival lifestyle in soil and consequences for estimating microbial rates

John E. Hobbie^{1*} and Erik A. Hobbie²

¹ The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA, USA

² Earth Systems Research Center, University of New Hampshire, Durham, NH, USA

Edited by:

Johannes Rousk, Lund University, Sweden

Reviewed by:

Michael Zubkov, National Oceanography Centre, UK
Johannes Rousk, Lund University, Sweden

*Correspondence:

John E. Hobbie, The Ecosystems Center, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543, USA
e-mail: jhobbie@mbi.edu

Understanding microbial transformations in soils is important for predicting future carbon sequestration and nutrient cycling. This review questions some methods of assessing one key microbial process, the uptake of labile organic compounds. First, soil microbes have a starving-survival life style of dormancy, arrested activity, and low activity. Yet they are very abundant and remain poised to completely take up all substrates that become available. As a result, dilution assays with the addition of labeled substrates cannot be used. When labeled substrates are transformed into $^{14}\text{CO}_2$, the first part of the biphasic release follows metabolic rules and is not affected by the environment. As a consequence, when identical amounts of isotopically substrates are added to soils from different climate zones, the same percentage of the substrate is respired and the same half-life of the respired $^{14}\text{CO}_2$ from the labeled substrate is estimated. Second, when soils are sampled by a variety of methods from taking 10 cm diameter cores to millimeter-scale dialysis chambers, amino acids (and other organic compounds) appear to be released by the severing of fine roots and mycorrhizal networks as well as from pressing or centrifuging treatments. As a result of disturbance as well as of natural root release, concentrations of individual amino acids of $\sim 10 \mu\text{M}$ are measured. This contrasts with concentrations of a few nanomolar found in aquatic systems and raises questions about possible differences in the bacterial strategy between aquatic and soil ecosystems. The small size of the hyphae (2–10 μm diameter) and of the fine roots (0.2–2 mm diameter), make it very difficult to sample any volume of soil without introducing artifacts. Third, when micromolar amounts of labeled amino acids are added to soil, some of the isotope enters plant roots. This may be an artifact of the high micromolar concentrations applied.

Keywords: microbes, soil, water, activity, labeled substrate, amino acids, sugars

INTRODUCTION

A major goal of microbial ecology is to connect the processes or functions occurring in aquatic and terrestrial ecosystems with the microbes present in those systems. A major function of heterotrophic bacteria and fungi in nature is to break down large organic molecules, transport low molecular weight (LMW) compounds into microbial cells, and use a portion of the LMW compounds for respiration and growth. There is agreement among authors that the concentration and supply rate of bioavailable organic material determine the growth of heterotrophic microbes (Coleman et al., 2004; Egli, 2010; Inselsbacher and Näsholm, 2012). These heterotrophic microbes and the methods used to study them are the subject of this review. In particular, this review deals with methods of measuring microbial rates of use of LMW compounds in soil and compares these with similar methods developed for aquatic ecosystems. This comparison between terrestrial and aquatic ecosystems is based on the premise that metabolic and stoichiometric constraints on microbial metabolism are very similar across ecosystems (Sinsabaugh et al., 2013). Three types

of commonly used soil methods appear to produce doubtful results and are described in Section “Observations and Concerns.”

AQUATIC MICROBES: MEASURES IN THE PLANKTON

For some questions, planktonic systems are easier to study than soil systems; they contain fewer microbes and lack the structure caused by roots and soil particles. The ideal method for measuring heterotrophic processing of LMW compounds in natural systems is to measure the concentration, uptake rate, and respiration rate of the compounds of interest. Uptake of LMW compounds accounts for the majority of carbon used by heterotrophic bacteria. For example, Kirchman (2003) reported that the flux of free amino acids or of glucose alone can support most or all of the bacterial growth. With high pressure liquid chromatography, individual LMW compounds such as sugars and amino acids can be measured to concentrations as low as a few nanomoles per liter. Uptake and respiration rates in aquatic systems are easily measured by adding ^{14}C - or ^{13}C -labeled compounds to samples from nature and incubating for a few hours.

Subsequently, the quantity incorporated into microbes caught on filters (as fine as 0.2 μm pore size) and released as CO_2 is measured. Turnover times of individual LMW compounds may be calculated as the concentration divided by the rate of uptake; when actual incorporation into protein is measured, the bacterial growth rate may be estimated (Fuhrman and Azam, 1982; Kirchman, 2012).

Bacterial abundance is close to 10^6 ml^{-1} in the plankton of lakes, estuaries, and oceans. Growth rates vary greatly but likely average around 0.2 day^{-1} (Pomeroy et al., 2007); in oligotrophic waters rates are very slow, less than 0.01 day^{-1} , while in rich estuaries they may be as high as 1 day^{-1} (Crump et al., 2013). Concentrations of sugars and amino acids are very low, with individual free amino acids ranging from <1 to 20 nM and the total concentrations of amino acids usually $<100 \text{ nM}$ (Fuhrman, 1987; Kirchman, 2012); the concentration of glucose and other free neutral sugars is $<5 \text{ nM}$ (Kirchman, 2000). Microbes are adapted to the extremely low concentrations of amino acids and sugars. In fact, bacteria in aquatic systems likely control the concentration of LMW compounds (Nissen et al., 1984). The turnover time of minutes to hours is rapid enough to result in high fluxes. In a eutrophic estuary in southeastern United States, turnover times of amino acids were as low as 0.7 h at summer temperatures and 206 h at winter temperatures (Crawford et al., 1974).

SOIL BACTERIA

Bacterial abundance is close to 10^9 per gram or several magnitudes greater in soils than in plankton (Whitman et al., 1998). When the total respiration of unmodified soil is used to calculate microbial growth, generation times are estimated at 120–180 days (Coleman et al., 2004); these authors attributed the slow rate to the extreme limitations of available carbon compounds. Faster turnover times are estimated from the microbial incorporation of labeled leucine or thymidine to estimate bacterial growth. With these methods, generation times ranging mostly from 2 to 13 days have been found (Bååth, 1998; Kirchman, 2000; Rousk and Bååth, 2011). Thus, bacteria are abundant and active in soils. Yet, in contrast to aquatic microbes these organisms apparently do not hold the concentrations of LMW compounds at a very low level. For example, concentrations of free amino acids found in soils is high and rather constant. Total free amino acids in 40 soils from around the globe had a mean concentration of $23 \pm 5 \mu\text{M}$ (SE) or nearly 1000 times the concentration in natural waters (Jones et al., 2009).

It should be noted that fungi are also abundant in soils but rare in the plankton and anaerobic salt marsh soils. In terrestrial soils, fungal biomass can be close to that of bacterial biomass (Hobbie and Hobbie, 2012) and fungal turnover is tens to several hundred days (Rousk and Bååth, 2011). In this review, measures of microbial uptake, respiration, and growth are considered to include both bacteria and fungi.

OBSERVATIONS AND CONCERNS

The methods that we are concerned about fall into three categories. Each is first described in this section along with the concerns for the quality of the method. In the next sections, the explanations for the concerns and of the characteristics of microbes in nature are discussed:

- (1) There is a very rapid microbial uptake of any and all added LMW compounds in experiments. The concern is the fact that starving bacteria in soil exist at low levels of activity yet can take up any added and readily available substrate; this rapid removal is a result of the high numbers of bacteria and does not represent the natural occurring rates.
- (2) High concentrations of LMW compounds are measured in soils while extremely low concentrations are present in planktonic systems. Given the presumed ability of planktonic bacteria to draw down available concentrations to nanomolar concentrations, why then do soil bacteria exist in a medium where the concentration of LMW compounds are at micromolar levels? The seeming disparity in concentrations is probably caused in part by mixing of the soil or severe disturbance of fine roots and mycorrhizal networks during soil preparation that release LMW compounds previously unavailable to microbes.
- (3) High rates of release of labeled CO_2 are measured in experiments in which labeled LMW compounds are added. These rates are interpreted to be equivalent to respiration of LMW compounds in nature and the release is used to estimate a half-life of these compounds in the soil, which is usually a few hours. However, once the added substrate is taken up by microbes, then the release rate is controlled by the internal metabolic pathways and not by external environmental conditions. More realistic turnover times, measured in days, are estimated from the ^{14}C held in microbial biomass and not released in the first burst of high respiration.

THE STARVING-SURVIVAL LIFESTYLE

In his 1997 book, titled *Bacteria in Oligotrophic Environments: Starvation-Survival Lifestyles*, Richard Morita reviewed the marine and terrestrial literature on microbial ecology and concluded that most of the biosphere is oligotrophic – and that this should be considered the normal state of most environments. He exempted rich coastal regions of the oceans, eutrophic lakes, and the rhizosphere (see also a discussion of the same theme in *Fundamentals of Soil Ecology*; Coleman et al., 2004). Furthermore, he also believed that the microbial response to oligotrophy, a starvation-survival lifestyle, is the normal physiological state of microorganisms in nature (Morita, 1988, 1997). At any moment, some microbes are active, some are in a state of arrested activity, and some are in a dormant state. Even when in a low activity phase, microbes maintain the biochemical machinery necessary to use any exogenous substrate. A similar concept concludes that the soil microbial community is largely inactive (Kuzakov et al., 2009).

A major aspect of the starving-survival lifestyle is that large numbers of microbes with low activity are poised to respond quickly to added substrate (White, 1995). This aspect of the survival lifestyle is very important to consider when the response (such as CO_2 release) of large numbers of activated soil microbes is measured. This response is less important for measurements in aquatic systems where there are many-fold fewer microbes. Soil microbes are physiologically adapted to respond rapidly. When soil was held in the laboratory for many months (Brookes et al., 1983; De Nobili et al., 2001) microbes maintained ATP and an adenylate charge ratio (AEC) of 0.8, typical of exponentially

growing microbes *in vitro*. The AEC is defined as the quantity $(\text{ATP} + 0.5 \text{ ADP})/(\text{ATP} + \text{ADP} + \text{AMP})$. The rapidity of the respiration response of soil microbes, just a few minutes, to very high micromolar amounts of added glucose and glycine is illustrated in **Figure 1** (Jones and Murphy, 2007).

After an amino acid or a sugar is taken up by the microbial cell, the subsequent release of $^{14}\text{CO}_2$ is controlled by the metabolic pathways within the cell (details in Hobbie and Hobbie, 2012). Because the fundamental pathway leading to respiration of a given amino acid is generally the same for most microbes, the percentage of substrate carbon converted into CO_2 for a given amino acid is the same for many microbial communities and is likely a function of the length of the catabolic pathway (Hobbie and Crawford, 1969). For example, the proportion of amino acid that was respired after addition to a freshwater pond and an estuary for glutamic acid and aspartic acid were always 50–60% while those of basic amino acids, such as leucine at 14% and lysine at 12–14%, were low (Hobbie and Crawford, 1969). These percentages for the individual amino acid taken up into microbial cells were confirmed in a eutrophic lake (Wright, 1974). It is now known that these percentages are the same for microbes in productive freshwaters and estuaries but the percentage of leucine respired increased up to 82% in ultraoligotrophic ocean waters (Alonso-Sáez et al., 2007). Data from the few soil respiration measures are very similar to the aquatic values (Rousk et al., 2011; Hobbie and Hobbie, 2012). It is important to note that in aquatic systems the percent respired is of the amount of the isotope taken up into the cell while in soil all the added substrate is taken up by microbes so the percent respired is of the amount added in the experiment. Thus 45% and 20% of added glutamic acid and lysine, respectively, were respired in a Spanish farm soil (Vinolas et al., 2001) and 50% of added aspartic acid was respired in tundra soils in Alaska (Nordin et al., 2004). In a careful comparison of 40 soils from around the world (polar, tropic, and temperate zones, cultivated, grassland, and forest soils), a mix of 15 amino

acids added to soil produced the respiration of exactly the same percentage of amino acid carbon (of that added) in all the soils (Jones et al., 2009).

A similar conclusion to the above is given, in somewhat different words, in a description of the biphasic pattern of $^{14}\text{CO}_2$ evolution in mineralization studies (Oburger and Jones, 2009). They describe a rapid mineralization phase (phase 1) that is largely independent of the experimental conditions (our metabolic control). A subsequent second phase (phase 2) had a much longer release time for substrate and was significantly affected by incubation conditions. There is a question, however, about what organic compound the ^{14}C is in during the long release period.

LMW COMPOUNDS IN OCEANS, LAKES, AND SOILS: CONCENTRATIONS AND SOURCES
SUGARS, ACETATE, AND AMINO ACIDS IN AQUATIC AND TERRESTRIAL ECOSYSTEMS

The concentrations of sugars, such as glucose and other free neutral sugars, is extremely low in planktonic aquatic systems. In general, these systems contained $<5\text{ nM}$ for individual sugars (Kirchman, 2000) while 2–15 nM of glucose were measured in surface waters of the Gulf of Mexico (Skoog et al., 1999). Somewhat higher concentrations, 20–60 nM, have been found in the equatorial Pacific (Rich et al., 1996).

Sugar concentrations in soils appear to be much higher than in aquatic systems, although the variety of methods used makes exact comparisons difficult. For example, 54 μM glucose was reported in an agricultural soil in North Wales, with sugars extracted by centrifugation of soil samples at 4000 g (Hill et al., 2008). Sugars extracted from soil of temperate forest plantations with 0.25 M sulfuric acid for 16 h averaged 244 mg kg^{-1} soil (equivalent to 4.5 mM, assuming 0.3 g water g^{-1} soil; Johnson and Pregitzer, 2007), whereas concentrations from water leachates of only 2.4 μM sugars ($\sim 30\%$ glucose) were reported from an agricultural soil (Fischer et al., 2007).

A similar picture is found for dissolved free amino acids (**Tables 1 and 2**); the concentration in the upper waters of aquatic

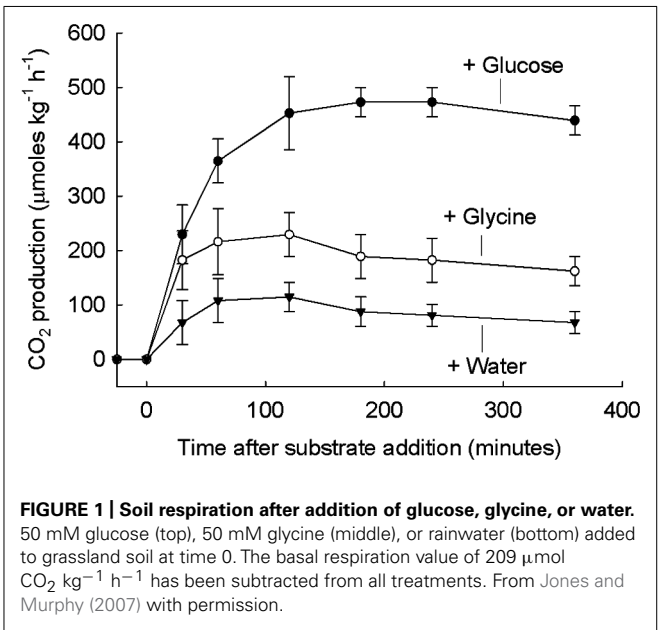


Table 1 | Concentrations of dissolved amino acids measured in lakes, estuaries, and oceans.

Location	Amino acid concentration (nM)	Notes
Ocean ^a	0.1–50	Individual amino acids
Coastal Ocean (New York Bight) ^b	1–15	Individual amino acids
Estuary (North Carolina) ^c	300–500	12 amino acids
Productive lakes ^d	78–277 ^e	Total for five sampling days

^aWilliams (2000).
^bFuhrman and Ferguson (1986).
^cCrawford et al. (1974).
^dJørgensen (1987).
^e1200 and 1500 nM found once.

Table 2 | Total dissolved amino acid concentration measured in soil water or soil water extracts or KCl extract.

System studied	Amino acid concentration (μM)	Notes
40 soils worldwide ^a	23 \pm 5	OPA fluorometry
Boreal forest, Sweden ^b	42–106	Upper organic layers OPA fluorometry
	5–20	Lower layers OPA fluorometry
Boreal forest, Sweden ^c	133	Water extraction,
Agricultural land, Sweden ^d	0.1–12.7	Small tension lysimeters, 2–9 cm depth
Temperate grassland, Wales ^e	23–58	Total for 15 different amino acids, Monthly for 6 mo by HPLC
Pine forest, California ^f	35	Leachate of O horizon, by HPLC
Temperate forest, U.S. ^g	301	Organic horizon
	59.9	Mineral horizon

OPA, ortho-phthaldialdehyde.

^a Polar, temperate, tropical, agriculture, non-agriculture (Jones et al., 2009).

^b van Hees et al. (2008).

^c Inselsbacher and Näsholm (2012). 0.3 g water assumed per g soil.

^d Jämtgård et al. (2010).

^e Jones et al. (2005b).

^f Yu et al. (2002).

^g Maple, ash, oak, beech, hemlock. Soil extracted with KCl immediately after collection, and amino acids assessed by ninhydrin method. For calculation of concentration, 0.3 g water assumed per g soil and published bulk density used (Gallet-Budynek et al., 2009).

systems is a few nanomolar while soil water concentrations are in the micromolar range. As seen in **Table 1**, the concentration is higher in the more eutrophic waters (coastal, estuarine) than in open ocean waters.

If, as widely believed, the microbes in soil are carbon and energy limited (Coleman et al., 2004), how can the micromolar concentrations of LMW compounds remain so high and not be removed? Are aquatic and soil microbes so fundamentally different that one group can live at concentrations of LMW compounds that are four orders of magnitude less than the other? One possibility is that the measured concentrations are correct and that the microbes have adapted to a life of plenty by developing transport systems with a relatively high K_m and that there is no competition among microbial species. A second possibility is that the diffusion of LMW compounds is very slow in soils and the amount of substrate that reaches the cells is low relative to bulk concentrations. A third possibility is that substrates are released from micropores, from severing of fine roots or the networks of mycorrhizal hyphae, or from release from loose attachment with soil particles when the sample of soil is sieved or mixed. Different ways of extracting organic

substrates from soil give different concentrations; for example, Darrouzet-Nardi and Weintraub (personal communication) found that samples from arctic soils extracted with water contained five times more labile N substrates than samples collected with a lysimeter.

In fact, there is evidence for a rapid microbial response to sampling, presumably to the LMW compounds newly made available to microbes. One bit of evidence is the burst of unusually high microbial respiration when cores are first collected (G. Shaver, personal communication). Also, when grassland soil is extracted with distilled water or with K_2SO_4 and ^{14}C -labeled amino acids and sugars are added to the solution, the organic compounds are quickly lost from solution with a maximum rate of 90% loss after 15 min (Rousk and Jones, 2010).

ARE LMW COMPOUNDS PRODUCED FROM DESTRUCTION OF FINE ROOTS AND FUNGAL NETWORKS?

Aquatic sediments are useful for investigating this question because they are anaerobic and therefore lack mycorrhizal fungi. In beds of the salt marsh grass, *Spartina alterniflora*, dissolved organic carbon (DOC) concentrations in the anaerobic sediments are sampled by pounding in a corer (e.g., 6.4 cm diameter), extruding the core, and extracting the water from the core by squeezing or centrifugation. A marsh in Massachusetts yielded DOC concentrations of 4–6 mM (Howes et al., 1985). Because of its importance in the anaerobic pathway of decomposition, acetate was chosen for detailed study; water extracted from a 6.4 cm diameter core by squeezing or centrifugation had acetate concentrations greater than 100 μM (Hines et al., 1994). In contrast, non-destructive collection methods found less than 10 μM acetate. These non-destructive methods either used an *in situ* Teflon sipper deployed several days before the first sampling (Hines et al., 1989) or collected water from an extruded core using a syringe and needle. In an experiment to test if the roots were the source of the acetate, all sediment was first washed from a large core and then the remaining roots were cored and destructively sampled; 75% of the acetate found in intact cores was recovered (Hines et al., 1994). Thus, in this case the roots were certainly the source of the acetate found in the core.

We now consider the evidence for production of LMW compounds from vegetated soils when bacteria, mycorrhizal fungi, and fine roots are all present. All these organisms are potential sources of the organic compounds found in soil water; it was estimated by one group that free amino acids have a concentration of 10 mM within roots (Jones and Darrah, 1994; Farrar et al., 2003; Jones et al., 2005a). Another estimate (Marschner, 1995) was that phloem sap has an amino acid concentration of 50–200 mM. In recent efforts to avoid the disruption to roots and microbes of coring or of other destructive collection and preparation manipulations, a microdialysis chamber sampled nitrogen (N) compounds (ammonium, nitrate, and amino acids) at a depth of 1 cm in the soil solution of a Swedish pine forest. This chamber, made from a semi-permeable membrane 10 mm long and 0.5 mm in diameter, received a continuous flow of deionized water at 5 $\mu\text{l min}^{-1}$ for 30 min (Inselsbacher et al., 2011; Inselsbacher and Näsholm, 2012). After water extraction of the soil by standard methods, concentrations of free

N were dominated by ammonium, up to 79% of the free N, while amino acids and nitrate made up 11 and 10%, respectively (Table 2). In the diffusive flux of N into the dialysis chamber, amino acids were 80% of the free N while ammonium and nitrate each contributed 10%. The authors stated that this approach measures the potential N supply rates in a system where N compounds are continually removed from solution. They suggest that this technique should give a more accurate representation of soil N supply than traditional soil sampling measures of concentration.

One potential problem with the microdialysis method as presented is that disruption of fine roots or fungal hyphae during insertion of the dialysis probe could release amino acids. However, this does not seem to be the case because E. Inselsbacher (personal communication) has a report in preparation showing that samples collected at a number of periods after the insertion of the dialysis probe into the soil show no changes in the time course of concentration.

Another type of evidence that the fungi and fine roots may be a source of the amino acids sampled during microdialysis comes from the compositional profile of amino acids sampled in ectomycorrhizal fungi and in the pine forest soils. The following data come from an experiment in which individual *Pinus sylvestris* seedlings were inoculated with four different taxa of ectomycorrhizal fungus (Finlay et al., 1988). Fungal hyphae subsequently grew from the root tip across a barrier into peat to which ^{15}N -labeled ammonium was added. Labeled free amino acid pools stemming from the hyphal uptake of the label were then measured in the hyphae, the mycorrhizal root tip, the roots, and needles. Labeled nitrogen was found in all four free amino acid pools principally as glutamine/glutamic acid but significant amounts of asparagine/aspartic acid were also found (Finlay et al., 1988). When microdialysis and tension lysimeters were used to sample homogenized boreal forest soils where ectomycorrhizal fungi of pine trees were certainly abundant, the most abundant amino acid was also glutamine followed by valine, alanine, and glutamic acid (Inselsbacher et al., 2011). When the soil amino acids were sampled across a successional sequence of boreal forest plants (willow, alder, balsam poplar, white spruce, and black spruce), the amino acid pool was dominated by glutamic acid, glutamine, aspartic acid, asparagines, alanine, and histidine in every case (Werderin-Pfisterer et al., 2009). Glutamine was also the dominant amino acid in the xylem pool of ectomycorrhizal plants (Pfautsch et al., 2009). We suggest that it is possible that the microdialysis methods as well as other methods of sampling amino acids in soils, such as tension lysimeters, are measuring mostly amino acids released from fine roots and mycorrhizal hyphae. This question about the source of the amino acids in the soil water should be extensively tested.

Finally, what are implications for soil studies generally of the possibility that the breakage of fine roots and mycorrhizal networks add large amounts of LMW compounds to soils during sampling and handling? Certainly the possibility of handling error is not considered in descriptions of methods. An example is the method of studying gross N-cycling rates of aggregates in a non-rhizosphere system (Muruganandam et al., 2010) described in a chapter in Methods in Enzymology (Myrold et al., 2011). After the

soil was collected it was air dried for days and then sieved. The ^{15}N -labeled ammonium or nitrate was added, the soil rehydrated to 60% of water holding capacity, and the soil incubated for a week. An isotope ratio mass spectrometer was used for analysis and the net rates of change calculated from the isotope pool dilution. No mention was made about any potential errors introduced during sampling of the soil or of the effect of increased microbial activity from labile organic compounds added during sampling and preparation.

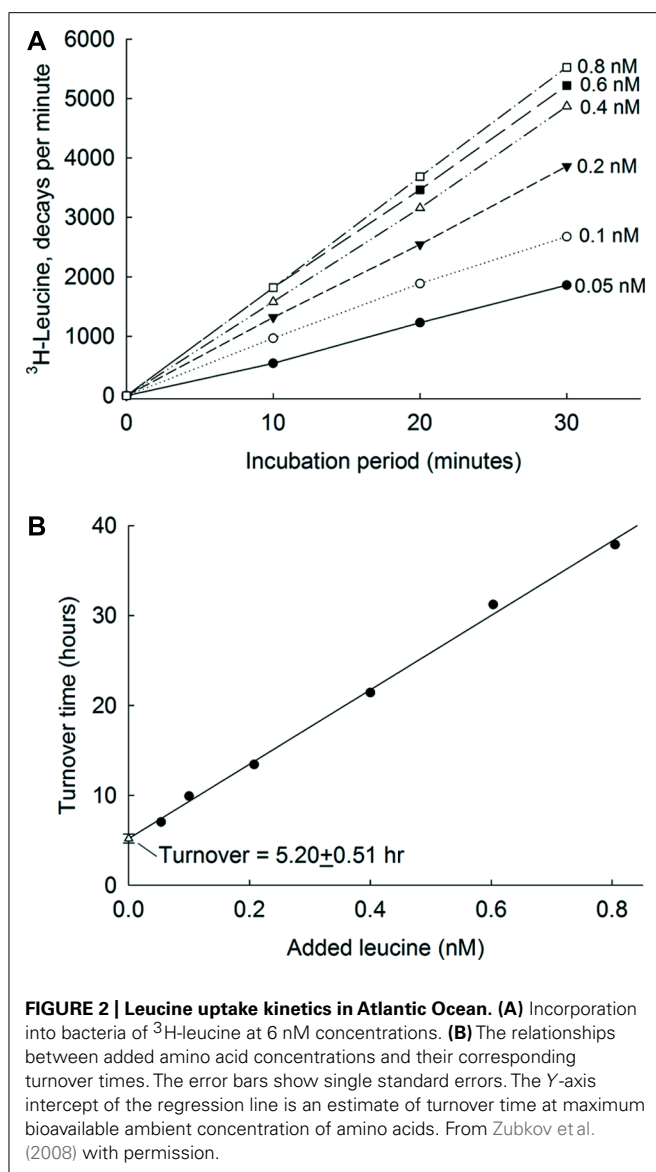
TURNOVER OF LMW COMPOUNDS

AQUATIC METHODS: ISOTOPE DILUTION MEASURES OF UPTAKE VELOCITY WORKS WHEN CONCENTRATION OF THE ADDED SUBSTRATE IS NEAR NATURAL LEVEL

In planktonic systems, the turnover of organic compounds is defined as the substrate concentration (S) divided by the uptake velocity (v). This may be measured by a short-term incubation of the sample with an array of different concentrations of a labeled substrate. The incubation must take place while uptake of the bacterioplankton is directly proportional to time (as shown in Figure 2A). The uptake velocity is sometimes increased by including the carbon respired during the experiment. When the concentration of substrate added (A) is close to the concentration S , the uptake follows Michaelis–Menten kinetics and the experiment can be analyzed as a dilution bioassay (Figure 2B). When the uptake velocity, v , is measured at various concentrations of A , each result can be plotted as a turnover time for that amount (A) of added substrate plus an unknown natural level of substrate (S). The extrapolation to zero added substrate (the Y intercept) is then the turnover at the natural level of substrate (Wright and Hobbie, 1966). In ultraoligotrophic ocean waters picomolar (pM) concentrations of leucine were added for uptake studies (Zubkov et al., 2008). The kinetic analysis of water from the Atlantic Ocean incubated for 30 min (Figure 2) shows that leucine was present in very low concentrations (0.1–0.2 nM and that the turnover time, the intercept on the Y axis, was around 5 h. See also Hobbie and Hobbie (2012) for a detailed explanation of the derivation of the equations.

SOILS METHODS: ISOTOPE DILUTION FAILS WHEN ALL ADDED SUBSTRATE IS IMMEDIATELY TAKEN UP

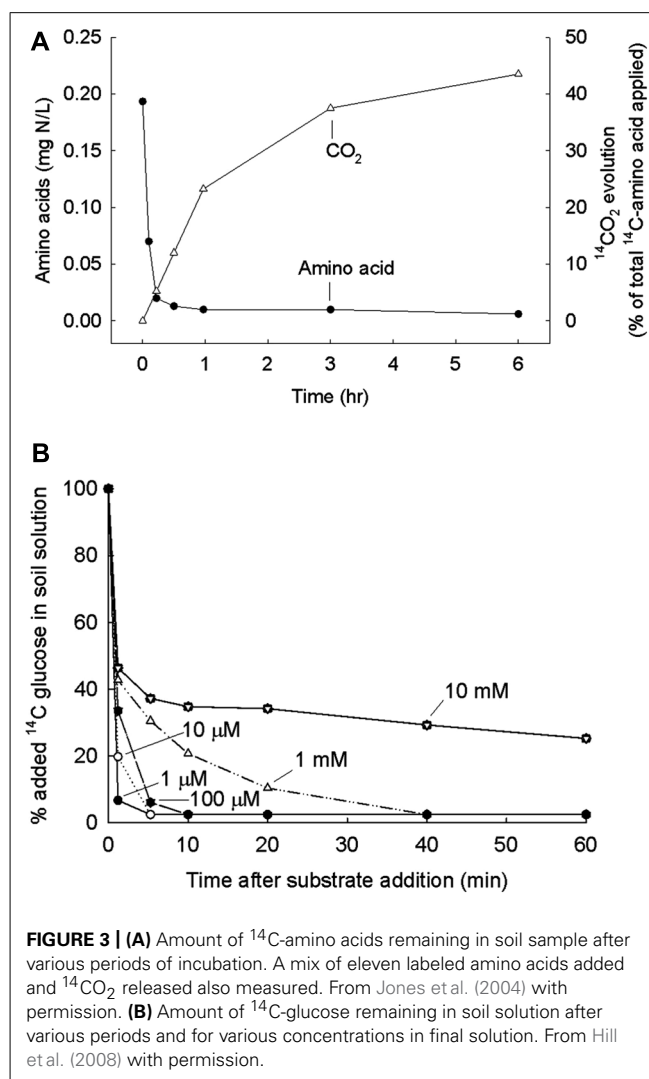
The methodology devised to examine the turnover of LMW compounds in soil is quite different from the dilution analysis that works in the plankton community. The necessity for the different methodology is shown in Figure 3A. In soil there are large numbers of low-activity microbes poised to respond quickly to added substrates. Because of this, uptake is immediate and complete for all of the added substrates, both amino acids and sugars; respiration of substrates to $^{14}\text{CO}_2$ also begins immediately which, incidentally, is proof that microbes are involved and not inorganic processes. Figure 3B shows that the rate of uptake into microbes is not affected by the concentration of the added substrate, at least at concentrations below 1 mM. Isotope dilution with different quantities of added glucose or amino acid does not work when all the substrate is immediately taken up. A modification that does work is to add additional water, to use homogenization, and centrifugation to extract bacteria from the soil matrix before adding



the labeled leucine, and to measure incorporation of leucine into cellular protein (Bååth, 1994, 1998; Rousk and Bååth, 2011).

SOILS METHODS: $^{14}\text{CO}_2$ PRODUCTION FROM ADDED SUBSTRATE MEASURES WITHIN-CELL PROCESSES ONLY AND NOT ENVIRONMENTAL EFFECTS

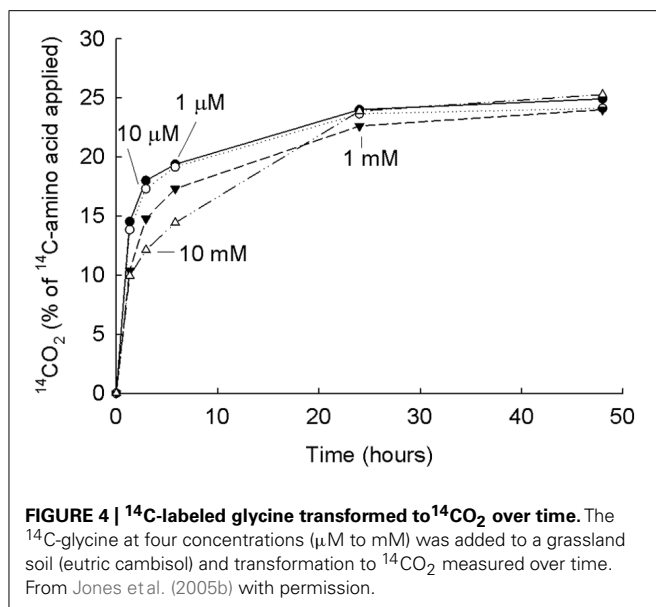
A different way of estimating the turnover or mineralization in soil is to measure the rate of $^{14}\text{CO}_2$ production after the substrate is taken up (e.g., Figure 4 for labeled glycine). This popular method makes use of concentrations of LMW labeled compounds added to produce concentrations in the micromolar range. The result of the measurements is estimations (Hill et al., 2008) of the half-life of the total amount of $^{14}\text{CO}_2$ formed from the labeled substrate; in this paper labeled glucose was added to produce a series of concentrations from 1 to 10,000 μM . The range of the calculated half-life range was 8–11 min and the assumption was made by the authors that this is equivalent to 10–1000 turnovers of the soil



glucose per day. Several papers have pointed out that the $^{14}\text{CO}_2$ is released in two phases (Hill et al., 2008; Oburger and Jones, 2009; Glanville et al., 2012), that is, an initial rapid phase of evolution (phase 1) followed by a slower phase (phase 2).

What actually is being measured in this method? The method neither measures the actual rate of respiration nor the turnover rate of substrate in soil. It does measure the time at which half of the total respired substrate has been respired; for example, if 60% of applied glucose is ultimately respired, the time at which 30% of the glucose has been respired is estimated. It thus measures the half-life for microbial respiration of the labeled substrate. Then the assumption is made that this equals the half-life of the amino acid or sugar in the soil. The actual turnover time in the unmodified soil is not measured; we suggest that it is the turnover time for labeled substrate inside the cell that is measured. In nature, many different environmental variables would change the actual turnover time such as number of microbes, their activity, and the concentration of amino acids or sugars.

What are the characteristic of soil microbes that cause difficulty with this method of estimating substrate turnover? The first



characteristic is that the microbes under energy and carbon limitation are poised to immediately take up the LMW compounds when they become available. In fact, the microbial physiology changes so that many substrates are taken up (the mixed substrate growth described by Egli, 2010). When labeled LMW compounds are added to soil, they are all immediately taken up by microbes no matter what the concentration added (from nanomolar to high micromolar levels; **Figure 3A**).

SOIL METHODS: A GIVEN SUBSTRATE HAS THE SAME TURNOVER TIME IRRESPECTIVE OF THE ENVIRONMENT

The second characteristic, that applies to the first phase of release, is that the release rate of $^{14}\text{CO}_2$ is controlled by the biochemical efficiency of pathways within the cell (see earlier discussion in the section “The Starving-Survival Lifestyle”) and not by events in the external environment. If the release rate of $^{14}\text{CO}_2$ is controlled by fundamental metabolic pathways common to all heterotrophic microbes and not by the activity of the microbial community of an individual soil, then soil samples treated with the same amount of labeled amino acid will always produce similar estimates of the half-time for mineralization. This was the exact finding for 40 soils collected worldwide (Jones et al., 2009); the mean global concentration of total amino acid was $23 \pm 5 \mu\text{M}$ and the half-life was $1.8 \pm 0.1 \text{ h}$. In addition, for all 40 soils an average of 71% of the substrate (a single addition of 15 amino acids totaling $20 \mu\text{M}$) was retained in the microbial cells and 29% was respired. Zoe Cardon and John Stark (personal communication) point out that this is exactly the summed percentage of 15 amino acids taken up and then respired in an aquatic study (Hobbie and Crawford, 1969) where each amino acid was added individually to sub-samples of a rich pond. The key factor is that microbes in both aquatic and soil systems processed the added labeled amino acids by the same fundamental biochemical pathways such as that leading to the citric acid cycle for respiration. We suggest that results from ponds and soils are so

similar because the same biochemical pathways dominate microbial processing across these two environments. A similar result was found for mineralization of amino acids and sugars across a soil pH gradient; the mineralization process was the same across the gradient while the microbial communities differed dramatically (Rousk et al., 2011).

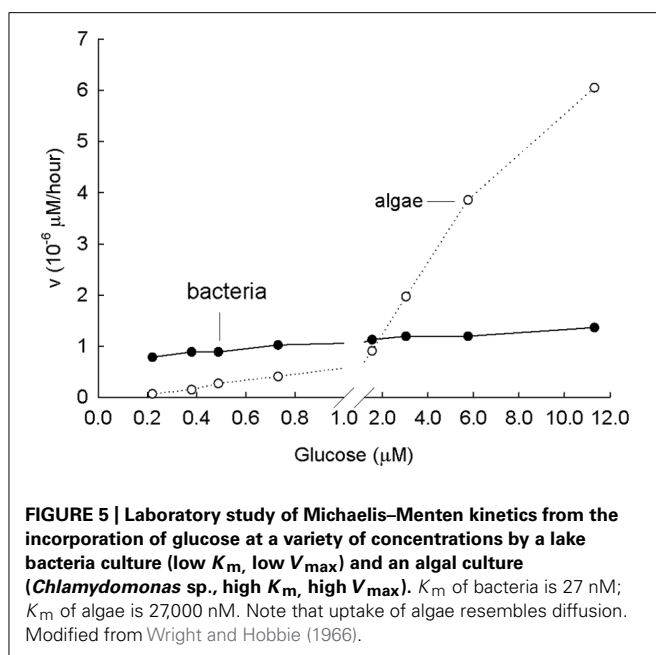
SOILS METHODS: LONG-TERM SLOW RELEASE OF $^{14}\text{CO}_2$ FROM ADDED SUBSTRATE MEASURES BREAKDOWN OF MICROBIALLY CREATED COMPOUNDS, NOT TURNOVER OF ORIGINAL SUBSTRATE IN NATURE

A different approach to measuring the turnover of LMW compounds in soil is to add low amounts of labeled compounds and to follow the release of $^{14}\text{CO}_2$ for 7 days (Glanville et al., 2012). In this experiment, 31 different labeled compounds were added individually to small chambers (6.1 cm^2) formed by pushing plastic cylinders $\sim 2 \text{ cm}$ into the soil of grassland in North Wales. The label ($< 10 \text{ nM}$) was added in 0.5 ml of water gently placed on the top of the soil. The $^{14}\text{CO}_2$ was collected in a NaOH trap inside the chamber. The total substrate half-life was estimated as ranging from 1 to 40 days with most substrates from 5 to 30 days. This range, which is the sum of that for phase 1 and phase 2, is much longer than estimates of several hours obtained when high (μM) concentrations of labeled substrate were added and only the phase 1 half-lives reported. There is, however, a conceptual problem with phase 2. That is, exactly what compound is being followed when all the isotope is in microbial biomass? Does the release of $^{14}\text{CO}_2$ reflect a rate of use of the original (added) compound? Yet, the general method of low concentrations and long incubations has potential for giving a more detailed understanding of how microbes process individual LMW compounds in the soil. One unanswered question that comes from our discussion of possible causes of high concentrations of LMW compounds in soil samples is about the possible effects of disrupting hyphal networks and fine roots. As a result of this disruption, measured concentrations of LMW compounds might increase thereby affecting calculations of turnover rates.

IS THERE AN IMPORTANT TRANSFER OF DISSOLVED ORGANIC NITROGEN COMPOUNDS FROM AQUATIC AND SOIL ENVIRONMENTS INTO INVERTEBRATES AND PLANTS?

In this review, we have argued that microbes quickly assimilate all available substrates and hold the concentration of amino acids and sugars at very low concentrations. Although the argument is backed by chemical measurement in planktonic aquatic systems, where measured amounts are in the nanomolar range, measured concentrations in soil waters appear to be much higher, in the micromolar range. We argue that the high concentrations were not available to microbes and came from the destruction of soil structure, perhaps the mycorrhizal hyphal network or fine roots, during sample preparation (Hobbie and Hobbie, 2012).

In the literature, the present understanding is that LMW compounds are present in micromolar concentrations in the soil. What happens in experiments when these high concentrations of labeled amino acids or sugars are added and when plants or animals are present? Fundamental information on the topic comes from studies in aquatic systems and with aquatic organisms. For example, the kinetics of the uptake of glucose in freshwaters was investigated

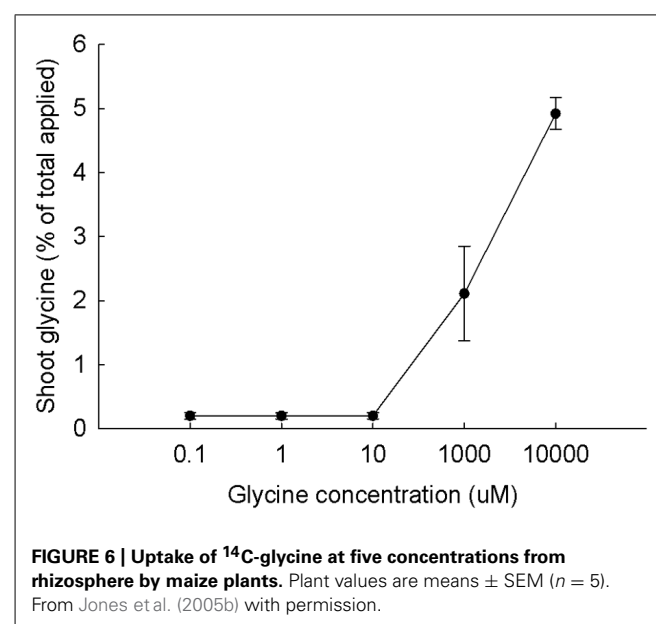


(Figure 5) with a bacterial culture (K_m of 27 nM) and an algal culture of *Chlamydomonas* sp. (K_m of 27 μ M) over a range of low concentrations (Wright and Hobbie, 1966). The bacterial culture was freshly isolated; the algae grew either in the light or on high concentrations of glucose in the dark (Bennett and Hobbie, 1972). In the experiment in Figure 5, at low concentrations ($<1 \mu$ M), the bacterial uptake rises to V_{max} as the transport systems become saturated. Over the range 0.3 to 11 μ M glucose (0.05–2 mg l⁻¹) algal uptake increased linearly. This linear increase over the entire range of expected glucose concentrations indicates that a diffusion-like process is driving the uptake. Therefore, if the labeled glucose is added at only one relatively high concentration, which is typical of most soil measurements made, there is no recognition of the importance of concentration added and algal uptake is believed to outcompete bacterial uptake. However, the ecological question is not whether LMW compounds enter the cell but rather whether the contribution of sugars and amino acids is important to the energy and growth requirement of these cells? The value of studying uptake at a number of concentrations of the added substrate is obvious.

Research on the uptake of organic substance into marine invertebrates (Gomme, 2001) has gone through several cycles since it began in the 1870s. Over time, methods have finally improved enough that it is recognized that inshore waters hold a total of 0.1–1 μ M free amino acids and that a net uptake of amino acids and glucose by the integument of soft-bodied marine invertebrates does occur at concentrations of $\sim 1 \mu$ M (Stephens, 1988). Influx across epidermal membranes was found to be saturable and occurred by means of several substrate-specific pathways (Gomme, 2001). Today, energy budget studies have concluded that dissolved organic matter in the coastal ocean is at least a supplementary energy source for marine invertebrates. However, we note that this conclusion holds only for coastal regions with the highest measured concentrations of amino acids (Table 1).

The evidence presented about the fate of LMW compounds in aquatic systems shows that sugars and amino acids commonly pass through cell membranes and enter eukaryotes both via diffusion and by transport systems. What is the evidence for effect of concentrations of LMW compounds on uptake into plant roots? The only published study we know of is that for glycine uptake in maize plants (Jones et al., 2005b; Figure 6). Uptake was very low until the glycine concentration exceeded 10 μ M. In a similar study, Campbell (2010) grew cucumber plants on sterile sand and Hoagland's solution and tested the uptake of ¹⁴C-leucine into roots at 0.1, 1, 10, 100, and 150 μ M. Incorporation was very low until leucine concentrations of 100 and 150 μ M were reached. Significant uptake was also measured for uptake of labeled amino acids into tomato roots at concentrations of 10–20 μ M (Ge et al., 2009). It is likely that labeled amino acids will enter the roots of many species of plants when uptake is measured at tens and hundreds of micromolar concentrations of substrate.

We are left with uncertainty about uptake rates at the concentrations of amino acids and sugars that are actually available to plant roots. One problem in this type of experiment has already been described because the soil microbes may take up all the added substrate. For example, in a 24 h experiment, labeled amino acids at 100 μ M were added to a microcosm with both microbes and wheat roots present; only 6% of the label ended up in the plant roots and microbes removed the rest (Owen and Jones, 2001). In a direct injection of ¹⁵N-glycine into an arctic soil, after 1 day up to 80% of the ¹⁵N was found in microbes and 2% in tree roots (Sorensen et al., 2008). The percent in the tree roots was unchanged a year later. Virtually the same results were found when ¹⁵N-glycine was added to a deciduous forest soil; after 45 min only 0.07% of the added ¹⁵N was in fine roots and 46% in microbial biomass (McFarland et al., 2002). The percentage in fine roots increased steadily with time, reaching 1.6% after 2 weeks of incubation.



Experimental additions of isotopically labeled amino acids at the same high concentrations that amino acids are chemically measured in the soil have led to the conclusion that plants take up significant amounts of amino acids from soil (Neff et al., 2003). Yet, the ecological significance of organic N uptake for plant N nutrition is still a matter of discussion (Näsholm et al., 2009). As we have discussed, one overlooked topic in almost all publications considering the importance of direct plant uptake of organic N is the ability of soil microbes to remove LMW compounds from soil solution. The typical experiment with a single relatively high concentration of added amino acid and no time course of transfer leaves many questions about rapidity of the removal and of the actual concentrations available to the roots. Once the question about the actual uptake rate is solved, then the larger question can be approached: is the organic nitrogen entering roots from the soil pool an important part of the total nitrogen budget of the plant?

CONCLUSION

- (1) Microbes in natural ecosystems, such as lakes, oceans, and soils, have a starving-survival life style of dormancy, and low activity yet are able to quickly respond to added substrate. While bacteria in planktonic systems keep sugars and amino acids at the nanomolar level, in soil the concentrations of these compounds are measured at the micromolar level. Another difference among ecosystems is that soils have several orders of magnitude more bacteria per unit of volume than do aquatic systems. Fungal hyphae are also abundant in soils and not in the plankton. This disparity of LMW compound concentration and of biomass leads to the conclusion that the high concentrations of LMW compounds in soil are not available to microbes.
- (2) The high concentrations of LMW compounds in soil are most likely caused by sampling-induced release of LMW compounds from disturbed soil structures and from damaged roots and mycorrhizal hyphae. The small size of the hyphae and of the fine roots make it very difficult to sample any volume of soil without introducing artifacts.
- (3) Kinetic analysis of uptake and turnover of LMW compounds is carried out by isotope dilution in planktonic systems. In soils, however, all added substrate is immediately taken up so dilution analysis is not possible. Instead the biphasic rate of production of $^{14}\text{CO}_2$ over time has been used to estimate the half-lives of the labeled compounds. Most of the labeled compound is rapidly respired (phase 1) and the percent respired and the rate follows metabolic rules that apply to most microbes. Thus the phase 1 results are independent of the environment and cannot be used to measure half-lives of compounds. In contrast, the longer-term and slow phase 2 release is affected by the environment and could be useful in understanding cycling of individual LMW compounds.
- (4) It is possible that the effect of the increased concentrations of LMW compounds caused by sampling disturbance is not confined to carbon cycling methods. Are measures of the rates of *in situ* nitrogen turnover also too high?
- (5) Dissolved organic compounds move into cells of plants, fungi, and larval animals by diffusion as well as by various transport mechanisms. If the experimental concentrations of labeled amino acids or sugars greatly exceed natural concentrations, then measured rates of dissolved organic compound use will be higher than the natural rates. Therefore, the effects of different concentrations of substrates used in experiments must be measured. The importance of dissolved organic matter in the carbon and nitrogen budgets of algae, fungi, plants, and even larval animals is still under discussion.
- (6) Field experiments where labeled amino acids and sugars are added to soils and the transport of the isotope into trees is measured must also include time-course measures of the rates of uptake into both bacteria and fungi. It is probable that the added substrate or the label passed through microbes before entering trees.

ACKNOWLEDGMENTS

We thank Zoe Cardon, Ben Colman, and John Stark for helpful comments. This work was supported by the National Science Foundation's Office of Polar Programs (1108074), Division of Environmental Biology (1026843 and 0423385), and Division of Ocean Sciences (OCE-1238212).

REFERENCES

- Alonso-Sáez, L., Gasol, J. M., Aristegui, J., Vilas, J. C., Vaque, D., Duarte, C. M., et al. (2007). Large-scale variability in surface bacterial carbon demand and growth efficiency in the subtropical northeast Atlantic Ocean. *Limnol. Oceanogr.* 52, 533–546. doi: 10.4319/lo.2007.52.2.0533
- Bååth, E. (1994). Measurement of protein synthesis by soil bacterial assemblages with the leucine incorporation technique. *Biol. Fertil. Soils* 7, 147–153. doi: 10.1007/BF00337747
- Bååth, E. (1998). Growth rates of bacterial communities in soils at varying pH: a comparison of the thymidine and leucine incorporation techniques. *Microb. Ecol.* 36, 316–327. doi: 10.1007/s002489900118
- Bennett, M. E., and Hobbie, J. E. (1972). The uptake of glucose by *Chlamydomonas* sp. *J. Phycol.* 8, 392–398.
- Brookes, P. C., Tate, K. R., and Jenkinson, D. S. (1983). The adenylate energy-charge of the soil microbial biomass. *Soil Biol. Biochem.* 15, 9–16. doi: 10.1016/0038-0717(83)90112-90118
- Campbell, M. (2010). *Amino Acids from Soil Do Enter Roots but are Not Important for Trees in Nature*. Semester in Environmental Science report. Woods Hole: Marine Biological Laboratory.
- Coleman, D. C., Crossley, D. A. Jr., and Hendrix, P. F. (2004). *Fundamentals of Soil Ecology*, 2nd Edn. Amsterdam: Elsevier.
- Crawford, C. C., Hobbie, J. E., and Webb, K. L. (1974). The utilization of dissolved free amino acids by estuarine microorganisms. *Ecology* 55, 551–563. doi: 10.2307/1935146
- Crump, B. C., Ducklow, H. W., and Hobbie, J. E. (2013). "Estuarine microbial food webs", in *Estuarine Ecology*, 2nd Edn, eds J. W. Day, Jr., B. C. Crump, W. M. Kemp, and A. Yáñez-Arancibia (Hoboken, NJ: Wiley-Blackwell), 263–284.
- De Nobili, M., Contin, M., Mondini, C., and Brookes, P. C. (2001). Soil microbial biomass is triggered into activity by trace amounts of substrate. *Soil Biol. Biochem.* 33, 1163–1170. doi: 10.1016/S0038-0717(01)00020-7
- Egli, T. (2010). How to live at very low substrate concentration. *Water Res.* 44, 4826–4837. doi: 10.1016/j.watres.2010.07.023
- Farrar, J. F., Hawes, M., Jones, D. L., and Lindow, S. (2003). How roots control the flux of carbon to the rhizosphere. *Ecology* 84, 827–837. doi: 10.1890/0012-9658(2003)084[0827:HRCTFO]2.0.CO;2
- Finlay, R. D., Ek, H., Odham, G., and Söderström, B. (1988). Mycelial uptake, translocation and assimilation of nitrogen from ^{15}N -labelled ammonium by *Pinus sylvestris* plants infected with four different ectomycorrhizal fungi. *New Phytol.* 110, 59–66. doi: 10.1111/j.1469-8137.1988.tb00237.x
- Fischer, H., Meyer, A., Fischer, K., and Kuzyakov, Y. (2007). Carbohydrate and amino acid composition of dissolved organic matter leached from soil. *Soil Biol. Biochem.* 39, 2926–2935. doi: 10.1016/j.soilbio.2007.06.014

- Fuhrman, J. (1987). Close coupling between release and uptake of dissolved free amino acids in seawater studied by an isotope dilution approach. *Mar. Ecol. Prog. Ser.* 37, 45–52. doi: 10.3354/meps037045
- Fuhrman, J., and Ferguson, R. L. (1986). Nanomolar concentrations and rapid turnover of dissolved free amino acids in seawater: agreement between chemical and microbiological measurements. *Mar. Ecol. Prog. Ser.* 33, 237–242. doi: 10.3354/meps033237
- Fuhrman, J. A., and Azam, F. (1982). Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters – evaluation and field results. *Mar. Biol.* 66, 109–120. doi: 10.1007/BF00397184
- Gallet-Budynek, A., Brzostek, E., Rodgers, V. L., Talbot, J. M., Hyzy, S., and Finzi, A. C. (2009). Intact amino acid uptake by northern hardwood and conifer trees. *Oecologia* 160, 129–138. doi: 10.1007/s00442-009-1284-2
- Ge, T., Song, S., Roberts, P., Jones, D. L., Huang, D., and Iwasaki, K. (2009). Amino acids as a nitrogen source for tomato seedlings: the use of dual-labeled (^{13}C , ^{15}N) glycine to test for direct uptake by tomato seedlings. *Environ. Exp. Bot.* 66, 357–361. doi: 10.1016/j.envexpbot.2009.05.004
- Glanville, H., Rousk, J., Golyshtin, P., and Jones, D. L. (2012). Mineralization of low molecular weight carbon substrates in soil solution under laboratory and field conditions. *Soil Biol. Biochem.* 48, 88–95. doi: 10.1016/j.soilbio.2012.01.015
- Gomme, J. (2001). Transport of exogenous organic substances by invertebrate integuments: the field revisited. *J. Exp. Zool.* 289, 254–265. doi: 10.1002/1097-010X(20010401/30)289:4<254::AID-JEZ6>3.0.CO;2-F
- Hill, P. W., Farrar, J. F., and Jones, D. L. (2008). Decoupling of microbial glucose uptake and mineralization in soil. *Soil Biol. Biochem.* 40, 616–624. doi: 10.1016/j.soilbio.2007.09.008
- Hines, M. E., Banta, G. T., Giblin, A. E., Hobbie, J. E., and Tugel, J. B. (1994). Acetate concentrations and oxidation in salt-marsh sediments. *Limnol. Oceanogr.* 39, 140–148. doi: 10.4319/lo.1994.39.1.0140
- Hines, M. E., Knollmeyer, S. L., and Tugel, J. B. (1989). Sulfate reduction and other sedimentary biogeochemistry in a northern New England salt marsh. *Limnol. Oceanogr.* 34, 578–590. doi: 10.4319/lo.1989.34.3.0578
- Hobbie, J. E., and Crawford, C. C. (1969). Respiration corrections for bacterial uptake of dissolved organic compounds in natural waters. *Limnol. Oceanogr.* 14, 528–532. doi: 10.4319/lo.1969.14.4.0528
- Hobbie, J. E., and Hobbie, E. A. (2012). Amino acid cycling in plankton and soil microbes studied with radioisotopes: measured amino acids in soil do not reflect bioavailability. *Biogeochemistry* 107, 339–360. doi: 10.1007/s10533-010-9556-9
- Howes, B. L., Dacey, J. W. H., and Wakeham, S. G. (1985). Effects of sampling technique on measurements of porewater constituents in salt marsh sediments. *Limnol. Oceanogr.* 46, 1358–1369. doi: 10.4319/lo.1985.30.1.0221
- Inselsbacher, E., and Näsholm, T. (2012). The below-ground perspective of forest plants: soil provides mainly organic nitrogen for plants and mycorrhizal fungi. *New Phytol.* 195, 329–334. doi: 10.1111/j.1469-8137.2012.04169.x
- Inselsbacher, E., Öhlund, J., Jämtgård, S., Huss-Danell, K., and Näsholm, T. (2011). The potential of microdialysis to monitor organic and inorganic nitrogen compounds in soil. *Soil Biol. Biochem.* 43, 1321–1332. doi: 10.1016/j.soilbio.2011.03.003
- Jämtgård, S., Näsholm, T., and Huss-Danell, K. (2010). Nitrogen compounds in soil solutions of agricultural land. *Soil Biol. Biochem.* 42, 2325–2330. doi: 10.1016/j.soilbio.2010.09.011
- Johnson, R. M., and Pregitzer, K. S. (2007). Concentration of sugars, phenolic acids, and amino acids in forest soils exposed to elevated atmospheric CO_2 and O_3 . *Soil Biol. Biochem.* 39, 3159–3166. doi: 10.1016/j.soilbio.2007.07.010
- Jones, D. L., and Darragh, P. R. (1994). Influx and efflux of amino acids from *Zea mays* L. roots and its implications in the rhizosphere and N nutrition. *Plant Soil* 163, 1–12.
- Jones, D. L., Healey, J. R., Willett, V. B., Farrar, J. F., and Hodge, A. (2005a). Dissolved organic nitrogen uptake by plants – an important N uptake pathway? *Soil Biol. Biochem.* 37, 413–423. doi: 10.1016/j.soilbio.2004.08.008
- Jones, D. L., Shannon, D., Junvee-Fortune, T., and Farrar, J. F. (2005b). Plant capture of free amino acids is maximized under high soil amino acid concentrations. *Soil Biol. Biochem.* 37, 179–181. doi: 10.1016/j.soilbio.2004.07.021
- Jones, D. L., Kielland, K., Sinclair, F. L., Dahlgren, R. A., Newsham, K. K., Farrar, J. F., et al. (2009). Soil organic nitrogen mineralization across a global latitudinal gradient. *Glob. Biogeochem. Cycles* 23, 1016. doi: 10.1029/2008GB003250
- Jones, D. L., and Murphy, D. V. (2007). Microbial response time to sugar and amino acid additions to soil. *Soil Biol. Biochem.* 39, 2178–2182. doi: 10.1016/j.soilbio.2007.03.017
- Jones, D. L., Shannon, D., Murphy, D. V., and Farrar, J. (2004). Role of dissolved organic nitrogen (DON) in soil N cycling in grassland soils. *Soil Biol. Biochem.* 36, 749–756. doi: 10.1016/j.soilbio.2004.01.003
- Jørgensen, N. O. G. (1987). Free amino acid in lakes: concentrations and assimilation rates in relation to phytoplankton and bacterial production. *Limnol. Oceanogr.* 32, 97–111. doi: 10.4319/lo.1987.32.1.0097
- Kirchman, D. L. (2000). “Uptake and regeneration of inorganic nutrients by marine heterotrophic bacteria,” in *Microbial Ecology of the Oceans*, ed. D. L. Kirchman (New York: Wiley-Liss), 261–288.
- Kirchman, D. L. (2003). “The contribution of monomers and other low molecular weight compounds to the flux of DOM in aquatic ecosystems,” in *Aquatic Ecosystems – Dissolved Organic Matter*, eds S. Findlay and R. L. Sinsabaugh (New York: Academic Press), 217–241.
- Kirchman, D. L. (2012). *Processes in Microbial Ecology*. New York: Oxford University Press.
- Kuzyakov, Y., Blagodatskaya, E., and Blagodatsky, S. (2009). Comments on the paper by Kemmitt et al. (2008), Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass – A new perspective [Soil Biol. Biochem. 40, 61–73]: the biology of the regulatory gate. *Soil Biol. Biochem.* 41, 435–439. doi: 10.1016/j.soilbio.2008.07.023
- Marschner, H. (1995). *Mineral Nutrition of Higher Plants*. New York: Academic Press.
- McFarland, J. W., Ruess, R. W., Kielland, K., and Doyle, A. P. (2002). Cycling dynamics of NH_4^{++} and amino acid nitrogen in soils of a deciduous boreal forest ecosystem. *Ecosystems* 5, 775–788.
- Morita, R. Y. (1988). Bioavailability of energy and its relationship to growth and starvation survival in nature. *Can. J. Microbiol.* 34, 436–441. doi: 10.1139/m88-076
- Morita, R. Y. (1997). *Bacteria in Oligotrophic Environments: Starvation-Survival Lifestyle*. New York: Chapman Hall.
- Myrold, D. D., Pett-Ridge, J., and Bottomley, P. J. (2011). “Nitrogen mineralization and assimilation at millimeter scales,” in *Methods in Enzymology, Research on Nitrification and Related Processes*, eds M. G. Klotz and L. Y. Stein (San Diego, CA: Academic Press), 91–114.
- Muruganandam, S., Israel, D., and Robarge, W. P. (2010). Nitrogen transformations and microbial communities in soil aggregates from three tillage systems. *Soil Sci. Soc. Am. J.* 74, 120–129. doi: 10.2136/sssaj2009.0006
- Näsholm, T., Kielland, K., and Ganeteg, U. (2009). Uptake of organic nitrogen by plants. *New Phytol.* 182, 31–48. doi: 10.1111/j.1469-8137.2008.02751.x
- Neff, J. C., Chapin, F. S., and Vitousek, P. M. (2003). Breaks in the cycle: dissolved organic nitrogen in terrestrial ecosystems. *Front. Ecol. Environ.* 1:205–211. doi: 10.1890/1540-9295(2003)001[0205:BITCDO]2.0.CO;2
- Nissen, H., Nissen, P., and Azam, F. (1984). Multiphasic uptake of D-glucose by an oligotrophic marine bacterium. *Mar. Ecol. Prog. Ser.* 16, 155–160. doi: 10.3354/meps016155
- Nordin, A., Schmidt, I. K., and Shaver, G. R. (2004). Nitrogen uptake by arctic soil microbes and plants in relation to soil nitrogen supply. *Ecology* 85, 955–962. doi: 10.1890/03-0084
- Oburger, E., and Jones, D. L. (2009). Substrate mineralization studies in the laboratory show different microbial C partitioning dynamics than in the field. *Soil Biol. Biochem.* 41, 1951–1956. doi: 10.1016/j.soilbio.2009.06.020
- Owen, A. G., and Jones, D. L. (2001). Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. *Soil Biol. Biochem.* 33, 651–657. doi: 10.1016/S0038-0717(00)00209-1
- Pfautsch, S., Gessler, A., Adams, M. A., and Rennenberg, H. (2009). Using amino-nitrogen pools and fluxes to identify contributions of understory *Acacia* spp. to overstory *Eucalyptus regnans* and stand nitrogen uptake in temperate Australia. *New Phytol.* 183, 1097–1113. doi: 10.1111/j.1469-8137.2009.02909.x
- Pomeroy, L. R., Williams, P. J., Azam, F., and Hobbie, J. E. (2007). The microbial loop. *Oceanography* 20, 28–33. doi: 10.5670/oceanog.2007.45
- Rich, J. H., Ducklow, H. W., and Kirchman, D. L. (1996). Concentrations and uptake of neutral monosaccharides along 140° W in the equatorial Pacific: contribution

- of glucose to heterotrophic bacterial activity and the DOM flux. *Limnol. Oceanogr.* 41, 595–604. doi: 10.4319/lo.1996.41.4.0595
- Rousk, J., and Bååth, E. (2011). Growth of saprotrophic fungi and bacteria in soil. *FEMS Microbiol. Ecol.* 78, 17–30. doi: 10.1111/j.1574-6941.2011.01106.x
- Rousk, J., and Jones, D. L. (2010). Loss of low molecular weight dissolved organic carbon (DOC) and nitrogen (DON) in H₂O and 0.5 M K₂SO₄ soil extracts. *Soil Biol. Biochem.* 42, 2331–2335. doi: 10.1016/j.soilbio.2010.08.017
- Rousk, J., Brookes, P. C., Glanville, H. C., and Jones, D. L. (2011). Lack of correlation between turnover of low-molecular-weight dissolved organic carbon and differences in microbial community composition or growth across a soil pH gradient. *Appl. Environ. Microbiol.* 77, 2791–2795. doi: 10.1128/AEM.02870-10
- Sinsabaugh, R. L., Manzoni, S., Moorhead, D. L., and Richter, A. (2013). Carbon use efficiency of microbial communities: Stoichiometry, methodology and modeling. *Ecol. Lett.* 16, 930–939. doi: 10.1111/ele.12113
- Skoog, A., Biddanda, B., and Benner, R. (1999). Bacterial utilization of dissolved glucose in the upper water column of the Gulf of Mexico. *Limnol. Oceanogr.* 44, 1625–1633. doi: 10.4319/lo.1999.44.7.1625
- Sorensen, P. L., Michelsen, A., and Jonasson, S. (2008). Ecosystem partitioning of ¹⁵N-glycine after long-term climate and nutrient manipulations, plant clipping and addition of labile carbon in a subarctic heath tundra. *Soil Biol. Biochem.* 40, 2344–2350. doi: 10.1016/j.soilbio.2008.05.013
- Stephens, G. C. (1988). Epidermal amino acid transport in marine invertebrates. *Biochim. Biophys. Acta* 947, 113–138. doi: 10.1016/0304-4157(88)90022-6
- van Hees, P. A. W., Johansson, E., and Jones, D. L. (2008). Dynamics of simple carbon compounds in two forest soils as revealed by soil solution concentrations and biodegradation kinetics. *Plant Soil* 310, 11–23. doi: 10.1007/s11104-008-9623-3
- Vinolas, L. C., Vallejo, V. R., and Jones, D. L. (2001). Control of amino acid mineralization and microbial metabolism by temperature. *Soil Biol. Biochem.* 33, 1137–1140. doi: 10.1016/S0038-0717(00)00243-1
- Werdin-Pfisterer, N. R., Kielland, K., and Boone, R. D. (2009). Soil amino acid composition across a boreal forest successional sequence. *Soil Biol. Biochem.* 41, 1210–1220. doi: 10.1016/j.soilbio.2009.03.001
- White, D. C. (1995). Chemical ecology: possible linkage between macro- and microbial ecology. *Oikos* 74, 177–184. doi: 10.2307/3545646
- Whitman, W. B., Coleman, D. C., and Wiebe, W. (1998). Perspective: prokaryotes: the unseen majority. *Proc. Natl. Acad. Sci. U.S.A.* 95, 6578–6583. doi: 10.1073/pnas.95.12.6578
- Williams, P. J. L. (2000). “Heterotrophic bacteria and the dynamics of dissolved organic matter.” in *Microbial Ecology of the Oceans*, ed. D. L. Kirchman (New York: Wiley-Liss), 153–200.
- Wright, R. T. (1974). “Mineralization of organic solutes by heterotrophic bacteria.” in *The Effect of the Ocean Environment on Microbial Activities*, eds R. R. Colwell, and R. Y. Morita (Baltimore: University Park Press), 546–565.
- Wright, R. T., and Hobbie, J. E. (1966). Use of glucose and acetate by bacteria and algae in aquatic ecosystems. *Ecology* 47, 447–464. doi: 10.2307/1932984
- Yu, Z., Zhang, Q., Kraus, T. E. C., Dahlgren, R. A., Anastasio, C., and Zasoski, R. J. (2002). Contribution of amino compounds to dissolved organic nitrogen in forest soils. *Biogeochemistry* 61, 173–198. doi: 10.1023/A:1020221528515
- Zubkov, M. V., Tarran, G. A., Mary, I., and Fuchs, B. M. (2008). Differential microbial uptake of dissolved amino acids and amino sugars in surface waters of the Atlantic Ocean. *J. Plankton Res.* 30, 211–220. doi: 10.1093/plankt/fbm091

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 15 May 2013; accepted: 14 October 2013; published online: 12 November 2013.

Citation: Hobbie JE and Hobbie EA (2013) Microbes in nature are limited by carbon and energy: the starving-survival lifestyle in soil and consequences for estimating microbial rates. *Front. Microbiol.* 4:324. doi: 10.3389/fmicb.2013.00324

This article was submitted to *Terrestrial Microbiology*, a section of the journal *Frontiers in Microbiology*.

Copyright © 2013 Hobbie and Hobbie. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Specificity of plant-microbe interactions in the tree mycorrhizosphere biome and consequences for soil C cycling

Carolyn Churchland and Sue J. Grayston *

Belowground Ecosystem Group, Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, BC, Canada

Edited by:

Per Bengtson, Lund University, Sweden

Reviewed by:

Maarja Öpik, University of Tartu, Estonia

Erin E. Nuccio, Lawrence Livermore National Laboratory, USA

*Correspondence:

Sue J. Grayston, Belowground Ecosystem Group, Department of Forest and Conservation Sciences, Forest Sciences Centre, University of British Columbia, 2424 Main Mall, Vancouver, BC V6T 1Z4, Canada
e-mail: sue.grayston@ubc.ca

Mycorrhizal associations are ubiquitous and form a substantial component of the microbial biomass in forest ecosystems and fluxes of C to these belowground organisms account for a substantial portion of carbon assimilated by forest vegetation. Climate change has been predicted to alter belowground plant-allocated C which may cause compositional shifts in soil microbial communities, and it has been hypothesized that this community change will influence C mitigation in forest ecosystems. Some 10,000 species of ectomycorrhizal fungi are currently recognized, some of which are host specific and will only associate with a single tree species, for example, *Suillus grevillei* with larch. Mycorrhizae are a strong sink for plant C, differences in mycorrhizal anatomy, particularly the presence and extent of emanating hyphae, can affect the amount of plant C allocated to these assemblages. Mycorrhizal morphology affects not only spatial distribution of C in forests, but also differences in the longevity of these diverse structures may have important consequences for C sequestration in soil. Mycorrhizal growth form has been used to group fungi into distinctive functional groups that vary qualitatively and spatially in their foraging and nutrient acquiring potential. Through new genomic techniques we are beginning to understand the mechanisms involved in the specificity and selection of ectomycorrhizal associations though much less is known about arbuscular mycorrhizal associations. In this review we examine evidence for tree species- mycorrhizal specificity, and the mechanisms involved (e.g., signal compounds). We also explore what is known about the effects of these associations and interactions with other soil organisms on the quality and quantity of C flow into the mycorrhizosphere (the area under the influence of mycorrhizal root tips), including spatial and seasonal variations. The enormity of the mycorrhizosphere biome in forests and its potential to sequester substantial C belowground highlights the vital importance of increasing our knowledge of the dynamics of the different mycorrhizal functional groups in diverse forests.

Keywords: mycorrhizosphere, root exudates, plant-microbe interactions, LMWOA, signaling, carbon cycling, ectomycorrhizae, arbuscular mycorrhizae

INTRODUCTION

Soil organic matter (SOM) is the largest carbon (C) pool in terrestrial ecosystems (Falkowski et al., 2000; Fontaine et al., 2003), greater than terrestrial biomass C and atmospheric C combined (Jobbágy and Jackson, 2000). Carbon enters the SOM pool via litter (leaves, coarse and fine roots), brash (branches and coarse woody debris) and root exudates. The proportion of recently photosynthesized C allocated to leaves, storage, metabolism and root exudates has important consequences for soil C storage and varies depending on the environment, plant type, age of the plant, microbial symbionts and nutrient availability (Litton et al., 2007; Epron et al., 2012). Belowground C allocation is notoriously difficult to measure and varies depending on the spatial heterogeneity of belowground structures, the assemblage of microorganisms in the rhizosphere and environmental conditions (Subke et al., 2009; Kuzyakov and Gavrichkova, 2010; Mencuccini and Holttä, 2010; Warren et al., 2012). Recent studies have challenged our

understanding of the mechanisms of C sequestration in soil. Clemmensen et al. (2013) showed that 50–70% of C stored in soil is derived from roots or root-associated microorganisms and that humus accumulation in boreal forests is regulated mainly by C allocation to roots and associated mycelium rather than decomposition of litter by saprophytes. Consequently, studies are beginning to focus on quantifying not only C allocation belowground, but also the spatial and temporal distribution of this C and how it is influenced by root-associated mycorrhizae (Litton and Giardina, 2008; Chapin et al., 2009; Warren et al., 2012).

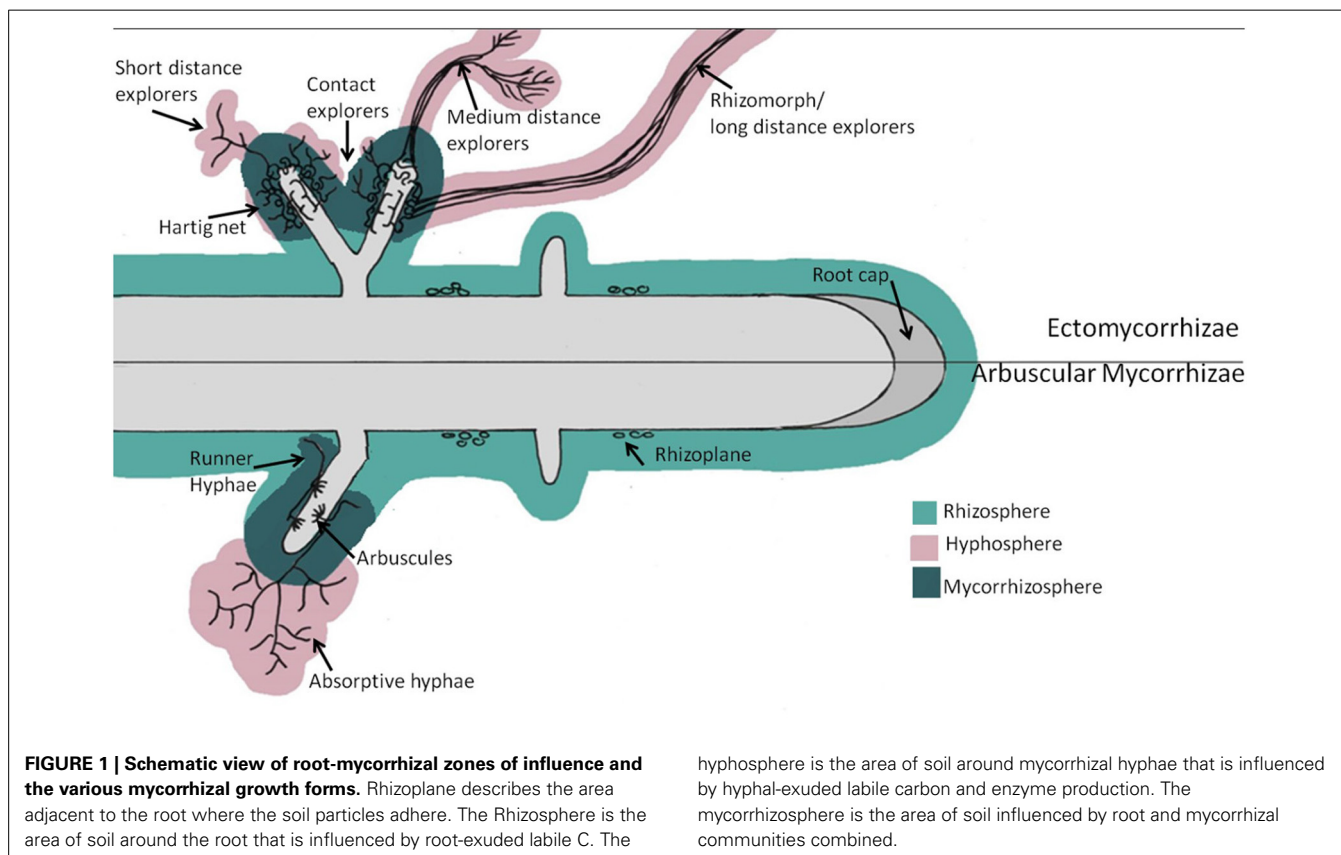
Ninety percent of vascular plants form symbiotic relationships with mycorrhizal fungi (Wang and Qui, 2006; Smith and Read, 2008). Mycorrhizae can be generalized into two groups, endomycorrhizae, where hyphae penetrate root cells, and ectomycorrhizae, which do not penetrate. There are several types of endomycorrhizae including ericoid, arbutoid, monotropoid, orchid and, by far the most prevalent, arbuscular (occurring in approximately

85% of plant species) (Smith and Read, 2008). Arbuscular mycorrhizae (AM) are generally Glomeromycota, and form vesicles or arbuscules after invaginating the cell membranes of root cells. Ectomycorrhizae (ECM) are typically Basidiomycetes, Ascomycetes and Zygomycetes, occurring in 10% of plant species (mostly trees and woody plants). Ectomycorrhizae create a hyphal mantle covering the root tip and form a Hartig net within the root cortex, surrounding the root cells. Although saprotrophic fungi and bacteria are the primary decomposers in the soil, plant acquisition of released nutrients, such as N and P, is achieved through their symbiotic relationships with mycorrhizae (Read and Perez-Moreno, 2003; Lindahl et al., 2007; Talbot et al., 2008).

Mycorrhizae are involved in a number of important soil processes including: weathering of mineral nutrients (Landeweert et al., 2001; Finlay and Rosling, 2006; Wallander, 2006), C cycling, mediating plant responses to stress (Finlay, 2008), and interacting with soil bacteria (both negatively e.g., pathogens and positively e.g., mycorrhization helper bacteria) (Johansson et al., 2004; Frey-Klett et al., 2007). Ectomycorrhizae have broad enzymatic capabilities; they can decompose labile and recalcitrant SOM, and some can mineralize organic N (Chalot and Brun, 1998). This allows the mycorrhizae to transfer large amounts of N directly to their host plants (Hobbie and Hobbie, 2006). Arbuscular mycorrhizal fungal enzymatic capabilities are not thought to be as extensive as ECM; AM can only transfer small amounts of N to their hosts when soil-N levels are high (Tobar et al., 1994; Hodge et al., 2000; Govindarajulu et al., 2005; Reynolds et al.,

2005). Arbuscular mycorrhizae mainly access inorganic N sources (Fellbaum et al., 2012), though organic N uptake by AM has been demonstrated in boreal forests (Whiteside et al., 2012). However, AM can transfer large amounts of P to their plant hosts (Smith and Read, 2008), either by hydrolysis of organic P from hyphal tips and subsequent transfer to the tree via arbuscules, or by uptake, conversion and transport of inorganic phosphorus along hyphae. Although some plant species can form symbiotic relationships with both AM and ECM, the dominance or presence of one over the other will alter tree-nutrient availability.

There are 10,000 ECM fungal species that are known to be associated with as many as 8,000 different plant species (Taylor and Alexander, 2005). Tree species select mycorrhizae and free-living microorganisms through exudation of distinct chemical signals into the rhizosphere (the area surrounding the root that is directly influenced by root exudates, **Figure 1**) (Pires et al., 2012; Shi et al., 2012). Specific exudates will trigger the expression of mycorrhization genes, which are associated with the initiation of hyphal growth toward the plant root rhizosphere (Martin et al., 2007; Podila et al., 2009). In addition, there is increasing evidence that tree-species-rhizosphere community differences are the result of the trees “selecting” for specific microbes through root exudates (Prescott and Grayston, 2013). Plants release several types of root exudates including: mucilage that maintains a constant moisture environment, metal chelators that mobilize iron and zinc, and various forms of C comprising of carbohydrates, amino acids, low-molecular-weight aliphatic- and



aromatic-acids, fatty acids, enzymes and hormones (Grayston et al., 1997; **Table 1**). The composition and quantity of root exudates will vary depending on tree species (Tuason and Arocena, 2009), and will also be modified within a given tree species depending on which mycorrhizal species colonize the tree roots (van Hees et al., 2005). Different ECM can increase root exudation of organic acid (van Hees et al., 2003, 2005; Johansson et al., 2009) and can change organic acid composition compared to non-mycorrhizal trees (Klugh and Cumming, 2003; van Hees et al., 2005). The variation in C allocated to ECM- and AM-roots, and subsequently ECM and AM root exudates is due, in part, to hyphal exudation from the mycorrhizae and mycorrhizal morphology. These hyphal exudates create an area of greater microbial biomass and activity, termed the mycorrhizosphere (area surrounding the mycorrhizal root tip) or hyphosphere (**Figure 1**) (Jones et al., 2004; Frey-Klett et al., 2007; Finlay, 2008; Nazir et al., 2010). Although bacteria and archaea are omnipresent in the rhizosphere and mycorrhizosphere, their role in ecosystem processes is only beginning to be understood.

This review focuses on describing host-specificity of soil microorganisms and fauna in the mycorrhizosphere of trees, the signals involved in establishing these interactions, and their impact on soil C flow and sequestration. We concentrate on interactions within the mycorrhizosphere, as this is the active site of root exudation, nutrient cycling, and plant nutrient uptake. The spatial enormity of the mycorrhizosphere biome in forests hints at its potential to sequester substantial amounts of C belowground. An understanding of the controls on C allocation belowground, and the movement of that C throughout the soil environment is a vital knowledge gap.

THE MYCORRHIZOSPHERE BIOME

TREE-MYCORRHIZAL SPECIFICITY

Many temperate forest tree species have ECM associations (including: pine, spruce, larch, hemlock, true firs, Douglas-fir, aspen, birch); some species have AM associations (e.g., cedar, maple, ash) and some have both (e.g., alder, poplar). Some tree species, such as Douglas fir (which associated with more than 2000 known ECM, Molina and Trappe, 1982) have high fungal receptivity, whereas other tree species such as alder (which only associate with 50 known ECM, Pritsch et al., 1997) have narrow fungal receptivity. It has been estimated that ECM mycelia can account for up to 80% of the fungal community and 30% of the total microbial biomass in forest soils (Högberg and Högberg, 2002; Wallander, 2006).

The presence and abundance of specific plant species can influence soil microbial community composition and function (Kourtev et al., 2002; Edwards and Zak, 2010; Eisenhauer et al., 2010), which can, in turn, impact soil C cycling and sequestration as mycorrhizal species differ in growth strategies and C demand. There is evidence of specificity in many plant-microbe interactions, suggesting both strong selective pressure and competition within the rhizosphere microbiome (Podila et al., 2009). There are many species of ECM fungi (Smith and Read, 2008) and though many ECM (e.g., *Lactarius*) have a broad host range some (e.g., *Suillus*) have only narrow host range (Bruns et al., 2002; Kennedy et al., 2003). The signaling specificity by host

tree species to engage ECM fungi has been well studied (Molina and Trappe, 1982; Ishida et al., 2007; Tedersoo et al., 2008). For example, distinct chemical signals (e.g., small-secreted proteins and hydrophobins) may enable trees such as *Populus* to recruit advantageous ectomycorrhizal fungi from the broad soil microbial community (Podila et al., 2009). Whole-genome sequencing is now enabling us to have a much greater understanding of the suite of important genes and signals involved in ECM symbiotic associations (Martin et al., 2008, 2010). However, we are only just beginning to understand the factors involved in specificity and selection in AM associations (Brachmann and Parniske, 2006; Bonfante and Genre, 2010).

MYCORRHIZAL MORPHOLOGY

Variations in extrametrical mycelium (EMM) hyphal pattern production and in mycorrhizae type may have consequences for C flow and carbon sequestration. ECM fungal taxa vary in the growth patterns of their EMM as a result of their multifarious foraging strategies (Agerer, 2001); the dominance of one morphological type over the other may have consequences for the spatial distribution of recent photosynthates belowground. Agerer (2001) describes the following ECM anatomies: contact explorers, convoy explorers, long-distance explorers, medium distance explorers and short-distance explorers (**Figure 1**). Contact explorers are EMM with a smooth mantle and few emanating hyphae (diffuse hyphal cords), the tips of which are often in close contact with dead leaves. Examples of contact explorers are *Lactarius* and *Russula* species that produce exudates throughout their hyphae. Convoy explorers are EMM that grow within rhizomorphs (aggregated parallel hyphal cords that can conduct nutrients over long distances) or mantles and produce haustoria in cortical cells of roots. Long-distance exploring EMM are smooth with highly differentiated rhizomorphs. For example, Boletales species are long distance hydrophilic hyphal explorers, and only exude compounds from their tips. Medium distance explorers have some rhizomorph formation and form 3 subtypes: fringe, mat, and smooth. Fringe subtype hyphae fan out from hairy rhizomorphs, which ramify and interconnect (e.g., *Dermocybe cinnamomeolutea*). Mat subtype hyphae have a limited range of exploration and rhizomorphs do not differentiate (e.g., *Hysterangium stoloniferum*). Smooth subtype hyphae have internally undifferentiated rhizomorphs with a central core of thick hyphae, with smooth mantles, and a few emanating hyphae (e.g., *Thelephora terrestris*) (Agerer, 2001). Short-distance explorers have a voluminous envelope of emanating hyphae without rhizomorph formation (e.g., *Quercirhiza squamosal*) (Agerer, 2001).

Hyphae have the ability to move carbon both horizontally, over long distances, extending well beyond the roots of trees and vertically, down the soil profile. Most ECM are found in the F and H soil layer (area of highly decomposed leaves beneath surface of forest floor, **Figure 2**), but also can be found in the mineral soil, whereas other ECM prefer decaying wood (*Amaranthus* and Perry, 1989; Tedersoo et al., 2003). Some ECM are able to mobilize minerals from rocks in soil (Landeweert et al., 2001), whereas others access nutrients from coarse woody debris (*Amaranthus* et al., 1994). Some ECM fungi also have saprophytic

Table 1 | Organic compounds and enzymes found in root exudates (Dakora and Phillips, 2002; Rasemann and Agrawal, 2008).

Amino acids	Organic acids	Fatty acids	Sugar	Sterols	Growth factors and Vitamins	Purines, Nucleosides	Enzymes	Inorganic Ions and Gases	Phytochemical compounds
α -alanine	Acetic	Linoleic	Arabinose	Compesterol	Biotin thiamine	Adenine	Acid/alkaline	HCO ₃ ⁻	Aldehyde
β -alanine	Aconitic	Linolenic	Deoxyribose	Cholesterol	Choline	Cytidine	Amylase	OH ⁻	Alkaloid
γ -aminobutyric acid	Aldonic	Oleic	Fructose	Sitosterol	Niacin	Guainin	Invertase	H ⁺	Cardenolide
α -aminoadipic Acid	Ascorbic	Palmitic	Galactose	Stigmasterol	Panthothenic	Uridine	Peroxidase	CO ₂	Cyanic
Arginine	Benzoic	Stearic	Glucose		Pantothenate		Photophatase	H ₂	Glucoside
Asparagine	Butyric		Maltose		Pyridoxine		Phenolase		Furanocoumarin
Aspartic	Caffeic		Mannose		Riboflavin		Polygalacturonase		Glcinosolate
Citrulline	Citric		Mucilage		p-amino benzoic acid		Protease		Glycoalkaloid
Cystathionine	Erythronic		Oligosacchirides		N-methyl nicotinic acid				Hydroxamic acid
Cysteine	Ferulic		Raffinose						Iridoid glycoside
Cystinemugineic	Formic		Rhamnose						Phytoecdysteroid
Deoxymugineic	Fumaric		Ribose						Pyrolizidine
3-epihydroxy	Glutaric		Sucrose						alakoid
Glutamate	Glycolic		Xylose						Polyphenol
Glycine	Glyoxilic								Resin
Histidine	Lactic								Tannin
Homoserine	Malic								Terpenoid
Isoleucine	Malonic								Triterpene
Leucine	Oxalic								
Lysine	Piscidic								
Methionine	Propionic								
Mugineic	Pyruvic								
Ornithine	Succinic								
Phenylalanine	Syringic								
Praline	Tartaric								
Proline	Tetronic								
Serine	Valeric								
Theronine	Vanillic								
Tryptophan	p-coumaric								
Tyrosine	Oxalacetic								
Valine	p-hydroxybenzoic								

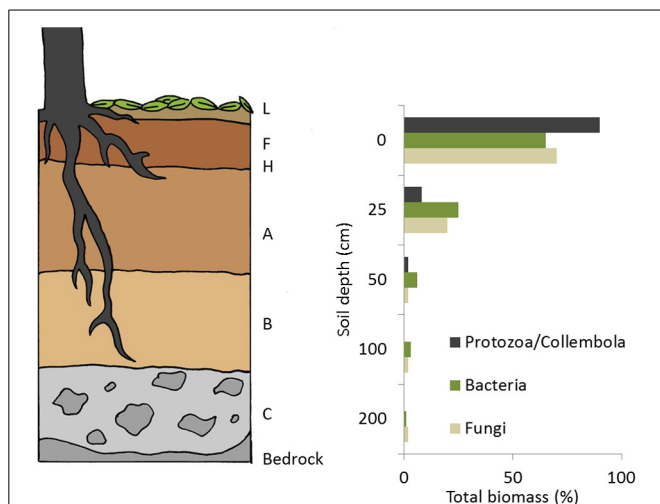


FIGURE 2 | Diagram of soil profiles with depth and the relative proportion of collembola/protozoa, bacteria and fungi at each of these depths. L, characterized by the accumulation of organic matter; F: characterized by the accumulation of partially decomposed organic matter; H: characterized by the accumulation of decomposed organic matter where the original structure is indiscernable. A: mineral horizon characterized by eluviation of materials in solution, or accumulation of organic matter, or both. B: mineral horizon characterized by enrichment of clay, organic matter, and iron and aluminium oxides or by *in situ* weathering. C: mineral horizon characterized by little or no alteration through the soil-forming processes, usually represents the parent material.

growth capabilities e.g., *Tomentella* sp. (Köljalg et al., 2000). It is hypothesized that these ECM may switch to a saprophytic lifestyle when photosynthate C becomes scarce e.g., during winter (Courty et al., 2008).

Arbuscular mycorrhizae do not form rhizomorphs and are considered to have five distinct hyphal architecture types (Figure 1). These include: infection networks, produced by spores and root fragments; germ tubes (only 20–30 mm long); hyphal bridges that connect runner-type hyphae and form patches of dense hyphal networks close to the root zone (Friesse and Allen, 1991; Dodd et al., 2000); runner-types that expand rapidly through the soil or along roots (Mosse, 1962), seeking out new segments of roots to infect (Friesse and Allen, 1991); and absorptive hyphal networks that explore the soil matrix for nutrients (Friesse and Allen, 1991). Absorptive hyphal networks can extend 4–7 centimeters into the soil. Each network can have up to 8 branching orders, with each branch extending approximately 5 millimeters (Allen, 2007). Bago et al. (1998) described a 6th architectural form, where absorptive hyphae can form from runner hyphae, extending the potential range of nutrient absorption well beyond 4–7 centimeters. However, ECM EMM can extend even further from the roots as a result of rhizomorph formation. ECM rhizomorphs live, on average, 11 months, but have been observed to live for up to 7 years (Treseder et al., 2005). In contrast, AM hyphae only live on average 5–6 days (Staddon et al., 2003), suggesting the ECM dominated forests have greater C storage potential. The following section describes tree-rhizosphere C flow in greater detail.

QUANTIFICATION AND CHARACTERISTICS OF MYCORRHIZOSPHERE C FLOW

TREE-RHIZOSPHERE C FLOW

Differences in root-associated fungi (both the presence/absence and type of fungal association) may be responsible for the large variation (10 X) in root exudation rates (Phillips et al., 2008, 2011). Exudation rates from root tips and hyphal tips tend to be greatest in the fine roots and in mycorrhizae that are allocated more C (Phillips et al., 2008, 2011). Carbon allocation to ECM hyphae will vary depending on ECM taxa (Bidartondo et al., 2001) and stage of colonization. For example, more C is allocated belowground during early stages of colonization (Cairney et al., 1989; Cairney and Alexander, 1992). Movement of recent photosynthates within EMM is not uniform, and will vary depending on the fungal species and their life stage (Cairney, 2012). Sun et al. (1999) demonstrated that ECM hyphal tips were active sites of exudation and re-adsorption of compounds, with little exudation along rhizomorphs. In addition, Leake et al. (2001) showed that more C was allocated to frontal tips of hyphae that occupied a hotspot of organic matter in soil. Infected ECM root tips may receive 42 times more carbon than uninfected root tips on the same plant (Cairney et al., 1989; Wu et al., 2002). Therefore there will be much patchiness in root exudate distribution in the forest floor, depending on root distribution and hyphal distribution.

Several techniques- including tree-girdling and stable-isotope labeling- have the potential to accurately measure the amount of C allocated belowground as well as the impact of root-exuded C on the microbial community. Tree girdling has demonstrated that labile C drives soil respiration (Högberg et al., 2001). Tree girdling stops the flow of photosynthates to tree roots, altering the availability and quality of C sources available to soil microbes in the rhizosphere (Subke et al., 2004; Högberg et al., 2007). However, how girdling affects the soil microbial community, particularly the bacterial community, is not consistent. Tree girdling caused significant decreases in the activity and biomass of the soil microbial community in boreal and temperate forests (Scott-Denton et al., 2006; Weintraub et al., 2007); this was mainly due to loss of ECM (45% decrease in ECM biomass relative to non-girdled plots) (Högberg and Högberg, 2002; Yarwood et al., 2009; Pena et al., 2010). The response of bacterial abundance and biomass to girdling has been marginal in boreal forests (Högberg et al., 2007; Yarwood et al., 2009) and in sub-tropical evergreen broadleaf forests (Li et al., 2009). Koranada et al. (2011) observed (using PLFA) a significant reduction in fungal biomass and Gram-positive bacterial biomass in girdled beech forests. As Gram-positive bacteria were less affected by exudates, Koranada et al. (2011) hypothesized that other effects of girdling treatments on rhizospheric conditions, such as alterations in oxygen supply, pH and redox potential (a result of the reduced root respiration or uptake of nutrients by plants) may have decreased Gram-positive bacterial populations. Other studies have shown no effect of girdling on microbial biomass or soil respiration; however in some of these studies trees re-sprouted (e.g., Eucalyptus), (Wu et al., 2011; Chen et al., 2012), and in other studies carbohydrates were still available in roots after girdling (Binkley et al., 2006). The increased availability of root carbohydrates may lead to a positive priming effects on SOM decomposition, increasing microbial

community biomass and activity in the short-term (Subke et al., 2004; Scott-Denton et al., 2006). The variability of tree-girdling results may be the result of variation in tree-mycorrhizal species associations, or may be due to priming effects. Consequently developing non-destructive techniques may provide more insight into C-flow in forest ecosystems.

Natural-abundance stable-isotope ratios have recently been used to non-destructively investigate the flux of C from trees to the soil microbial community. In a second-growth coastal western hemlock forests in B.C. eighty-year-old Douglas-fir and western hemlock trees supplied C to the mycorrhizal symbionts for a distance up to ten meters (Churchland et al., 2013). Similarly, labeling of young trees with ^{13}C -enriched CO_2 has also been used to assess spatial and temporal C flux belowground. Epron et al. (2011) showed that there was rapid transfer of recent photosynthates to the mycorrhizosphere of beech (0.5–1 day), oak (0.5–1 day) and pine (1–2 days), and that the patterns of carbon allocation belowground varied seasonally in pine and beech, according to the phenology of the species. Similarly, Esperschütz et al. (2009) demonstrated using $^{13}\text{CO}_2$ pulse-labeling and PLFA analysis, that the C in beech root exudates is first utilized by Gram-negative bacteria and mycorrhizal fungi. Stem-injection-labelling of mature trees has shown that C exudation from 22-year-old Sitka spruce in the field is rapid (24 h) and that these exudates are utilized first by fungi. The extent of influence of these trees exudates can be up to 20 m away from the base, and may, in part, be due to transport through EMM (Churchland et al., 2012). Although these techniques are too coarse to measure carbon movement in a single hypha, they show that C can move great distances away from the tree base and are utilized by the fungi and bacteria in the rhizosphere.

ROOT EXUDATES

Characterizing root exudation is challenging, but new techniques hold potential for breakthroughs. Most studies characterizing exudates released by different tree species have been microcosm studies conducted on seedlings in the laboratory under controlled conditions, either in hydroponic or sand systems, which do not scale up to mature trees and forests (Grayston et al., 1997). Hydroponic systems lack the physical substrates important for root growth; this affects exudation and can lead to re-uptake of exudates by plant roots. Studies in sand or soil systems are limited because of adsorption of exudates or degradation by the microbial community (Grayston et al., 1997, and references therein). There have been a few studies of tree root exudation in the field, mainly on young seedlings using either excavated root tips (which are surface-sterilized and placed in sterile tubes in the field) or soil extraction techniques. This latter approach has similar problems to the microcosms mentioned above (Phillips et al., 2008). In addition, it is difficult to extrapolate exudation rates from seedlings to mature trees, as a smaller portion (though, in total, a much greater amount) of recently-photosynthesized C is being allocated to the roots. Recently Shi et al. (2012) demonstrated an anion exchange membrane system that improved root exudate collection *in situ* from two-year-old radiata pine trees growing in large-scale biotrons. Because these anion exchange membranes rapidly adsorb root exudates there is little chance for

consumption by microbes present in the biotron soil. This technique may result in a better understanding of root exudation from mature trees and forest stands.

The amount of C allocated to roots, root exudates, mycorrhizae and other rhizosphere microorganisms can change under different nutrient regimes and increase in the presence of specific microorganisms (Grayston et al., 1997). Ectomycorrhizal fungi influence both the quantity of C allocated to their roots, and the chemical composition of those exudates (van Schöll et al., 2006; Rineau and Garbaye, 2010). For example, ECM trees will allocate a third more C to their roots than non ECM trees (Durall et al., 1994; Riewicz and Anderson, 1994; Qu et al., 2004), likely because EMM have a large C demand (Riewicz and Anderson, 1994; Cairney and Burke, 1996; Cairney, 2012). Laboratory studies have shown that up to 29% of plant-assimilated C can be allocated to EMM (Riewicz and Anderson, 1994; Ek, 1997; Bidartondo et al., 2001). Environmental conditions also influence the degree to which tree roots are colonized, and likely mediate fluxes of labile C in forest soils (Meier et al., 2013). For instance, loblolly pine mass-specific exudation rates can vary by over three orders of magnitude under varying CO_2 concentrations (Phillips et al., 2008, 2011). Plants have been observed to allocate more C to their roots and mycorrhizal symbionts under nutrient poor conditions (Zak et al., 1993; Franklin et al., 2012). In systems that are not N-limited, or in systems where N has been added, fungal biomass can decrease up to 45%, mainly due to decreased C allocation from trees to the mycorrhizal fungi (Högberg et al., 2007).

Root exudates represent semi-continuous input of labile C into soil, though exudation rates vary in time and space (Hinsinger et al., 2005), between deciduous and conifer species, over seasons (Collignon et al., 2011) and in different climates (Lin et al., 1999; Jones et al., 2004). Reviews on rhizodeposition from plants acknowledge the scant information on the character of exudates from trees (Grayston et al., 1997; Kuzyakov and Domanski, 2000; Neumann and Romheld, 2001; Jones et al., 2004). Plants are able to influence not only the quantity but also the composition of C exuded by their roots. This is thought to play a role in tree-microbe signaling and specificity in the rhizosphere. Production of enzymes, low-molecular-weight organic-acids (LMWOA), and other compounds support rhizosphere microbial communities (Bais et al., 2006). Root exudates also enhance nutrient availability by mobilizing poorly-soluble mineral-nutrients (Jones and Darrah, 1994; Marschner et al., 2011) and supplying labile-C substrates that increase rhizosphere microorganism activity and turnover (Phillips et al., 2012), ultimately influencing the decomposition of SOM (Rosling et al., 2004a,b). Most of the knowledge about the character of root exudates and how they may vary between tree species is on LMWOA and with ECM fungi (Cairney, 2012). In one of the few studies on carbohydrate characterization, Liebeke et al. (2009) used a gas-chromatograph-mass-spectrometer to reveal differences in the sugar content of soil extracts from different forest soils, demonstrating that oak soil contained mannitol and trehalose that was not present in beech soil. They hypothesized that the variation in sugar concentrations was responsible for differences in the bacterial communities under these tree species. There is increasing evidence that trees

can actively restrict carbohydrate flow to their fungal partners. This is done through control of sucrose export and hydrolysis if the fungal partner does not deliver sufficient mineral nutrients (see review by Nehls et al., 2010).

PLANT-MYCORRHIZAE SIGNALING MOLECULES

Several root exudates and hyphal exudates have the potential to induce mycorrhizal infection and change the microbial community structure of the rhizosphere. Secreted proteins, specifically a class of secreted proteins called effectors, have recently been established as plant-mycorrhizal signaling molecules (Lowe and Howlett, 2012). Effector proteins facilitate infection by suppressing immunity and/or inducing defense responses in plants (DeWit et al., 2009). For example, *Laccaria bicolor* was found to secrete the effector Mycorrhizal-Induced Small Secreted Protein 7 (MISSP7) during root colonization, in response to diffusible signals exuded from plant roots (Plett et al., 2011). Secretion and uptake of MISSP7 by the plant (via PI-3-P mediated endocytosis) affected cell wall chemistry, ultimately allowing hyphal penetration of the root apoplast. MISSP7 is the most upregulated protein during mycorrhization, and without it symbiosis does not occur (Plett et al., 2011). Following this discovery another effector protein, SP7, was uncovered (Maffei et al., 2012). Secreted by the AM fungi *Gigaspora intraradices*, SP7 interacts with a plant pathogenesis related transcription factor. SP7 was found to play a role in managing the formation of symbiosis with plant roots through the suppression of the plant immune system (Kloppholz et al., 2011). Plants have also been found to increase production of strigolactones under nutrient poor conditions (Maffei et al., 2012). Strigolactones have been found to induce fungal spore germination (Maffei et al., 2012) and hyphal branching (Bonfante and Requena, 2011), suggesting that plants might be signaling nearby mycorrhizae to promote infection. Much less is known about AM signaling, although recently it has been shown that AM fungi also produce active diffusible signals, similar to Nod factors released by rhizobia. These signals are needed for mycorrhizal formation (Bonfante and Requena, 2011). Similarly plant secreted effectors have also been found, which influence interactions between plant roots and free-living microorganisms (Hogenhout et al., 2009).

MODIFICATIONS BY ECM/AM ON EXUDATES AND SIGNALS

Mycorrhizae modify the amount and composition of root exudates (van Schöll et al., 2006; Johansson et al., 2008, 2009), affecting exudation into the mycorrhizosphere and hyphosphere (Sun et al., 1999; Ahonen-Jonnarth et al., 2000; Jones et al., 2004; Johansson et al., 2008, 2009). The tips of growing ECM hyphae have been found to exude sugars, polyols, amino acids, peptides, proteins, hydroxamate siderophores, various LMWOA and pigments (growing front; Table 1) (Sun et al., 1999; Ahonen-Jonnarth et al., 2000; Jones et al., 2004; Johansson et al., 2008, 2009). Different ECM taxa vary the amount and composition of compounds exuded (Lapeyrie et al., 1987; Griffiths et al., 1994; van Schöll et al., 2006; Johansson et al., 2009; Tuason and Arocena, 2009). In general, the presence of ECM increases organic acid exudation (Johansson et al., 2008, 2009) and/or changes the type of organic acid exuded (van Schöll et al., 2006; Table 2).

For example, van Hees et al. (2006a) found that *Hebeloma crustuliniforme* (ECM), when in symbiosis with *Pinus sylvestris*, exuded oxalate and ferrirocinnic acid, to a lesser extent, malonate and acetate which were absent from non-mycorrhizal Scots pine soil.

There is some evidence that hyphal exudates result in specific hyphosphere bacteria communities (Table 3; See Nazir et al., 2010 for list of bacterial-AM fungal relationships). It has been suggested that organic acids contribute to microbial selection in the mycorrhizosphere (de Boer et al., 2005). Differences in LMWOA ECM hyphal exudation are thought to be partially responsible for selecting specific microbial communities (Martin et al., 2008; Tuason and Arocena, 2009). Similarly, Toljander et al. (2007) found increased γ -proteobacteria abundance when extracted AM mycelial exudates were present, including formate, acetate, α and β glucose, and oligosaccharides. Trehalose has been reported to select specific bacterial communities in the mycorrhizosphere of several tree species including, Douglas-fir, Corsican pine and oak (Frey et al., 1997; Rangel-Castro et al., 2002; Izumi et al., 2006a,b; Uroz et al., 2007). Frey et al. (1997) suggested that the release of trehalose by the ECM fungus *Laccaria bicolor* exerts a nutrient-mediated selection on the surrounding bacteria. Specifically, trehalose has been found to have growth-promoting effects on the mycorrhization-helper bacteria (MHB), *Pseudomonas monteilii*, when inoculated with the ECM fungus *Pisolithus albus* in a plate-assay (Duponnois and Kisa, 2006). Trehalose released by the mycelium of *Laccaria bicolor* was shown to be a chemoattractant for *Pseudomonas fluorescens* BBc6R8 (Frey-Klett et al., 2007). At present it is not clear how hyphosphere microbial communities will impact a mycorrhizal's ability to acquire nutrients, but it is clear that exudation specificity has the potential to select for species-specific microbial communities.

SPATIAL AND SEASONAL VARIATION IN RHIZOSPHERE C FLOW

Rhizosphere C flow varies spatially down the soil profile and horizontally with changes in root and hyphal distribution. Carbon flow also fluctuates seasonally and differs between coniferous and deciduous trees. In forest soil there is soil microbial community-composition stratification with depth due to a decrease in root biomass, root exudates, available C and a shift in SOM composition (Grayston et al., 1997; Berg et al., 1998; Fritze et al., 2000; Leckie et al., 2004; Lejon et al., 2005). Fungi are typically found in upper soil layers (Litter > Formulating > Humified, Figure 2) (Gardes and Bruns, 1993; Hirose et al., 2004). In contrast, actinomycete abundance has been shown to increase with depth (Fritze et al., 2000) while Gram-negative bacterial distribution is linked to root distribution (Soderberg et al., 2004). However, soil respiration rates and microbial activity are related to proximity to trees and tree roots (Churchland et al., 2013). In a free-air carbon-dioxide-enrichment (FACE) study, Phillips et al. (2008) showed that exudation rates could be predicted by the number of roots and mycorrhizal fine root tips (Pritchard et al., 2008b). This suggests that recent tree-carbon can be transported over large distances via roots and hyphae, supporting microbial communities meters away from the tree base.

There are different seasonal and physiological effects on rhizosphere C flow for deciduous and evergreen tree species. In

Table 2 | Modification of low molecular weight organic acid (LMWOA) exudates from trees by different ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungi.

Tree species	ECM or AM	Mycorrhizal symbiont	ECM/AM effect on LMWOA exudation (vs. non ECM/AM roots)	Methodology	References
Scots pine	ECM	<i>Paxillus involutus</i>	↑ oxalic acid, formic acid	9-month-old inoculated seedlings were planted in sterilized soil collected from an E-horizon, and placed in climate controlled growth room. LMWOA were collected via suction from soil column and identified using capillary zone electrophoresis	van Hees et al., 2005
Norway spruce	ECM	<i>Suillus granulatus</i>	↑ citric acid		
		<i>Paxillus involutus</i>	↑ malonic acid		
Scots pine	ECM	<i>Suillus variegatus</i>	↑ oxalic acid	9–12-week-old inoculated seedlings were grown in petri dishes containing glass beads with a growth solution. LMWOA were analyzed using HPLC analysis	Ahonen-Jonnarth et al., 2000
		<i>Rhizopogon roseolus</i>	↑ oxalic acid		
		<i>Paxillus involutus</i>	↑ oxalic acid, malonic acid		
Scots Pine (under elevated CO ₂)	ECM	<i>Suillus variegatus</i> <i>Suillus bovinus</i> <i>Paxillus involutus</i> <i>Rhizopogon roseolus</i>	↑ oxalic acid	16-week-old inoculated seedlings were grown in petri dishes containing peat:vermiculite substrate with a growth solution. LMWOA were analyzed using HPLC analysis	Johansson et al., 2009
		<i>Hebelomavelutipes</i> <i>Piloderma byssinum</i>	↑ citric, fumaric, formic, malonic acid		
Scots pine	ECM	<i>Hebeloma longicaudum</i>	↓ malonic acid	21-week-old inoculated seedlings were grown on glass beads or sand with a growth solution. LMWOA were identified using capillary zone electrophoresis	van Schöll et al., 2006
		<i>Paxillus involutus</i> <i>Piloderma croceum</i>	↑ oxalic		
White spruce	ECM	<i>Not identified</i>	↑ malonic, oxalic, gluconic, succinic, protocatechuic acid	Soil collected <i>in situ</i> around trees that were 20–35 cm diameter at breast height. LMWOA were identified using capillary zone electrophoresis	Tuason and Arocena, 2009
Subalpine fir	ECM	<i>Not identified</i>	↑ malonic, oxalic, glutaric, isocitric acid		
Norway spruce	ECM	<i>Paxillus involutus</i>	↑ Malate, citric	9-month-old inoculated seedlings were planted in a soil-sand column system. LMWOA were collected using suction from soil column and analyzed using capillary zone electrophoresis	van Hees et al., 2003
Scots pine	ECM	<i>Hebeloma crustuliniforme</i>	↑ oxalic, citric, propionic acid	16-week-old inoculated seedlings were grown in a sand culture system. LMWOA were collected via suction from soil column and analyzed using capillary zone electrophoresis.	van Hees et al., 2006a
			↑ oxalic acid, ferrocrocic	16-week-old inoculated seedlings were grown in aseptic multi-compartment dishes containing sterile nutrient agar with stock nutrient solution. LMWOA were analyzed using capillary zone electrophoresis	van Hees et al., 2006b

(Continued)

Table 2 | Continued

Tree species	ECM or AM	Mycorrhizal symbiont	ECM/AM effect on LMWOA exudation (vs. non ECM/AM roots)	Methodology	References
Norway spruce	ECM	<i>Laccaria bicolor</i>	↑oxalic acid	8-week-old inoculated seedlings were grown in glass bead mesocosms with growth medium. LMWOA were analyzed using HPLC analysis	Eldhuset et al., 2007
Japanese red pine	ECM	<i>Pisolithus tinctorius</i>	↑citric acid	4-month-old inoculated seedlings were grown in perlite in pots. LMWOA were analyzed using an electroconductivity detection method	Tahara et al., 2005
Scots pine	ECM	<i>Amantia muscaria</i> <i>Hebeloma velutipes</i> <i>Piloderma fallax</i> <i>Suillus variegatus</i>	↑LMWOA exudation, individual OAs varied depending on N addition and elevated CO ₂	4-week-old inoculated seedlings were grown in petri dishes containing vermiculite and a growth medium. LMWOA were analyzed using capillary zone electrophoresis	Fransson and Johansson, 2010.
Scots pine	ECM	<i>Hebeloma velutipes</i> , <i>P. involutus</i> , <i>Piloderma byssinum</i> , <i>R. roseolus</i> , <i>S. bovinus</i> <i>S. variegatus</i>	↑LMWOA exudation, especially oxalic acid	16-week-old inoculated seedlings were grown in petri dishes containing peat:vermiculite and growth medium. LMWOA were analyzed using capillary zone electrophoresis	Johansson et al., 2008
Tulip poplar	AM	<i>Acaulospora morrowiae</i> <i>Glomus claroideum</i> <i>G. clarum</i> <i>Paraglomus brasilianum</i>	- - ↑ malate, citric acid -	5-month-old seedlings were grown in fungal inoculated sand. Roots were washed for organic acid profiles. LMWOA were identified using ion chromatography	Klugh and Cumming, 2007

↑, increase; -, no change; ↓, decrease; n/a, information not available.

a meta-analysis of C-allocation dynamics in trees Epron et al. (2012) showed that broadleaf trees exhibit, on average, 10 times higher rates of C transfer than coniferous species, although this varies depending on season. In spring (before bud break) and fall (during leaf senescence), broadleaves allocate a greater proportion of C to their roots (Epron et al., 2012). A number of studies have documented seasonal trends in soil microbial communities and activities in a variety of ecosystems (Allison and Treseder, 2008; Björk et al., 2008; Cruz-Martinez et al., 2009), including the coniferous forests of the Pacific Northwest (Brant et al., 2006; Moore-Kucera and Dick, 2008) and deciduous forests of Europe (Hibbard et al., 2005; Rasche et al., 2011). Studies specifically examining ECM fungi have found that their community structure, as well as enzymatic and metabolic capabilities, exhibit considerable temporal variation over a single year (Buée et al., 2005; Courty et al., 2007, 2008). This is likely related to differences in belowground C flow (Collignon et al., 2011). Burke et al. (2011) showed ECM, but not AM varied over a growing season in a mixed deciduous forest in Pennsylvania and that

ECM and AM were associated with different enzyme activities involved in nutrient cycling. Specifically, AM fungi were associated with leucine aminopeptidase and urease, both enzymes involved in N acquisition. Arbuscular mycorrhizae were not traditionally considered able to supply their host with significant amounts of N (Smith and Read, 2008), though there is recent evidence that AM fungi can access both inorganic N (Fellbaum et al., 2012) and organic N (Whiteside et al., 2012) sources in forests. ECM were associated with most measured enzymes involved in C and N acquisition, but only during the late summer (Burke et al., 2011). This indicates that mycorrhizal ability to breakdown recalcitrant C and provide their host with N may vary seasonally.

EFFECTS OF MYCORRHIZOSPHERE C FLOW ON OTHER ORGANISMS

FREE-LIVING FUNGI, BACTERIA AND ARCHAEA

It is clear that variation in the quality and quantity of C released in root and hyphal exudates produced by different tree species can

Table 3 | Examples of mycorrhization helper bacteria, with significant effects on ECM formation.

Fungi	Bacteria	MHB effect	Host plant	References
<i>Laccaria laccata</i>	<i>Agrobacterium radiobacter</i>	↑ mycorrhizal colonization	6-month-old pine and birch seedlings grown on sand-mica-rock substrate	Leyval and Berthelin, 1993
<i>Suillus grevillei</i>	<i>Pseudomonas fluorescens</i> strain 70 <i>Pseudomonas putida</i> strain 42	↑ Fungal growth	Fungi and bacteria were cultured from sporocarps found in European larch forest	Varese et al., 1996
<i>Geopora</i> species	<i>Sphingomonas</i> sp. 23L	↑ fungal inoculation, and tree growth	Willow tree cuttings potted in 1 kg of fly ash, bacterial inoculant was added	Hryniewicz et al., 2009
<i>Lactarius rufus</i> , <i>Laccaria bicolor</i> or <i>Suillus luteus</i>	<i>Paenibacillus</i> sp. EJP73, <i>Burkholderia</i> sp. EJP67, <i>Paenibacillus</i> sp. EJP73	Altered root branching ↑ <i>L. bicolor</i> mycorrhiza formation	Scots pine seedlings grown in vermiculite-peat moss microcosms	Aspray et al., 2006
<i>Suillus granulatus</i> <i>Cenococcum geophilum</i>	<i>Ralstonia basilensis</i> , <i>Bacillus subtilis</i>	Increased hyphal growth	1-week-old Japanese black pine was planted in autoclaved soil before inoculated with fungi	Kataoka et al., 2009
<i>Laccaria bicolor</i> S238N	<i>Pseudomonas fluorescens</i> BBc6R8	Promotes presymbiotic fungal-survival and increases radial growth, hyphal apex density and branching angle	Pre-symbiotic, grown on Pachlewski medium	Deveau et al., 2007
<i>Amantia muscaria</i> <i>Suillus bovinus</i>	<i>Streptomyces</i> nov. sp. 505 <i>Streptomyces annulatus</i> 1003 (AcH 1003)	1.2–1.7 fold increase in second-order root mycorrhizal rate	4-weeks-old Norway spruce and Scots pine seedlings were grown on autoclaved peatmoss and perlite before inoculation	Schrey et al., 2005
<i>Laccaria laccata</i>	<i>Pseudomonas</i> species, <i>Bacillus</i> species	↑ mycorrhizal colonization	Douglas-fir seeds were sown in inoculated vermiculite-peat moss polythene cells	Duponnois and Garbaye, 1991
<i>Laccaria fraterna</i> <i>Laccaria laccata</i>	<i>Bacillus</i> species <i>Pseudomonas</i> species	↑ mycorrhizal colonization	Eucalyptus seeds were sown in sphagnum peat-perlite before inoculation	Dunstan et al., 1998
<i>Lactarius rufus</i>	<i>Paenibacillus</i> species <i>Burkholderia</i> species	↑ mycorrhizal colonization	Sterile Scots pine seedlings grown on agar petri dishes were used for inoculation once roots were 4.5–6 cm long	Poole et al., 2001
<i>Pisolithus alba</i>	<i>Pseudomonas monteilli</i> <i>Pseudomonas resinovorans</i>	↑ mycorrhizal colonization	Soapbush seedlings were planted in autoclaved soapbush soil before inoculation	Founoune et al., 2002a
<i>Pisolithus</i> species	<i>Pseudomonas</i> species	↑ mycorrhizal colonization	Soapbush seedlings were planted in autoclaved soapbush soil before inoculation	Founoune et al., 2002b
<i>Rhizopogon luteolus</i>	Unidentified	↑ mycorrhizal colonization	Radiata pine seedlings were grown on autoclaved soil before inoculation	Garbaye and Bowen, 1989
<i>Scleroderma</i> species <i>Pisolithus</i> species	<i>Pseudomonas monteilli</i> strain HR13	↑ mycorrhizal colonization	Acacia seedlings were grown on sterilized sand before inoculation	Duponnois and Plenchette, 2003
<i>Suillus luteus</i>	<i>Bacillus</i> species	↑ root growth and mycorrhizal colonization	2-week-old Scots pine seedlings were grown on inoculated peat-vermiculate petri dishes	Bending et al., 2002

result in different rhizosphere and hyphosphere microbial communities and this varies between tree species associated with ECM and AM (Garbaye, 1991; Broeckling et al., 2008; Prescott and Grayston, 2013). Phillips and Fahey (2006) collected rhizosphere soil, bulk soil, and fine roots from the upper four centimeters of 12 monospecific tree species plots (six AM and six ECM tree species) planted on a common soil. The rhizosphere of AM trees and ECM trees were 10–12 and 25–30% more active (as measured by respired CO₂) than bulk soil, demonstrating that ECM trees have a greater rhizosphere effect than AM trees. The magnitude of rhizosphere effects was negatively correlated with the degree of mycorrhizal colonization in AM tree species and with fine root biomass in ECM tree species. This suggests that different factors influence rhizosphere effects in tree species forming AM vs. ECM associations (Phillips and Fahey, 2006). Hyphal exudates from ECM tips support a diverse population of bacteria, archaea and fungi (Frey-Klett et al., 2007; Tedersoo et al., 2009; Bomberg et al., 2011). High throughput sequencing methods developed over recent years are enabling us to obtain much greater phylogenetic resolution to our studies of mycorrhizosphere microbial communities. For example, Kluber et al. (2010) used DNA sequencing to identify the rhizomorphic ECM mat-forming taxa (*Hysterangium*, *Piloderma*, *Suillus* and *Russula* species) in the forest floor and the hydrophobic mat-forming taxa (*Gomphus* and *Ramaria* species) in the mineral soil in a Douglas-fir forest. The two ECM mat forms had enhanced enzyme activities, specifically chitinase, phosphatase and phenol oxidase compared to non-mat forms in adjacent locations (Kluber et al., 2010). It was not established if the enhanced enzyme activity in the mats was the result of the ECM themselves or the distinctive bacteria and fungi in their mycorrhizosphere (Kluber et al., 2011). Bomberg and Timonen (2007, 2009) demonstrated (using PCR-DGGE of archaeal 16S rRNA genes) that there were specific archaeal communities in the ectomycorrhizosphere of several common boreal forest trees and that the type of ECM had the most influence on archaeal diversity. Bomberg et al. (2011) found no evidence of archaea in bulk humus samples lacking tree roots or ECM, indicating archaea are dependent on plant-derived C for growth. Similarly, Pires et al. (2012) used pyrosequencing and PCR-DGGE to reveal differences in archaeal richness between two mangrove species. Uroz et al. (2012) revealed (pyrosequencing 16S rRNA) that *Alpha*-, *Beta*-, and *Gammaproteobacteria* were significantly higher in the ectomycorrhizosphere of oak than in bulk soil and the bacterial communities found in the ectomycorrhizosphere of *Xerocomus pruinatus* and *Scleroderma citrinum* on oak were similar at the genus level, but different at the OTU level, demonstrating the specificity of the ectomycorrhizosphere. In the future, further refinements to molecular techniques, enhanced bioinformatic analysis and development of novel methods to culture and study these newly revealed organisms should enable links between these organisms and their functions to be elucidated. To date most of our knowledge on the role of associated microorganisms in the ectomycorrhizosphere has been based on studies of culturable organisms. The spectrum of plant-microbe relationships in the rhizosphere can range from mutualistic to pathogenic (Bais et al., 2006). Plant-growth-promoting rhizobacteria (PGPR)—which are found in

the rhizosphere and mycorrhizosphere—benefit plants by creating biofilms that protect the root against pathogens (Akhtar and Siddiqui, 2009). These rhizosphere bacteria induce systemic acquired resistance (preparing the plant for attack; Pieterse et al., 2003) and enhance plant growth (Adesemoye et al., 2008; Yang et al., 2009). Several very good reviews have been written on PGPR (Vessey, 2003; Lugtenberg and Kamilova, 2009). There is some evidence of synergistic interactions between PGPR and mycorrhizal fungi, which may benefit the plants as a result of greater nutrient acquisition, inhibition of plant pathogens and greater mycorrhization (Artursson et al., 2006). Uroz et al. (2007) demonstrated positive interactions between ECM and bacteria that result in increased weathering of mineral nutrients, ultimately increasing nutrient uptake by the plant. AM have also been found to alter the structure of mycorrhizosphere microbial communities (Rillig and Mummey, 2006; Toljander et al., 2007; Welc et al., 2012). Isolation and identification of rhizobacteria found in the mycorrhizosphere around AM hyphae have shown bacteria with antagonistic properties toward soil-borne pathogens (Lioussanne et al., 2010), and antifungal properties (although they do not affect the AM symbiosis; Dwivedi et al., 2009). The N₂ fixing ability of some AM plants improves when mycorrhizae are present vs. when they are absent (Kucey and Paul, 1982; Fitter and Garbaye, 1994).

Greater mycorrhization effects have been attributed to one specific group of PGPR, the so-called mycorrhization-helper bacteria (MHB) (Garbaye, 1994). Three life-stages in mycorrhizal fungi have been recognized, the free-living saprotrophic, the pre-infection stage and the symbiotic, mycorrhization stage (Deveau et al., 2007; Courty et al., 2008). During the pre-infection “free-living stage,” mycorrhizal fungi can interact with specific bacteria (e.g., *Pseudomonas* species) that are thought to enhance mycorrhizal establishment (Garbaye, 1994; Pivato et al., 2009). These mycorrhization-helper bacteria (MHB) can increase mycorrhization of a plant 1.2–17.5 times (Frey-Klett et al., 2007). Mycorrhization-helper bacteria are not plant-specific, but may be fungal-specific (Garbaye, 1994; Pivato et al., 2009). For example, *Pseudomonas fluorescens* BBc6R8 promotes survival of ECM *Laccaria bicolor* S238N when in its free-living stage, increasing radial fungal growth, hyphal density and branching angle. Mycorrhization-helper bacteria also change mycelial physiology from the free-living saprotrophic state to a “pre-symbiotic” stage (Deveau et al., 2007). During mycorrhization, a proliferation of bacteria can improve the receptivity of roots (Aspray et al., 2006), accelerate germination of fungal propagules in soil (Garbaye, 1994), and increase production of compounds such as auxofurans (Tylka et al., 1991) which have been shown to affect fungal metabolism and gene expression (Riedlinger et al., 2006). Mycorrhization-helper bacterial strains identified thus far include: Gram-negative Proteobacteria, Gram-positive Firmicutes and Gram-positive Actinomycetes (Frey-Klett et al., 2007) (Table 3). How MHB encourage mycorrhization is only beginning to be unraveled. Most MHB increase fungal colonization of the roots via: stimulating mycelia extension and branching (Garbaye, 1994; Poole et al., 2001; Schrey et al., 2005), increasing root-fungus contacts/colonization, and influencing soil environmental conditions (Frey-Klett et al., 2007).

Mycorrhization-helper bacteria have been observed to stimulate spore germination of *Glomus mosseae* and *Glomus clarum* (AM) (Mosse, 1962; Xavier and Germida, 2003, respectively). In the case of *Glomus clarum* there may have been a complex bacterial consortium producing antagonistic volatiles (Tylka et al., 1991). The release of a number of different compounds, including gasses (Duponnois and Kisa, 2006) and secondary metabolites (e.g., auxofuran) (Keller et al., 2006; Riedlinger et al., 2006) by MHB have been shown to increase mycelial growth. Mycorrhization-helper bacteria are thought to reduce plant and mycorrhizal stress by detoxifying soil (e.g., Polyphenolic substances produced by *Paxillus involutus* are toxic to the fungus, but can be broken down by MHB; Duponnois and Garbaye, 1990). The potential for MHB to increase and support mycorrhizal infection has been demonstrated only under laboratory conditions. However, as in the case of PGPR, little is known about the effect these bacteria have on mycorrhization *in situ*.

TREE-MYCORRHIZAL-MICROBIAL AND FAUNAL INTERACTIONS

The term rhizosphere fauna has typically been used to refer to agricultural pests, specifically root herbivores (Bonkowski et al., 2009). However, rhizosphere fauna encompass a broad range of feeding types, including those that feed on bacteria, mycelium, and other fauna. Soil fauna influence the composition and activity of microbial populations by: directly grazing on bacteria and fungal hyphae, transporting fungal and microbial cells in their gut (thus facilitating microbial dispersion) and changing physical and chemical conditions of the soil (i.e., worm casts) (Oades, 2003). Several fungivorous collembola species have the capacity to influence development of Basidiomycete mycelia (Tordoff et al., 2008; Crowther et al., 2011b), and the extent of that influence is directly dependent on collembola density (Hanlon and Anderson, 1979; Kaneko et al., 1998). Setälä (1995) compared control soil (no fauna) and faunal-inoculated soil in Scots pine and silver birch microcosms. In all cases the presence of soil fauna reduced ECM abundance, reduced microbial biomass and increased shoot production. Faunal community impacts on decomposer fungi have also been shown to be density dependent, although there is evidence that the faunal community composition may have a greater impact on the microbial community. Crowther and A'Bear (2012) found that grazing pressures exerted by low-density woodlouse populations on saprotrophic fungi surpassed grazing pressures exerted by high density millipedes or high density collembola populations, ultimately limiting mycelial development. Grazing of mycelium not only influences microbial populations, but also has direct impacts on nutrient cycling because it increases enzyme release into the mycorrhizosphere (Crowther et al., 2011a), particularly in the presence of macrofauna (Crowther et al., 2011b). This increase will, in turn, affect soil nutrient availability (both N and P) and SOM turnover.

Mycophagous soil fauna grazing on AM and ECM in forests will affect C flow into the mycorrhizosphere by disrupting the movement of C along rhizomorphs and runner-type hyphae (Setälä, 1995; Coleman et al., 2004). Once removed from its C source, the growing hyphal front will stop releasing exudates,

stop growing and potentially die off or convert to a saprotrophic life stage. The amount of hyphal grazing varies with mycorrhizal species, as soil fauna have been shown to be selective in their feeding preferences (Klironomos and Kendrick, 1996; Crowther and A'Bear, 2012). Klironomos and Kendrick (1996) showed that mites and collembola preferentially graze fungi growing on litter. However, when offered only AM growing on maple, they consume the fine hyphae most distant from the root. Cesarz et al. (2013) demonstrated that ECM vs. AM mycorrhizal-tree identity had a major influence on belowground nematode communities. Ash, which forms AM symbiosis, had greater populations of bacterial-feeding nematodes and lesser populations of fungal-feeding nematodes. In contrast beech, which forms EM symbiosis, had enhanced fungal-feeding nematode populations. Grazing on hyphal mycorrhizal networks can also significantly influence plant-C allocation belowground, and may influence C sequestration (Johnson et al., 2005).

CONSEQUENCES OF THE MYCORRHIZOSPHERE ON SOIL C

Differences in tree-mycorrhizal symbiosis types may impact C-cycling and C sequestration because of differences in C allocation and longevity of these structures in soil. ECM trees with extensive mycelia have two to three times more C flux to the soil than AM trees (Finlay and Söderström, 1992; Phillips and Fahey, 2005; Pumpanen et al., 2009). This may be due to ECM roots being “more leaky,” possibly due to higher exudation rates (Phillips and Fahey, 2006). Pumpanen et al. (2009) demonstrated that roots and ECM growth account for 13–21% of recently assimilated C, whereas 9–26% of recently assimilated C is respired from the roots and rhizosphere. The turnover times of ECM and AM are also dramatically different; the turnover times of EMM and mycorrhizal fine roots are in the order of months to years (Cairney, 2012) and AM days to weeks (Langley and Hungate, 2003). The slower turnover of ECM is thought to be due to the chitin content of this fungal tissue, although in AM fungi the production of the glycoprotein glomalin can decrease AM hyphal turn over times significantly. Glomalin binds the soil matrix forming a soil aggregate within which AM hyphae are trapped and are slow to decompose, having an estimated residence time of 6–42 years (Rillig, 2004). These soil aggregates represent more the 5% of total soil C, significantly contributing to long term soil C sequestration (Wright and Upadhyaya, 1998; Rillig et al., 2001). However, the grazing of AM fungi is also higher than ECM because of the thin walls of AM fungi, which reduces residence times of this C in soil (Klironomos and Kendrick, 1996). Cheng et al. (2012) recently suggested that AM fungi diminish rather than enhance soil C pools in the short-term, as a result of accelerated decomposition of litter, when sites are exposed to elevated CO₂. Although ECM production of proteolytic and lignolytic enzymes enables increased degradation of SOM (releasing more C) relative to AM (Read, 1992; Chalot and Brun, 1998), the recent study by Clemmensen et al. (2013) has suggested that accumulation and preservation of root and root-associated fungal residues is responsible for up to two-thirds of the C sequestered in boreal forests. This suggests that ECM dominated soils are more likely to sequester soil C, at least in the short term. However, long-term effects (decadal) may be qualitatively different from short-term

effects; specifically there may be a long-term gain in recalcitrant compounds (Verbruggen et al., 2012).

ECM differ in nutrient uptake and transfer rates, altering the net primary production (NPP) of trees and may ultimately influence ecosystem C-cycling and C sequestration (Burgess et al., 1993). The ability of ECM to promote tree NPP varies depending on the extent of root colonization, the type of hyphae (Colpaert et al., 1992; Thomson et al., 1994) and the ability of the hyphae to acquire and transfer nutrients to the tree (Agerer, 2001). ECM fungi also have broad enzymatic capabilities (Chalot and Brun, 1998) that allow them to decompose labile and recalcitrant components of SOM, access organic sources of N, and transfer large amounts of N to host plants (Hobbie and Hobbie, 2006). AM fungi can also acquire substantial N from SOM (Hodge et al., 2010; Whiteside et al., 2012), although they do not have as broad an N-based enzymatic capability and appear to transfer only a small fraction of their host plants demand for N (Hodge and Fitter, 2010). This is particularly evident in dry soil conditions when N transport by roots is restricted, but soil N levels are still high (Tobar et al., 1994; Govindarajulu et al., 2005). There is some evidence that AM hyphae hydrolyze organic C at their root tips (Koide and Kabir, 2000), but there is limited evidence of AM derived phosphatases in the mycorrhizosphere along the hyphae (Joner et al., 2000). Belowground C allocation in AM-fungal-dominated ecosystems may not return sufficient N (or P) to offset the C investment by the tree, limiting the increase in NPP associated with greater atmospheric CO₂ concentrations (Drake et al., 2011). Turnover of SOM has been shown to be faster in forest stands with AM mycorrhizal associations compared to ECM (Vesterdal et al., 2012). Phillips et al. (2013) proposed that forests dominated by AM and ECM associated trees vary in their C cycling and nutrient acquisition and may respond to global changes in predictable ways. They have proposed a new framework for predicting these variations in biogeochemical processes between forests [the Mycorrhizal-Associated Nutrient Economy model (MANE)] using forest inventory analysis maintained by the US Forest Service and previously described mycorrhizal designations (Brundrett et al., 1990; Wang and Qui, 2006). AM-dominated forest stands will have an inorganic nutrient economy resulting from elevated rates of C, N, and P mineralization and high quality litter. In contrast, ECM-dominated forest stands will have an organic nutrient economy as a result of slow rates of C, N, and P turnover and a lower quality litter. Thus further supports the hypothesis that ECM dominant forests will sequester more C.

ECM have the potential to act as a strong C sink, acquiring large amounts of C from their plant hosts (Smith and Read, 2008). The ECM then move the plant C to their hyphal tips, generating new biomass and exuding various compounds for nutrient acquisition. This movement of C can be a significant transport of plant C beyond the rhizosphere (Norton et al., 1990; Erland et al., 1991; Finlay and Söderström, 1992), and the recalcitrant chitinous cell wall of the mycelium will remain in the soil for months (Setälä et al., 1999; Treseder and Allen, 2000). The life-span of ECM root tips may be anywhere from 3 to 22 months (Orlov, 1960; Majdi et al., 2001), and may increase with soil depth (Pritchard et al., 2008a; McCormack et al., 2010). The consequences of C movement throughout the soil via hyphae is only beginning to

be understood. Carbon will be transported out of the rhizosphere, moving as little as a few centimeters to as much as tens of meters (Gryta et al., 1997; Dunham et al., 2003; Murata et al., 2005; Churchland et al., 2012). However, long-distance, continuous, transport of C in hyphae is likely small as EMM are often fragmented, due to foraging by soil fauna (Dahlberg and Stenlid, 1995) and there are impermeable cell walls that form physiologically separated regions along hyphae (Olsson, 1999). However, C movement along hyphae would be very difficult to measure, and the potential impacts of the movement on C sequestration is large. Depending on forest type, climate and measurement methods, estimates of fungal biomass in ECM root tips can range from 20–10,000 kg/ha (Fogel and Hunt, 1979; Vogt et al., 1982; Dahlberg et al., 1997; Satomura et al., 2003; Sims et al., 2007; Helmisaari et al., 2009; Okada et al., 2011). The majority of this biomass is found in the forest floor and organic soil layers (Bååth et al., 2004; Wallander et al., 2004; Göransson et al., 2006), and constitute up to 1/3 of the total microbial biomass in forests (Swedish conifer forest; Höglberg and Höglberg, 2002). A recent study by Clemmensen et al. (2013) determined that 50–70% of stored C belowground was derived from root and root-associated microorganisms. Using ¹⁴C bomb-carbon modeling Clemmensen et al. (2013) found preservation of fungal residues in late-successional forests and in particular root-associated fungi, not saprotrophs, are the important regulators of ecosystem C dynamics. The sheer volume of tree C allocated belowground, and the ability of this C to move throughout the soil profile and soil ecosystem, shows how important it is to determine accurate C models of forests and other mycorrhizal-dominated soil ecosystems.

CONCLUSIONS

Carbon allocation to mycorrhizal hyphae enhances the degree to which tree C can impact soil microbial communities and soil C cycling. Different mycorrhizal morphotypes will vary the spatial distribution of this C considerably, although the vast, delicate nature of mycorrhizal hyphae makes this a difficult area of study. The greater C allocation to mycorrhizal roots, coupled with slower turnover times of mycorrhizal roots compared to non-mycorrhizal roots, hints at the potential of mycorrhizal associations to increase C sequestration in soil. However, differences between ECM and AM may impact soil C sequestration. ECM roots have longer turnover times than AM, and, due to their chitinous cell walls, are less likely to be grazed by fauna. Recent improvements in stable-isotope labeling and probing methods have resulted in a better understanding of the quantity and quality of C exuded belowground and spatial and temporal dynamics of C flow in forest soil. In addition whole-genome sequencing is showing us the large suite of important genes and signals involved in symbiotic associations. These new techniques should enable great strides to be made on our understanding of the role of different mycorrhizal functional groups in forest C cycling. We suggest the next step in developing this understanding would be tracing the flow C throughout the different hyphal morphotypes and measuring turnover times of this C to establish how ECM and AM distribute C in forest soil. This could be done through a combination of stable-isotope labeling and probing techniques conducted in large-scale controlled conditions, such as a biotron,

coupled with nanosims technology to increase sensitivity and isotopic detection at high spatial resolution. We may then be able to determine the consequences of these variations for C-cycling and C sequestration.

REFERENCES

- Adesemoye, A. O., Torbert, H. A., and Kloepper, J. W. (2008). Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. *Can. J. Microb.* 54, 876–886. doi: 10.1139/W08-081
- Agerer, R. (2001). Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* 11, 107–114. doi: 10.1007/s005720100108
- Ahonen-Jonnarh, U., van Hees, P. A. W., Lundström, U. S., and Finlay, R. D. (2000). Organic acids produced by mycorrhizal *Pinus sylvestris* exposed to elevated aluminium and heavy metal concentrations. *New Phyt.* 146, 557–567. doi: 10.1046/j.1469-8137.2000.00653.x
- Akhtar, M. S., and Siddiqui, Z. A. (2009). Use of plant growth-promoting rhizobacteria for the biocontrol of root-rot disease complex of chickpea. *Australas. Plant Path.* 38, 44–50. doi: 10.1071/AP08075
- Allen, M. F. (2007). Mycorrhizal fungi: highways for water and nutrients in arid soils. *Vadose Zone J.* 6, 291–297. doi: 10.2136/vzj2006.0068
- Allison, S. D., and Treseder, K. K. (2008). Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Glob. Change Biol.* 14, 2898–2909. doi: 10.1111/j.1365-2486.2008.01716.x
- Amaranthus, M. P., and Perry, D. A. (1989). Rapid root tip and mycorrhiza formation and increased survival of Douglas-fir seedlings after soil transfer. *New For.* 3, 259–264. doi: 10.1007/BF00028933
- Amaranthus, M., Trappe, J. M., Bednar, L., and Arthur, D. (1994). Hypogeous fungal production in mature Douglas-fir forest fragments and surrounding plantations and its relation to coarse woody debris and animal mycophagy. *Can. J. For. Res.* 24, 2157–2165. doi: 10.1139/x94-278
- Artursson, V., Finlay, R. D., and Jansson, J. K. (2006). Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. *Environ. Microbiol.* 8, 1–10. doi: 10.1111/j.1462-2920.2005.00942.x
- Aspray, T. J., Frey-Klett, P., Jones, J. E., Whipps, J. M., Garbaye, J., and Bending, G. D. (2006). Mycorrhization helper bacteria: a case of specificity for altering ectomycorrhiza architecture but not ectomycorrhiza formation. *Mycorrhiza* 16, 533–541. doi: 10.1007/s00572-006-0068-3
- Bååth, E., Nilsson, L. O., Göransson, H., and Wallander, H. (2004). Can the extent of degradation of soil fungal mycelium during soil incubation be used to estimate ectomycorrhizal biomass in soil? *Soil Biol. Biochem.* 26, 2105–2109. doi: 10.1016/j.soilbio.2004.06.004
- Bago, B., Azcon-Aguilar, C., and Piche, Y. (1998). Architecture and developmental dynamics of the external mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown under monoxenic conditions. *Mycologia* 90, 52–62. doi: 10.2307/3761011
- Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., and Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* 57, 233–266. doi: 10.1146/annurev.arplant.57.032905.105159
- Bending, G. D., Poole, E. J., Whipps, J. M., and Read, D. J. (2002). Characterisation of bacteria from *Pinus sylvestris*-*Suillus luteus* mycorrhizas and their effects on root-fungus interactions and plant growth. *FEMS Microbiol. Ecol.* 39, 219–227. doi: 10.1016/S0168-6496(01)00215-X
- Berg, B., Johansson, M., Meentemeyer, V., and Kratz, W. (1998). Decomposition of tree root litter in a climatic transect of coniferous forests in northern Europe: a synthesis. *Scand. J. For. Res.* 13, 402–412. doi: 10.1080/02827589809383000
- Bidartondo, M. I., Ek, H., Wallander, H., and Söderström, B. (2001). Do nutrient additions alter carbon sink strength of ectomycorrhizal fungi? *New Phyt.* 151, 543–550. doi: 10.1046/j.1469-8137.2001.00180.x
- Binkley, D., Stape, J. L., Takahashi, E. N., and Ryan, M. G. (2006). Tree-girdling to separate root and heterotrophic respiration in two eucalyptus stands in Brazil. *Oecologia* 148, 447–454. doi: 10.1007/s00442-006-0383-6
- Björk, R. G., Björkman, M. P., Andersson, M. X., and Klemetsson, L. (2008). Temporal variation in soil microbial communities in Alpine tundra. *Soil Biol. Biochem.* 40, 266–268. doi: 10.1016/j.soilbio.2007.07.017
- Bomberg, M., Münster, U., Pumpanen, J., Ilvesniemi, H., and Heinonsalo, J. (2011). Archaeal communities in boreal forest tree rhizospheres respond to changing soil temperatures. *Microb. Ecol.* 62, 205–217. doi: 10.1007/s00248-011-9837-4
- Bomberg, M., and Timonen, S. (2007). Distribution of cren- and Euryarchaeota in Scots pine mycorrhizospheres and boreal forest humus. *Microb. Ecol.* 54, 406–416. doi: 10.1007/s00248-007-9232-3
- Bomberg, M., and Timonen, S. (2009). Effect of tree species and mycorrhizal colonization on the archaeal population of boreal forest rhizospheres. *Appl. Environ. Microbiol.* 75, 308–315. doi: 10.1128/AEM.01739-08
- Bonfante, P., and Genre, A. (2010). Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. *Nat. Commun.* 1, 48. doi: 10.1038/ncomms1046
- Bonfante, P., and Requena, N. (2011). Dating in the dark: how roots respond to fungal signals to establish arbuscular mycorrhizal symbiosis. *Curr. Opin. Plant Biol.* 14, 451–457. doi: 10.1016/j.pbi.2011.03.014
- Bonkowski, M., Villenave, C., and Giffiths, B. (2009). Rhizosphere faunal: the functional and structural diversity of intimate interactions of soil fauna with plant roots. *Plant Soil* 321, 213–233. doi: 10.1007/s11104-009-0013-2
- Brachmann, A., and Parniske, M. (2006). The most widespread symbiosis on earth. *PLoS Biol.* 4:e239. doi: 10.1371/journal.pbio.0040239
- Brant, J. B., Sulzman, E. W., and Myrold, D. D. (2006). Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. *Soil Biol. Biochem.* 38, 2219–2232. doi: 10.1016/j.soilbio.2006.01.022
- Broeckling, C. D., Broz, A. K., Bergelson, J., Manter, D. K., and Vivanco, J. M. (2008). Root exudates regulate soil fungal community composition and diversity. *Appl. Environ. Microbiol.* 74, 738–744. doi: 10.1128/AEM.02188-07
- Brundrett, M., Murase, G., and Kendrick, B. (1990). Comparative anatomy of roots and mycorrhizae of common Ontario trees. *Can. J. Bot.* 68, 551–578. doi: 10.1139/b90-076
- Bruns, T. D., Bidartondo, M. I., and Taylor, D. L. (2002). Host specificity in ectomycorrhizal communities: what do the exceptions tell us? *Integr. Comp. Biol.* 42, 352–359. doi: 10.1093/icb/42.2.352
- Buée, M., Vairelles, D., and Garbaye, J. (2005). Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (*Fagus sylvatica*) forest subjected to two thinning regimes. *Mycorrhiza* 15, 235–245. doi: 10.1007/s00572-004-0313-6
- Burgess, T. I., Malajczuk, N., and Groves, T. S. (1993). The ability of 16 ectomycorrhizal fungi to increase growth and phosphorus uptake of *Eucalyptus globulus* Labill. and *E. diversicolor* F. Muell. *Plant Soil* 153, 155–164. doi: 10.1007/BF00012988
- Burke, D. J., Weintraub, M. N., Hewins, C. R., and Jalisz, S. (2011). Relationship between soil enzyme activities, nutrient cycling and soil fungal communities in a northern hardwood forest. *Soil Biol. Biochem.* 43, 795–803. doi: 10.1016/j.soilbio.2010.12.014
- Cairney, J. W. G. (2012). Extramatrical mycelia of ectomycorrhizal fungi as moderators of carbon dynamics in forest soil. *Soil Biol. Biochem.* 47, 198–208. doi: 10.1016/j.soilbio.2011.12.029
- Cairney, J. W. G., and Alexander, I. J. (1992). A study of ageing of spruce [*Picea sitchensis* (Bong.) Carr.] ectomycorrhizas. II. carbohydrate allocation in ageing *Picea sitchensis*/Tylospora fibrillosa (Burt.) Donk ectomycorrhizas. *New Phyt.* 122, 153–158. doi: 10.1111/j.1469-8137.1992.tb00061.x
- Cairney, J. W. G., Ashford, A. E., and Allway, W. G. (1989). Distribution of photosynthetically fixed carbon within root systems of *Eucalyptus pilularis* ectomycorrhizal with *Pisolithus tictorius*. *New Phyt.* 112, 495–500. doi: 10.1111/j.1469-8137.1989.tb00343.x
- Cairney, J. W. G., and Burke, R. M. (1996). Physiological heterogeneity within fungal mycelia: an important concept for a functional understanding of the ectomycorrhizal symbiosis. *New Phyt.* 134, 685–695. doi: 10.1111/j.1469-8137.1996.tb04934.x
- Cesarz, S., Ruess, L., Jacob, M., Schaefer, M., and Scheu, S. (2013). Tree species diversity versus tree species identity: driving forces in structuring forest food webs as indicated by soil nematodes. *Soil Biol. Biochem.* 62, 36–45. doi: 10.1016/j.soilbio.2013.02.020
- Chalot, M., and Brun, A. (1998). Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas. *FEMS Microb. Rev.* 22, 21–44. doi: 10.1111/j.1574-6976.1998.tb00359.x

- Chapin, I., Stuart, F., MacFarland, J., McGuire, A. D., Euskirchen, E. S., Ruess, R. W., et al. (2009). The changing global carbon cycle: linking plant-soil carbon dynamics to global consequences. *J. Ecol.* 97, 840–850. doi: 10.1111/j.1365-2745.2009.01529.x
- Chen, D. M., Zhou, L. X., Wu, J. P., Hsu, J. N., Lin, Y. B., and Fu, S. L. (2012). Tree girdling affects the soil microbial community by modifying resource availability in two subtropical plantations. *Appl. Soil Ecol.* 53, 108–115. doi: 10.1016/j.apsoil.2011.10.014
- Cheng, L., Booker, F. L., Tu, C., Burkey, K. O., Zhou, L., Shew, H. D., et al. (2012). Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO₂. *Science* 337, 1084–1087. doi: 10.1126/science.1224304
- Churchland, C., Grayston, S. J., and Bengtson, P. (2013). Spatial variability of soil fungal and bacterial abundance: consequences for carbon turnover along a transition from a forested to clear-cut site. *Soil Biol. Biochem.* 63, 5–13. doi: 10.1016/j.soilbio.2013.03.015
- Churchland, C., Weatherall, A., Briones, M. J. I., and Grayston, S. J. (2012). Stable-isotope labeling and probing of recent photosynthates into respired CO₂, soil microbes and soil mesofauna using a xylem and phloem stem-injection technique on Sitka spruce (*Picea sitchensis*). *Rapid Comm. Mass Spec.* 26, 2493–2501. doi: 10.1002/rcm.6368
- Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., et al. (2013). Roots and associated fungi drive long-term carbon sequestration in boreal forests. *Science* 339, 1615–1618. doi: 10.1126/science.1231923
- Coleman, D. C., Crossley, D. A. Jr., and Hendrix, P. F. (2004). *Fundamentals of Soil Ecology*, 2nd Edn. London: Elsevier inc.
- Collignon, C., Uroz, S., Turpault, M. P., and Frey-Klett, P. (2011). Seasons differently impact the structure of mineral weathering bacterial communities in beech and spruce stands. *Soil Biol. Biochem.* 43, 2012–2022. doi: 10.1016/j.soilbio.2011.05.008
- Colpaert, J. V., Vanassche, J. A., and Luijckens, K. (1992). The growth of the extramatrical mycelium of ectomycorrhizal fungi and the growth-response of *Pinus sylvestris* L. *New Phyt.* 120, 127–135. doi: 10.1111/j.1469-8137.1992.tb01065.x
- Courty, P. E., Breda, N., and Garbaye, J. (2007). Relationship between oak tree phenology and the secretion of organic matter degrading enzymes by *Lactarius quietus* ectomycorrhizas before and during bud break. *Soil Biol. Biochem.* 39, 1655–1663. doi: 10.1016/j.soilbio.2007.01.017
- Courty, P. E., Franc, A., Pierrat, J. C., and Garbaye, J. (2008). Temporal changes in the ectomycorrhizal community in two soil horizons of a temperate oak forest. *App. Environ. Microb.* 74, 5792–5801. doi: 10.1128/AEM.01592-08
- Crowther, T. W., and A'Bear, A. D. (2012). Impacts of grazing soil fauna on decomposer fungi are species-specific and density-dependent. *Fungal Ecol.* 5, 277–281. doi: 10.1016/j.funeco.2011.07.006
- Crowther, T. W., Boddy, L., and Jones, T. H. (2011b). Species-specific effects of soil fauna on fungal foraging and decomposition. *Oecologia* 167, 535–545. doi: 10.1007/s00442-011-2005-1
- Crowther, T. W., Jones, T. H., Boddy, L., and Baldrian, P. (2011a). Invertebrate grazing determines enzyme production by basidiomycete fungi. *Soil Biol. Biochem.* 43, 2060–2068. doi: 10.1016/j.soilbio.2011.06.003
- Cruz-Martinez, K., Suttle, K. B., Brodie, E. L., Power, M. E., Andersen, G. L., and Banfield, J. F. (2009). Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. *ISME J.* 3, 738–744. doi: 10.1038/ismej.2009.16
- Dahlberg, A., Jonsson, L., and Nylund, J. E. (1997). Species diversity and distribution of biomass above and belowground among ectomycorrhizal fungi in an old-growth Norway spruce forest in south Sweden. *Can. J. Bot.* 75, 1323–1335. doi: 10.1139/b97-844
- Dahlberg, A., and Stenlid, J. (1995). Spatiotemporal patterns in ectomycorrhizal populations. *Can. J. Bot.* 73, S1222–S1230. doi: 10.1139/b95-382
- Dakora, F. D., and Phillips, D. A. (2002). Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant Soil* 245, 35–47. doi: 10.1023/A:1020809400075
- de Boer, W., Folman, L. B., Summerbell, R. C., and Boddy, L. (2005). Living in a fungal world: impact of fungi on soil bacteria niche development. *FEMS Microb. Rev.* 29, 795–811. doi: 10.1016/j.femsre.2004.11.005
- Deveau, A., Palin, B., Delaruelle, C., Peter, M., Kohler, A., Pierrat, J. C., et al. (2007). The mycorrhiza helper *Pseudomonas fluorescens* BbC6R8 has a specific priming effect on the growth, morphology and gene expression of the ectomycorrhizal fungus *Laccaria bicolor* S238N. *New Phytol.* 1175, 743–755. doi: 10.1111/j.1469-8137.2007.02148.x
- DeWit, P. J. G. M., Mehrabi, R., Van Den Burg, H. A., and Stergiopoulos, I. (2009). Fungal effector proteins: past present and future. *Mol. Plant Pathol.* 10, 735–747. doi: 10.1111/j.1364-3703.2009.00591.x
- Dodd, J. C., Boddington, C. L., Rodriguez, A., Gonzalez-Chavez, V., and Mansur, I. (2000). Mycelium of arbuscular mycorrhizal fungi (AMF) from different genera: form, function and detection. *Plant Soil* 226, 131–151. doi: 10.1023/A:1026574828169
- Drake, J. E., Gallet-Budynek, A., Hofmockel, K. S., Bernhardt, E. S., Billings, S. A., Jackson, R. B., et al. (2011). Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO₂. *Ecol. Lett.* 14, 349–357. doi: 10.1111/j.1461-0248.2011.01593.x
- Dunham, S. M., Kretzer, A., and Pfrender, M. E. (2003). Characterization of pacific golden chanterelle (*Cantarellus formosus*) genet size using co-dominant microsatellite markers. *Mol. Ecol.* 12, 1607–1618. doi: 10.1046/j.1365-294X.2003.01837.x
- Dunstan, W. A., Malajczuk, N., and Dell, B. (1998). Effects of bacteria on mycorrhizal development and growth of container grown *Eucalyptus diversicolor* F. Muell. seedlings. *Plant Soil* 201, 241–249. doi: 10.1023/A:1004329626763
- Duponnois, R., and Garbaye, J. (1990). Some mechanisms involved in growth simulation of ectomycorrhizal fungi by bacteria. *Can. J. Bot.* 68, 2148–2152. doi: 10.1139/b90-280
- Duponnois, R., and Garbaye, J. (1991). Mycorrhization helper bacteria associated with the Douglas-fir laccaria-laccata symbiosis: effects in aseptic and in glasshouse conditions. *Annal. Sci. For.* 48, 239–251. doi: 10.1051/forest:19910301
- Duponnois, R., and Kisa, M. (2006). The possible roles of trehalose in the mycorrhiza helper effect. *Can. J. Bot.* 84, 1005–1008. doi: 10.1139/b06-053
- Duponnois, R., and Plenchette, C. (2003). A mycorrhiza helper bacterium enhances ectomycorrhizal and endomycorrhizal symbiosis of Australian *Acacia* species. *Mycorrhiza* 13, 85–91. doi: 10.1007/s00572-002-0204-7
- Durall, D. M., Jones, M. D., and Tinker, P. B. (1994). Allocation of ¹⁴C-carbon in ectomycorrhizal willow. *New Phyt.* 128, 109–114. doi: 10.1111/j.1469-8137.1994.tb03993.x
- Dwivedi, D., Johri, B. N., Ineichen, K., Wray, V., and Wiemken, A. (2009). Impact of antifungals producing rhizobacteria on the performance of *Vigna radiata* in the presence of arbuscular mycorrhizal fungi. *Mycorrhiza* 19, 559–570. doi: 10.1007/s00572-009-0253-2
- Edwards, I. P., and Zak, D. R. (2010). Chronic simulated atmospheric N deposition alters actinobacterial community composition in forest floor and surface soil. *Soil Sci. Soc. Am. J.* 74, 1157–1166. doi: 10.2136/sssaj2009.0240
- Eisenhauer, S. D., Beßler, H., Engels, C., Gleixner, G., Habekost, H., Milcu, A., et al. (2010). Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology* 91, 485–496. doi: 10.1890/08-2338.1
- Ek, H. (1997). The influence of nitrogen fertilization on the carbon economy of *Paxillus involutus* in ectomycorrhizal association with *Betula pendula*. *New Phyt.* 135, 133–142. doi: 10.1046/j.1469-8137.1997.00621.x
- Eldhuset, T. D., Swensen, B., Wickström, T., and Wollebaek, G. (2007). Organic acids in root exudates from *Picea abies* seedlings influenced by mycorrhiza and aluminum. *J. Plant Nut. Soil Sci.* 170, 645–648. doi: 10.1002/jpln.200700005
- Epron, D., Bahn, M., Derrien, D., Lattanzi, F. A., Pumpanen, J., Gessler, A., et al. (2012). Pulse-labelling trees to study carbon allocation dynamics: a review of methods, current knowledge and future prospects. *Tree Physiol.* 32, 776–798. doi: 10.1093/treephys/tps057
- Epron, D., Ngao, J., Dannoura, M., Bakker, M. R., Zeller, B., Bazot, S., et al. (2011). Seasonal variation of belowground carbon transfer assessed by *in situ* (CO₂)-C-13 pulse labelling of trees. *Biogeosciences* 8, 1153–1168. doi: 10.5194/bg-8-1153-2011
- Erland, S., Finlay, R., and Söderström, B. (1991). The influence of substrate pH on carbon translation in ectomycorrhizal and non-mycorrhizal pine seedlings. *New Phyt.* 119, 235–242. doi: 10.1111/j.1469-8137.1991.tb01026.x
- Esperschütz, J. A., Gattinger, A., Buegger, F., Lang, H., Munch, J., Schloter, M., et al. (2009). A continuous labelling approach to recover photosynthetically fixed carbon in plant tissue and rhizosphere organisms of young beech trees (*Fagus sylvatica* L.) using ¹³C depleted CO₂. *Plant Soil* 323, 21–29. doi: 10.1007/s11104-009-9998-9
- Falkowski, P., Scholes, R. J., Boyle, E., Canadell, J., Canfield, D., Elser, J., et al. (2000). The global carbon cycle: a test of our knowledge of earth as a system. *Science* 290, 291–296. doi: 10.1126/science.290.5490.291

- Fellbaum, C. R., Gachomo, E. W., Beesetty, Y., Choudhari, S., Strahan, G. D., Pfeffer, P. E., et al. (2012). Carbon availability triggers fungal nitrogen uptake and transport in arbuscular mycorrhizal symbiosis. *Proc. Nat. Acad. Sci. U.S.A.* 109, 2666–2671. doi: 10.1073/pnas.1118650109
- Finlay, R. D. (2008). Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. *J. Exp. Biol.* 59, 1115–1126. doi: 10.1093/jxb/ern059
- Finlay, R. D., and Rosling, A. (2006). “Integrated nutrient cycles in forest ecosystems, the role of ectomycorrhizal fungi,” in *Fungi in Biogeochemical Cycles*, ed G. M. Gadd (Cambridge: Cambridge University Press), 28–50. doi: 10.1017/CBO9780511550522.003
- Finlay, R., and Söderström, B. (1992). “Mycorrhiza and carbon flow to the soil,” in *Mycorrhizal Functioning: and Integrative Plant-Fungal Process*, ed M. F. Allen (New York, NY: Chapman and Hall), 134–160.
- Fitter, A. H. and Garbaye, J. (1994). Interactions between mycorrhizal fungi and other soil organisms. *Plant Soil* 159, 123–132.
- Fogel, R., and Hunt, G. (1979). Fungal and arboreal biomass in western oregon douglas-fir ecosystems: distribution patterns and turnover. *Can. J. For. Res.* 9, 245–256. doi: 10.1139/x79-041
- Fontaine, S., Mariotti, A., and Abbadié, L. (2003). The priming effect of organic matter: a question of microbial competition? *Soil Biol. Biochem.* 35, 837–843. doi: 10.1016/S0038-0717(03)00123-8
- Founoune, H., Duponnois, R., Ba, A. M., Sall, S., Branget, I., Lorquin, J., et al. (2002a). Mycorrhiza helper bacteria stimulated ectomycorrhizal symbiosis of *Acacia holosericea* with *Pisolithus alba*. *New Phytol.* 153, 81–89. doi: 10.1046/j.0028-646X.2001.00284.x
- Founoune, H., Duponnois, R., Meyer, J. M., Thioulouse, J., Masse, D., Chotte, J. L., et al. (2002b). Interactions between ectomycorrhizal symbiosis and fluorescent pseudomonads on *Acacia holosericea*: isolation of mycorrhiza helper bacteria (MHB) from a Soudano-Sahelian soil. *FEMS Microbiol. Ecol.* 41, 37–46. doi: 10.1111/j.1574-6941.2002.tb00964.x
- Franklin, O., Johansson, J., Dewar, R. C., Dieckmann, U., McMurtrie, R. E., Brännström, Å., et al. (2012). Modeling carbon allocation in trees: a search for principles. *Tree Physiol.* 32, 648–666. doi: 10.1093/treephys/tp138
- Fransson, P. M. A., and Johansson, E. M. (2010). Elevated CO₂ and nitrogen influence exudation of soluble organic compounds by ectomycorrhizal root systems. *FEMS Microbiol. Ecol.* 71, 186–196. doi: 10.1111/j.1574-6941.2009.00795.x
- Frey, P., Frey-Klett, P., Garbaye, J., Berge, O., and Heulin, T. (1997). Metabolic and genotypic fingerprinting of fluorescent pseudomonas associated with the Douglas-fir-*Laccaria bicolor* mycorrhizosphere. *Appl. Environ. Microb.* 63, 1852–1860.
- Frey-Klett, P., Garbaye, J., and Tarkka, M. (2007). The mycorrhiza helper bacteria revisited. *New Phyt.* 176, 22–36. doi: 10.1111/j.1469-8137.2007.02191.x
- Friese, C. E., and Allen, M. F. (1991). The spread of VA mycorrhizal fungal hyphae in the soil—Inoculum types and external hyphal architecture. *Mycologia* 83, 409–418. doi: 10.2307/3760351
- Fritze, H., Pietikainen, J., and Pennanen, T. (2000). Distribution of microbial biomass and phospholipid fatty acids in Podzol profiles under coniferous forest. *Eur. J. Soil Sci.* 51, 565–573. doi: 10.1046/j.1365-2389.2000.00346.x
- Garbaye, J. (1991). Biological interactions in the mycorrhizosphere. *Experientia* 47, 370–375. doi: 10.1007/BF01972079
- Garbaye, J. (1994). Helper bacteria—a new dimension to the mycorrhizal symbiosis. *New Phyt.* 128, 197–210. doi: 10.1111/j.1469-8137.1994.tb04003.x
- Garbaye, J., and Bowen, G. D. (1989). Stimulation of ectomycorrhizal infection of *Pinus radiata* by some microorganisms associated with the mantle of ectomycorrhizas. *New Phytol.* 112, 383–388. doi: 10.1111/j.1469-8137.1989.tb00327.x
- Gardes, M., and Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Mol. Ecol.* 2, 113–118. doi: 10.1111/j.1365-294X.1993.tb00005.x
- Göransson, H., Wallander, H., Ingerslev, M., and Rosengren, U. (2006). Estimating the relative nutrient uptake from different soil depths in *Quercus robur*, *Fagus sylvatica* and *Picea abies*. *Plant Soil* 286, 87–97. doi: 10.1007/s11104-006-9028-0
- Govindarajulu, M., Pfeffer, P. E., Jin, H. R., Abubaker, J., Douds, D. D., Allen, J. W., et al. (2005). Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature* 435, 819–823. doi: 10.1038/nature03610
- Grayston, S. J., Vaughan, D., and Jones, D. (1997). Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. *App. Soil Ecol.* 5, 29–56. doi: 10.1016/S0929-1393(96)00126-6
- Griffiths, R. P., Baham, J. E., and Caldwell, B. A. (1994). Soil solution chemistry of ectomycorrhizal mats in forest soil. *Soil Biol. Biochem.* 26, 331–337. doi: 10.1016/0038-0717(94)90282-8
- Gryta, H., Debaud, J.-C., Effosse, A., Gay, G., and Marmeisse, R. (1997). Fine-Scale structure of populations of the ectomycorrhizal fungus *Hebeloma cylindrosporum* in coastal sand dune forest ecosystems. *Mol. Ecol.* 6, 353–364. doi: 10.1046/j.1365-294X.1997.00200.x
- Hanlon, R. D. G., and Anderson, J. M. (1979). Effects of collembola grazing on microbial activity in decomposing leaf litter. *Oecologia* 38, 93–99. doi: 10.1007/BF00347827
- Helmisaari, H. S., Ostonen, I., Lohmus, K., Derome, J., Lindroos, A. J., Merilä, P., et al. (2009). Ectomycorrhizal root tips in relation to site and stand characteristics in Norway spruce and Scots pine stands in boreal forests. *Tree Physiol.* 29, 445–456. doi: 10.1093/treephys/tpn042
- Hibbard, K. A., Law, B. E., Reichstein, M., and Sulzman, J. (2005). An analysis of soil respiration across northern hemisphere temperate ecosystems. *Biogeochem* 73, 29–70. doi: 10.1007/s10533-004-2946-0
- Hinsinger, P., Gobran, G. R., Gergory, P. J., and Wenzel, W. W. (2005). Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. *New Phyt.* 168, 293–303. doi: 10.1111/j.1469-8137.2005.01512.x
- Hirose, D., Kikuchi, J., Kanzaki, N., and Futai, K. (2004). Genet distribution of sporocarps and ectomycorrhizas of *Sullis pictus* in a Japanese white pine plantation. *New Phyt.* 164, 527–554. doi: 10.1111/j.1469-8137.2004.01188.x
- Hobbie, J. E., and Hobbie, E. A. (2006). N-15 in symbiotic fungi and plants estimates nitrogen and carbon flux rates in Arctic tundra. *Ecology* 87, 816–822. doi: 10.1890/0012-9658(2006)87[816:NISFAP]2.0.CO;2
- Hodge, A., and Fitter, A. H. (2010). Substantial nitrogen acquisition by arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen direction from organic material. *Nature* 413, 297–299. doi: 10.1038/35095041
- Hodge, A., Helgason, T., and Fitter, A. H. (2010). Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecol.* 3, 267–273. doi: 10.1016/j.funeco.2010.02.002
- Hodge, A., Robinson, D., and Fitter, A. H. (2000). An arbuscular mycorrhizal inoculum enhances root proliferation in, but not nitrogen capture from, nutrient-rich patches in soil. *New Phyt.* 145, 575–584. doi: 10.1046/j.1469-8137.2000.00602.x
- Högberg, M. N., and Högberg, P. (2002). Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phyt.* 154, 791–795. doi: 10.1046/j.1469-8137.2002.00417.x
- Högberg, M. N., Högberg, P., and Myrold, D. D. (2007). Is microbial community composition in Boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? *Oecologia* 150, 590–601. doi: 10.1007/s00442-006-0562-5
- Högberg, P., Nordgren, A., Buchmann, N., Taylor, A. F. S., Ekblad, A., Högberg, M. N., et al. (2001). Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* 411, 789–792. doi: 10.1038/35081058
- Hogenhout, S. A., Van der Hoorn, R. A. L., Terauchi, F., and Kamoun, S. (2009). Emerging concepts in effector biology of plant-associated organisms. *Mol. Plant Microbe Interact.* 22, 115–122. doi: 10.1094/MPMI-22-2-0115
- Hryniewicz, K., Baum, C., Niedojadlo, J., and Dahm, H. (2009). Promotion of mycorrhiza formation and growth of willows by the bacterial strain *Sphingomonas* sp. 23L on fly ash. *Biol. Fertil. Soils* 45, 385–394. doi: 10.1007/s00374-008-0346-7
- Ishida, T. A., Nara, K., and Hogetsu, T. (2007). Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer-broadleaf forests. *New Phyt.* 174, 430–440. doi: 10.1111/j.1469-8137.2007.02016.x
- Izumi, H., Anderson, I. C., Alexander, I. J., Killam, K., and Moore, E. R. B. (2006a). Diversity and expression of nitrogenase genes (nifH) from ectomycorrhizas of Corsican pine (*Pinus nigra*). *Environ. Microbiol.* 8, 2224–2230. doi: 10.1111/j.1462-2920.2006.01104.x
- Izumi, H., Anderson, I. C., Alexander, I. J., Killam, K., and Moore, E. R. B. (2006b). Endobacteria in some ectomycorrhiza of Scots pine (*Pinus sylvestris*). *FEMS Microb. Ecol.* 56, 34–43. doi: 10.1111/j.1574-6941.2005.00048.x
- Jobbágy, E. G., and Jackson, R. B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol. App.* 10, 423–436. doi: 10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2
- Johansson, E. M., Fransson, P. M. A., Finlay, R. D., and van Hees, P. A. W. (2009). Quantitative analysis of soluble exudates produced by ectomycorrhizal roots as

- a response to ambient and elevated CO₂. *Soil Biol. Biochem.* 41, 1111–1116. doi: 10.1016/j.soilbio.2009.02.016
- Johansson, J. F., Fransson, P. M. A., Finlay, R. D., and van Hees, P. A. W. (2008). Quantitative analysis of root and ectomycorrhizal exudates as a response to Pb, Cd and As stress. *Plant Soil* 313, 39–54. doi: 10.1007/s11104-008-9678-1
- Johansson, J. F., Paul, L. R., and Finlay, R. D. (2004). Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microb. Ecol.* 48, 1–13. doi: 10.1016/j.femsec.2003.11.012
- Johnson, D., Krsek, M., Wellington, M. H., Stott, A. W., Cole, L., Bardgett, R. D., et al. (2005). Soil invertebrates disrupt carbon flow through fungal networks. *Science* 309, 1047. doi: 10.1126/science.1114769
- Joner, E. J., van Aarle, I. M., and Vosatka, M. (2000). Phosphatase activity of extraradical arbuscular mycorrhizal hyphae: a review. *Plant Soil* 226, 199–210. doi: 10.1023/A:1026582207192
- Jones, D. L., and Darrah, P. R. (1994). Role of root derived organic-acids in the mobilization of nutrients from the rhizosphere. *Plant Soil* 313, 39–54.
- Jones, D. L., Hodge, A., and Kuzyakov, Y. (2004). Plant and mycorrhizal regulation of rhizodeposition. *New Phyt.* 163, 459–480. doi: 10.1111/j.1469-8137.2004.01130.x
- Kaneko, N., McLean, M. A., and Parkinson, D. (1998). Do mites and Collembola affect pine litter fungal biomass and microbial respiration? *Appl. Soil Ecol.* 9, 209–213. doi: 10.1016/S0929-1393(98)00077-8
- Kataoka, R., Siddiqui, Z. A., Taniguchi, T., and Futai, K. (2009). Quantification of *Wautersia [Ralstonia] basileensis* in the mycorrhizosphere of *Pinus thunbergii* Parl. and its effect on mycorrhizal formation. *Soil Biol. Biochem.* 41, 2147–2152. doi: 10.1016/j.soilbio.2009.07.027
- Keller, S., Schneider, K., and Suessmuth, R. D. (2006). Structure elucidation of auxofuran, a metabolite involved in stimulating growth of fly agaric, produced by the mycorrhiza helper bacterial *streptomyces* AcH 505. *J. Antibiot.* 59, 801–803. doi: 10.1038/ja.2006.106
- Kennedy, P. G., Izzo, A. D., and Bruns, T. D. (2003). There is high potential for the formation of common mycorrhizal networks between understory and canopy trees in a mixed evergreen forest. *J. Ecol.* 91, 1071–1080. doi: 10.1046/j.1365-2745.2003.00829.x
- Klironomos, J. N., and Kendrick, W. B. (1996). Palatability of microfungi to soil arthropods in relation to the functioning of arbuscular mycorrhizae. *Biol. Fert. Soils* 21, 43–52. doi: 10.1007/BF00335992
- Kloppholz, S., Kuhn, H., and Requena, N. (2011). A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. *Curr. Biol.* 21, 1204–1209. doi: 10.1016/j.cub.2011.06.044
- Kluber, L. A., Smith, J. E., and Myrold, D. D. (2011). Distinctive fungal and bacterial communities are associated with mats formed by ectomycorrhizal fungi. *Soil Biol. Biochem.* 43, 1042–1050. doi: 10.1016/j.soilbio.2011.01.022
- Kluber, L. A., Tinnesand, K. M., Caldwell, B. A., Dunham, S. M., Yarwood, R. R., Bottomley, P. J., et al. (2010). Ectomycorrhizal mats alter forest soil biogeochemistry. *Soil Biol. Biochem.* 42, 1607–1613. doi: 10.1016/j.soilbio.2010.06.001
- Klugh, K. R., and Cumming, J. R. (2003). Variation in organic acid exudates among mycorrhizal species colonizing *Liriodendron tulipifera* L. (yellow-poplar) in the presence of aluminum. 88th Annual Meeting of the Ecological Society of America held jointly with the International Society for Ecological Modeling-North American Chapter. *Ecol. Soc. Am. Ann. Meet. Abstr.* 88, 186.
- Klugh, K. R., and Cumming, J. R. (2007). Variations in organic acid exudation and aluminum resistance among arbuscular mycorrhizal species colonizing *Liriodendron tulipifera*. *Tree Physiol.* 27, 1103–1112.
- Koide, R. T., and Kabir, Z. (2000). Extraradical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate. *New Phyt.* 148, 511–517. doi: 10.1046/j.1469-8137.2000.00776.x
- Köljal, U., Dahlberg, A., Taylor, A. F. S., Larsson, E., Hallenberg, N., Stenlid, J., et al. (2000). Diversity and abundance of *Resupinate thelephoroid* fungi as ectomycorrhizal symbionts in Swedish boreal forests. *Mol. Ecol.* 9, 1985–1996. doi: 10.1046/j.1365-294X.2000.01105.x
- Koranda, M., Schnecker, J., Kaiser, C., Fuchsluger, L., Kitzler, B., Stange, C. E., et al. (2011). Microbial processes and community composition in the rhizosphere of European beech—The influence of plant C exudates. *Soil Biol. Biochem.* 43, 551–558. doi: 10.1016/j.soilbio.2010.11.022
- Kourtev, P. S., Ehrenfeld, J. G., and Haggblom, M. (2002). Exotic plant species alter microbial structure and function in the soil. *Ecology* 83, 3152–3166. doi: 10.1890/0012-9658(2002)083[3152:EPSATM]2.0.CO;2
- Kucey, R. M. N., and Paul, E. A. (1982). Carbon flow, photosynthesis and N₂ fixation in mycorrhizal and nodulated faba beans (*Vicia faba* L.). *Soil Biol. Biochem.* 14, 407–412. doi: 10.1016/0038-0717(82)90013-X
- Kuzyakov, Y., and Domanski, G. (2000). Carbon input by plants into the soil. Review. *J. Plant Nutr. Soil Sci.* 4, 421–431. doi: 10.1002/1522-2624(200008)163:4<421::AID-JPLN421>3.0.CO;2-R
- Kuzyakov, Y., and Gavrichkova, O. (2010). Time lag between photosynthesis and CO₂ efflux from soil: a review of mechanisms and controls. *Glob. Change Biol.* 16, 3386–3406. doi: 10.1111/j.1365-2486.2010.02179.x
- Landeweert, R., Hofflund, E., Finlay, R. D., and van Breeman, N. (2001). Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. *Trends Ecol. Evol.* 16, 248–254. doi: 10.1016/S0169-5347(01)02122-X
- Langley, J. A., and Hungate, B. A. (2003). Mycorrhizal controls on belowground litter quality. *Ecol.* 84, 2301–2312. doi: 10.1890/02-0282
- Lapeyrie, F., Chilvers, G. A., and Bhem, C. A. (1987). Oxalic acid synthesis by the ectomycorrhizal fungus *Paxillus involutus*. *New Phyt.* 106, 139–146. doi: 10.1111/j.1469-8137.1987.tb04797.x
- Leake, J. R., Donnelly, D. P., Saunders, E. M., Boddy, L., and Read, D. J. (2001). Rates and quantities of carbon flux to ectomycorrhizal mycelium following 14C pulse labeling of *Pinus sylvestris* seedlings: effects of litter patches and interaction with a wood-decomposer fungus. *Tree Physiol.* 21, 71–82. doi: 10.1093/treephys/21.2-3.71
- Leckie, S. E., Prescott, C. E., Grayston, S. J., Neufeld, J. D., and Mohn, W. W. (2004). Characterization of humus microbial communities in adjacent forest types that differ in nitrogen availability. *Microb. Ecol.* 48, 29–40. doi: 10.1007/s00248-003-1020-0
- Lejon, D. P. H., Chausson, R., Ranger, J., and Ramjard, L. (2005). Microbial community structure and density under different tree species in an acid forest soil. *Microb. Ecol.* 50, 614–625. doi: 10.1007/s00248-005-5130-8
- Leyval, C., and Berthelin, J. (1993). Rhizodeposition and net release of soluble organic-compounds by pine and beech seedlings inoculated with rhizobacteria and ectomycorrhizal fungi. *Biol. Fert. Soils* 15, 259–267. doi: 10.1007/BF00337210
- Li, Y. J., Yang, X. D., Zou, X. M., and Wu, J. H. (2009). Responses of soil nematode communities to tree girdling in a subtropical evergreen broad-leaved forest of southwest China. *Soil Biol. Biochem.* 41, 877–882. doi: 10.1016/j.soilbio.2008.07.031
- Liebeke, M., Brozel, V. S., Hecker, M., and Lalk, M. (2009). Chemical characterization of soil extract as growth media for the ecophysiological study of bacteria. *Appl. Microb. Biotech.* 83, 161–173. doi: 10.1007/s00253-009-1965-0
- Lin, G., Ehleringer, J. R., Rygielwicz, P. T., Johnson, M. G., and Tingey, D. T. (1999). Elevated CO₂ and temperature impacts on different components of soil CO₂ efflux in Douglas-fir terracosms. *Glob. Change Biol.* 5, 157–168. doi: 10.1046/j.1365-2486.1999.00211.x
- Lindahl, B. D., Ihrmark, K., Boberg, J., Trumbore, S., Höglberg, P., Stenlid, J., et al. (2007). Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in boreal forests. *New Phyt.* 173, 611–620. doi: 10.1111/j.1469-8137.2006.01936.x
- Lioussanne, L., Perrault, F., Jolicœur, M., and St-Arnaud, M. (2010). The bacterial community of tomato rhizosphere is modified by inoculation with arbuscular mycorrhizal fungi but unaffected by soil enrichment with mycorrhizal root exudates or inoculation with *Phytophthora nicotianae*. *Soil Biol. Biochem.* 42, 473–483. doi: 10.1016/j.soilbio.2009.11.034
- Litton, C. M., and Giardina, C. P. (2008). Below-ground carbon flux and partitioning: global patterns and response to temperature. *Func. Ecol.* 22, 941–954. doi: 10.1111/j.1365-2435.2008.01479.x
- Litton, C. M., Raich, J. W., and Ryan, M. G. (2007). Carbon allocation in forest ecosystems. *Glob. Change Biol.* 13, 2089–2109. doi: 10.1111/j.1365-2486.2007.01420.x
- Lowe, R. G. T., and Howlett, B. J. (2012). Indifferent, affectionate or deceitful: lifestyles and secretomes of fungi. *PLoS Pathog.* 8:e1002515. doi: 10.1371/journal.ppat.1002515
- Lugtenberg, B., and Kamilova, F. (2009). Plant-growth-promoting-rhizobacteria. *Annu. Rev. Microb.* 63, 541–556. doi: 10.1146/annurev.micro.62.081307.162918
- Maffei, M. E., Arimura, G. I., and Mithofer, A. (2012). Natural elicitors, effectors and modulators of plant responses. *Nat. Prod. Rep.* 29, 1288–1303. doi: 10.1039/c2np20053h

- Majdi, H., Damm, E., and Nylund, J. E. (2001). Longevity of mycorrhizal roots depends on branching order and nutrient availability. *New Phyt.* 150, 195–202. doi: 10.1046/j.1469-8137.2001.00065.x
- Marschner, P., Crowley, D., and Rengel, Z. (2011). Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis—model and research methods. *Soil Biol. Biochem.* 43, 883–894. doi: 10.1016/j.soilbio.2011.01.005
- Martin, F., Aerts, A., Ahren, D., Brun, A., Duchaussoy, F., Kohler, A., et al. (2008). The genome sequence of the basidiomycete fungus *Laccaria bicolor* provides insights into the mycorrhizal symbiosis. *Nature* 452, 88–92. doi: 10.1038/nature06556
- Martin, F., Kohler, A., and Duplessis, S. (2007). Living in harmony in the wood underground: ectomycorrhizal genomics. *Curr. Opin. Plant Biol.* 10, 204–210. doi: 10.1016/j.pbi.2007.01.006
- Martin, F., Kohler, A., Murat, C., Balestrini, R., Coutinho, P. M., Jaillon, O., et al. (2010). Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. *Nature* 464, 1033–1038. doi: 10.1038/nature08867
- McCormack, M. L., Pritchard, S. G., Breland, S., Davis, M. A., Prior, S. A., Runion, B., et al. (2010). Soil fungi respond more strongly than fine roots to elevated CO₂ in a model regenerating longleaf pine-wiregrass ecosystem. *Ecosystems* 13, 901–916. doi: 10.1007/s10021-010-9360-3
- Meier, I. C., Avids, P. G., and Phillips, R. P. (2013). Fungal communities influence root exudation rates in pine seedlings. *FEMS Microb. Ecol.* 83, 585–595. doi: 10.1111/1574-6941.12016
- Mencuccini, M., and Holtta, T. (2010). The significance of phloem transport for the speed with which canopy photosynthesis and belowground respiration are linked. *New Phyt.* 185, 189–203. doi: 10.1111/j.1469-8137.2009.03050.x
- Molina, R., and Trappe, J. M. (1982). Patterns of Ectomycorrhizal host specificity and potential among Pacific Northwest conifers and fungi. *For. Sci.* 28, 423–458.
- Moore-Kucera, J., and Dick, R. P. (2008). PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. *Microb. Ecol.* 55, 500–511. doi: 10.1007/s00248-007-9295-1
- Mosse, B. (1962). The establishment of vesicular-arbuscular mycorrhiza under aseptic conditions. *J. Gen. Microb.* 27, 509–520. doi: 10.1099/00221287-27-3-509
- Murata, H., Ohta, A., Yamada, A., Narimatsu, M., and Futamura, N. (2005). Genetic mosaics in the massive persisting rhizosphere colony “shiro” of the ectomycorrhizal basidiomycete *Tricholoma matsutake*. *Mycorrhiza* 15, 505–512. doi: 10.1007/s00572-005-0358-1
- Nazir, R., Warmink, J. A., Boersma, H., and van Elsas, J. D. (2010). Mechanisms that promote bacterial fitness in fungal-affected soil microhabitats. *FEMS Microb. Ecol.* 71, 169–185. doi: 10.1111/j.1574-6941.2009.00807.x
- Nehls, U., Gohringer, F., Wittulsky, S., and Dietz, S. (2010). Fungal carbohydrate support in the ectomycorrhizal symbiosis: a review. *Plant Biol.* 12, 292–301. doi: 10.1111/j.1438-8677.2009.00312.x
- Neumann, G., and Romheld, V. (2001). “The release of root exudates as affect by the plant’s physiological status,” in *The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface*, eds R. Pinto, Z. Varanini, and P. Nannipieri (New York, NY: Dekker), 41–93.
- Norton, J. M., Smith, J. L., and Firestone, M. K. (1990). Carbon flow in the rhizosphere of ponderosa pine seedlings. *Soil Biol. Biochem.* 22, 449–455. doi: 10.1016/0038-0717(90)90177-2
- Oades, J. M. (2003). *The Role of Biology in the Formation, Stabilization and Degradation of Soil Structure*. Wageningen: International workshop on methods of research in soil structure, soil biota interrelationships in Wageningen.
- Okada, K., Okada, S., Yasue, K., Fukuda, M., and Yamada, A. (2011). Six-year monitoring of pine ectomycorrhizal biomass under a temperate monsoon climate indicates significant annual fluctuations in relation to climatic factors. *Ecol. Res.* 26, 411–419. doi: 10.1007/s11284-011-0800-0
- Olsson, S. (1999). “Nutrient translocation and electrical signalling in mycelia,” in *The Fungal Colony*, eds N. A. R. Gow, G. D. Robson, and G. M. Gadd (Cambridge: Cambridge University Press), 25–48. doi: 10.1017/CBO9780511549694.003
- Orlov, A. (1960). Growth and changes with age of feeder roots of *Picea excelsa* Link. *Bot. Z.* 45, 888–896.
- Pena, R., Offermann, C., Simon, J., Naumann, P. S., Gessler, A., Hlost, J., et al. (2010). Girdling affects ectomycorrhizal fungi (EMF) diversity and reveals functional differences in EMF community composition in a beech forest. *App. Environ. Microb.* 76, 1831–1841. doi: 10.1128/AEM.01703-09
- Phillips, R. P., Brzostek, E., and Midgley, M. G. (2013). The mycorrhizal associated nutrient economy: a new framework for predicting carbon-nutrient couplings in temperate forests. *New Phytol.* 199, 41–51. doi: 10.1111/nph.12221
- Phillips, R. P., Erlitz, Y., Bier, R., and Bernhardt, E. S. (2008). New approach for capturing soluble root exudate in forest soils. *Func. Ecol.* 22, 990–999. doi: 10.1111/j.1365-2435.2008.01495.x
- Phillips, R. P., and Fahey, T. J. (2005). Patterns of rhizosphere carbon flux in sugar maple (*Acer saccharum*) and yellow birch (*Betula allegheniensis*) saplings. *Glob. Change Biol.* 11, 983–995. doi: 10.1111/j.1365-2486.2005.00959.x
- Phillips, R. P., and Fahey, T. J. (2006). Tree species and mycorrhizal associations influence the magnitude of rhizosphere effects. *Ecology* 87, 1302–1313. doi: 10.1890/0012-9658(2006)87[1302:TSAMAI]2.0.CO;2
- Phillips, R. P., Finzi, A. C., and Bernhardt, E. S. (2011). Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO₂ fumigation. *Ecol. Lett.* 14, 1887–1894. doi: 10.1111/j.1461-0248.2010.01570.x
- Phillips, R. P., Meier, I. C., Bernhardt, E. S., Grandy, A. S., Wickings, K., and Finzi, A. C. (2012). Roots and fungi accelerate carbon and nitrogen cycling in forest exposed to elevated CO₂. *Ecol. Lett.* 15, 1042–1049. doi: 10.1111/j.1461-0248.2012.01827.x
- Pieterse, C. M. J., Van Pelt, J. A., Verhagen, B. W. M., Ton, J., van Wees, S. C. M., Leon-Kloosterziel, K. M., et al. (2003). Induced systemic resistance by plant growth-promoting rhizobacteria. *Symbiosis* 35, 39–54.
- Pires, A. C. C., Cleary, D. F. R., Almeida, A., Cunha, A., Dealtry, S., Mendonça-Hagler, L. C. S., et al. (2012). Denaturing gradient gel electrophoresis and barcoded pyrosequencing reveal unprecedented archaeal diversity in mangrove sediment and rhizosphere samples. *App. Environ. Microb.* 78, 5520–5528. doi: 10.1128/AEM.00386-12
- Pivato, B., Offre, P., Marchelli, S., Barbonaglia, B., Mougél, C., Lemanceau, P., et al. (2009). Bacterial effects on arbuscular mycorrhizal fungi and mycorrhiza development as influenced by the bacteria, fungi, and host plant. *Mycorrhiza* 19, 81–90. doi: 10.1007/s00572-008-0205-2
- Plett, J. M., Kemppainen, M., Kale, S. D., Kohler, A., Legue, V., Brun, A., et al. (2011). A secreted effector protein of *Laccaria bicolor* is required for symbiosis development. *Curr. Biol.* 21, 1197–1203. doi: 10.1016/j.cub.2011.05.033
- Podila, G. K., Sreedasyam, A., and Muratet, M. A. (2009). *Populus* rhizosphere and the ectomycorrhizal interactome. *Crit. Rev. Plant Sci.* 28, 359–367. doi: 10.1080/07352680903241220
- Poole, E. J., Bending, G. D., Whipps, J. M., and Read, D. J. (2001). Bacteria associated with *Pinus sylvestris*-*Lactarius rufus* ectomycorrhizas and their effects on mycorrhiza formation *in vitro*. *New Phyt.* 151, 743–751. doi: 10.1046/j.0028-646x.2001.00219.x
- Prescott, C. E., and Grayston, S. J. (2013). Tree species influence on microbial communities in litter and soil: current knowledge and research needs. *For. Ecol. Manag.* 309, 19–27. doi: 10.1016/j.foreco.2013.02.034
- Pritchard, S. G., Strand, A. E., McCormack, M. L., Davis, M. A., Finzi, A. C., Jackson, R. B., et al. (2008b). Fine root dynamics in a loblolly pine forest are influenced by Free-Air-CO₂-Enrichment (FACE): a six year minirhizotron study. *Glob Change Biol.* 7, 829–837. doi: 10.1111/j.1365-2486.2007.01523.x
- Pritchard, S. G., Strand, A. E., McCormack, M. L., Davis, M. A., and Oren, R. (2008a). Mycorrhizal and rhizomorph dynamics in a loblolly pine forest during 5 years of free-air-CO₂-enrichment. *Glob. Change Biol.* 14, 1–13. doi: 10.1111/j.1365-2486.2008.01567.x
- Pritsch, K., Munch, J. C., and Buscot, F. (1997). Morphological and anatomical characterization of black alder *Alnus glutinosa* (L.) Gaertn. ectomycorrhizas. *Mycorrhiza* 7, 201–216. doi: 10.1007/s005720050182
- Pumpanten, J. S., Heinonsalo, J., Rasilo, T., Hurme, K., and Ilvesniemi, H. (2009). Carbon balance and allocation of assimilated CO₂ in Scots pine, Norway spruce, and Silver birch seedlings determined with gas exchange measurements and C-14 pulse labelling. *Tree Struct. Func.* 23, 611–621. doi: 10.1007/s00468-008-0306-8
- Qu, L. Y., Shinano, T., Quresji, A. M., Tamai, Y., Osaki, M., and Koike, T. (2004). Allocation of ¹⁴C carbon in two species of larch seedlings infected with ectomycorrhizal fungi. *Tree Physiol.* 24, 1369–1376. doi: 10.1093/treephys/24.12.1369
- Rangel-Castro, J. I., Dannell, E., and Pfeffer, P. E. (2002). A C-13-NMR study of exudation and storage of carbohydrates and amino acids in the ectomycorrhizal edible mushroom *Chanterelle cibarius*. *Mycologia* 94, 190–199. doi: 10.2307/3761795
- Rasche, F., Knapp, D., Kaiser, C., Koranada, M., Kitzler, B., Zechmeister-Boltenstern, S., et al. (2011). Seasonality and resource availability control

- bacterial and archeal communities in soils of a temperate beech forest. *ISME J.* 5, 389–402. doi: 10.1038/ismej.2010.138
- Rasmann, S., and Agrawal, A. A. (2008). In defense of roots: a research agenda for studying plant resistance to belowground herbivory. *Plant Physiol.* 146, 875–880. doi: 10.1104/pp.107.112045
- Read, D. J. (1992). “The mycorrhizal mycelium,” in *Mycorrhizal Functioning: an Integrative Plant-Fungus Process*, ed M. J. Allen (New York, NY: Chapman and Hall), 102–133.
- Read, D. J., and Perez-Moreno, J. (2003). Mycorrhizas and nutrient cycling in ecosystems—a journey towards relevance? *New Phyt.* 157, 475–492. doi: 10.1046/j.1469-8137.2003.00704.x
- Reynolds, H. L., Hartley, A. E., Vogelsang, K. M., Bever, J. D., and Schultz, P. A. (2005). Arbuscular mycorrhizal fungi do not enhance nitrogen acquisition and growth of old-field perennials under low nitrogen supply in glasshouse culture. *New Phyt.* 167, 869–880. doi: 10.1111/j.1469-8137.2005.01455.x
- Riedlinger, J., Schrey, S. D., Tarkka, M. T., Hampp, R., Kapur, M., and Fiedler, H. P. (2006). Auxofuran, a novel substance stimulating growth of fly agaric produced by the mycorrhiza helper bacteria *Streptomyces* ACh 505. *App. Environ. Micro.* 72, 3550–3557. doi: 10.1128/AEM.72.5.3550-3557.2006
- Rillig, M. C. (2004). Arbuscular Mycorrhizae and terrestrial ecosystem processes. *Ecol. Lett.* 7, 740–754. doi: 10.1111/j.1461-0248.2004.00620.x
- Rillig, M. C., and Mummey, D. L. (2006). Mycorrhizas and soil structure. *New Phyt.* 171, 41–53. doi: 10.1111/j.1469-8137.2006.01750.x
- Rillig, M. C., Wright, S. F., Nicholls, K. A., Schmidt, W. F., and Torn, M. S. (2001). Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant Soil.* 233, 167–177. doi: 10.1023/A:1010364221169
- Rineau, F., and Garbaye, J. (2010). Effects of liming on potential oxalate secretion and iron chelation of beech ectomycorrhizal root tips. *Microb. Ecol.* 60, 331–339. doi: 10.1007/s00248-010-9697-3
- Rosling, A., Lindahl, B. D., and Finlay, R. D. (2004a). Carbon allocation to ectomycorrhizal roots and mycelium colonizing different mineral substrates. *New Phyt.* 162, 795–802. doi: 10.1111/j.1469-8137.2004.01080.x
- Rosling, A., Lindahl, B. D., Taylor, A. F. S., and Finlay, R. D. (2004b). Mycelia growth and substrate acidification of ectomycorrhizal fungi in response to different minerals. *FEMS Microb. Ecol.* 47, 31–37. doi: 10.1016/S0168-6496(03)00222-8
- Rygielwicz, P. T., and Anderson, C. P. (1994). Mycorrhizae alter quality and quantity of carbon allocated below ground. *Nature* 369, 58–60. doi: 10.1038/369058a0
- Satomura, T., Nakatsubo, T., and Horikoshi, T. (2003). Estimation of the biomass of fine roots and mycorrhizal fungi: a cases study in a Japanese red pine (*Pinus densiflora*) stand. *J. For. Res.* 8, 221–225. doi: 10.1007/s10310-002-0023-x
- Schrey, S. D., Schellhammer, M., Ecke, M., Hampp, R., and Tarkka, M. T. (2005). Mycorrhiza helper bacterium *Streptomyces* sp. induces differential gene expression in the ectomycorrhizal fungus *Amanita muscaria*. *New Phyt.* 168, 205–216. doi: 10.1111/j.1469-8137.2005.01518.x
- Scott-Denton, L. E., Rosensteel, T. N., and Monson, R. K. (2006). Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Glob. Change Biol.* 12, 205–216. doi: 10.1111/j.1365-2486.2005.01064.x
- Setälä, H. (1995). Growth of birch and pine seedlings in relation to grazing by soil fauna on ectomycorrhizal fungi. *Ecology* 76, 1844–1851. doi: 10.2307/1940716
- Setälä, H., Kulmala, P., Mikola, J., and Markkula, A. M. (1999). Influence of ectomycorrhiza on the structure of detrital food webs in pine rhizosphere. *Oikos* 87, 113–122. doi: 10.2307/3547002
- Shi, S. J., O’Callaghan, M., Jones, E. E., Richardson, A. E., Walter, A., Stewart, A., et al. (2012). Investigation of organic anions in tree root exudates and rhizosphere microbial communities using *in situ* and destructive sampling techniques. *Plant Soil* 359, 149–163. doi: 10.1007/s11104-012-1198-3
- Sims, S. F., Hendricks, J. J., Mitchell, R. J., Kuehn, K. A., and Pecot, S. D. (2007). Nitrogen decreases and precipitation increases ectomycorrhizal extrametrical mycelia production in a longleaf pine forest. *Mycorrhiza* 17, 299–309. doi: 10.1007/s00572-007-0105-x
- Smith, S. E. and Read, D. J. (2008). *Mycorrhizal Symbiosis*. New York, NY: Academic Press.
- Soderberg, K. H., Probanza, A., Jumpponen, A., and Bååth, E. (2004). The microbial community in the rhizosphere determined by community-level physiological profiles (CLPP) and direct soil- and cfu-PLFA techniques. *App. Soil Ecol.* 25, 135–145. doi: 10.1016/j.apsoil.2003.08.005
- Staddon, P. L., Ramsey, C. B., Ostle, N., Ineson, P., and Fitter, A. H. (2003). Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of C-14. *Science* 300, 1138–1140. doi: 10.1126/science.1084269
- Subke, J. A., Hahn, V., Battipaglia, G., Linder, S., Buchmann, N., and Cotrufo, M. F. (2004). Feedback interactions between needle litter decomposition and rhizosphere activity. *Oecologia* 139, 551–559. doi: 10.1007/s00442-004-1540-4
- Subke, J. A., Vallack, H. W., Magnusson, T., Keel, S. G., Metcalfe, D. B., Höglberg, P., et al. (2009). Short-term dynamics of abiotic and biotic soil ¹³CO₂ effluxes after *in situ* ¹³CO₂ pulse labelling of a boreal pine forest. *New Phyt.* 183, 349–357. doi: 10.1111/j.1469-8137.2009.02883.x
- Sun, Y.-P., Unestam, T., Lucas, S. D., Johanson, K. J., Kenne, L., and Finlay, R. D. (1999). Exudation-reabsorption in mycorrhizal fungi, the dynamic interface for interaction with soil and other microorganisms. *Mycorrhiza* 9, 137–144. doi: 10.1007/s005720050298
- Tahara, K., Norisada, M., Tange, T., Yagi, H., and Kojima, K. (2005). Ectomycorrhizal association enhances Al tolerance by inducing citrate secretion in *Pinus densiflora*. *Soil Sci. Plant Nut.* 51, 397–403. doi: 10.1111/j.1747-0765.2005.tb00045.x
- Talbot, J. M., Allison, S. D., and Treseder, K. K. (2008). Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. *Func. Ecol.* 22, 955–963. doi: 10.1111/j.1365-2435.2008.01402.x
- Taylor, A. F. S., and Alexander, I. (2005). The ectomycorrhizal symbiosis: life in the real world. *Mycologist* 19, 102–112. doi: 10.1017/S0269-915X(05)00303-4
- Tedersoo, L., Jairus, T., Horton, B. M., Abarenkov, K., Suvi, T., Saar, I., et al. (2008). Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. *New Phyt.* 180, 479–490. doi: 10.1111/j.1469-8137.2008.02561.x
- Tedersoo, L., Kõljalg, U., Hallenberg, N., and Larsson, K. H. (2003). Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. *New Phyt.* 159, 153–165. doi: 10.1046/j.1469-8137.2003.00792.x
- Tedersoo, L., Partel, K., Jairus, T., Gates, G., Poldmaa, K., and Tamm, H. (2009). Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the helotiales. *Environ. Microb.* 11, 3166–3178. doi: 10.1111/j.1462-2920.2009.02020.x
- Thomson, B. D., Grove, T. S., Malajczuk, N., and Hardy, G. E. S. (1994). The effectiveness of ectomycorrhizal fungi increasing the growth of *Eucalyptus globulus* Labill. In relation to root colonizing and hyphal development in soil. *New Phyt.* 126, 517–524. doi: 10.1111/j.1469-8137.1994.tb04250.x
- Tobar, R. M., Azcon, R., and Barea, J. M. (1994). The improvement of plant N acquisition from an ammonium-treated, drought-stressed soil by the fungal symbiont in arbuscular mycorrhizae. *Mycorrhiza* 4, 105–108. doi: 10.1007/BF00203769
- Toljander, J. F., Lindhal, B. D., Paul, L. R., Elfstrand, M., and Finlay, R. D. (2007). Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. *FEMS Microbiol. Ecol.* 61, 295–304. doi: 10.1111/j.1574-6941.2007.00337.x
- Tordoff, G. M., Boddy, L., and Jones, T. H. (2008). Species-specific impacts of collembola grazing on fungal foraging ecology. *Soil Biol. Biochem.* 40, 434–442. doi: 10.1016/j.soilbio.2007.09.006
- Treseder, K. K., and Allen, M. F. (2000). Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO₂ and nitrogen deposition. *New Phyt.* 147, 189–200. doi: 10.1046/j.1469-8137.2000.00690.x
- Treseder, K. K., Allen, M. F., Ruess, R. W., Pregitzer, K. S., and Hendrick, R. L. (2005). Lifespans of fungal rhizomorphs under nitrogen fertilization in a pinyon-juniper woodland. *Plant Soil* 270, 249–255. doi: 10.1007/s11104-004-1559-7
- Tuason, M. M. S., and Arocena, J. M. (2009). Calcium oxalate biomineralization by *Piloderma fallax* in response to various levels of calcium and phosphorus. *App. Environ. Microb.* 75, 7079–7085. doi: 10.1128/AEM.00325-09
- Tylk, G. L., Hussey, R. S., and Roncadori, R. W. (1991). Axenic germination of vesicular-arbuscular mycorrhizal fungi: effects of selected *Streptomyces* species. *Phytopathol.* 81, 754–759. doi: 10.1094/Phyto-81-754
- Uroz, S., Calvaruso, C., Turpault, M. P., Pierrat, J. C., Mustin, C., and Frey-Klett, P. (2007). Effects of the mycorrhizosphere on the genotypic and metabolic diversity of the soil bacterial communities involved in mineral weathering in a forest soil. *Appl. Environ. Microbiol.* 73, 3019–3027. doi: 10.1128/AEM.00121-07
- Uroz, S., Oger, P., Morin, E., and Frey-Klett, P. (2012). Distinct ectomycorrhizospheres share similar bacterial communities as revealed by

- pyrosequencing-based analysis of 16S rRNA genes. *Appl. Environ. Microbiol.* 78, 3019–3027. doi: 10.1128/AEM.06742-11
- van Hees, P. A. W., Godbold, D. L., Jentschke, G., and Jones, D. L. (2003). Impact of ectomycorrhizas on the concentration and biodegradation of simple organic acids in a forest soil. *Eur. J. Soil Sci.* 54, 697–706. doi: 10.1046/j.1351-0754.2003.0561.x
- van Hees, P. A. W., Jones, D. L., Finlay, R. D., Godbold, D. L., and Lundström, U. S. (2005). The carbon we do not see—the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. *Soil Biol. Biochem.* 37, 1–13. doi: 10.1016/j.soilbio.2004.06.010
- van Hees, P. A. W., Rosling, A., Essén, S., Godbold, D. L., Jones, D. L., and Finlay, R. D. (2006a). Oxalate and ferricrocin exudation by the extramatrical mycelium of an ectomycorrhizal fungus in symbiosis with *Pinus sylvestris*. *New Phyt.* 169, 367–377. doi: 10.1111/j.1469-8137.2005.01600.x
- van Hees, P. A. W., Rosling, A., Lundström, U. S., and Finlay, R. D. (2006b). The biogeochemical impact of ectomycorrhizal conifers on major soil elements (Al, Fe, K and Si). *Geoderma* 136, 364–377. doi: 10.1016/j.geoderma.2006.04.001
- van Schöll, L., Hoffland, E., and van Breenan, N. (2006). Organic acid exudation by ectomycorrhizal fungi and *Pinus sylvestris* in response to nutrient deficiencies. *New Phyt.* 170, 153–163. doi: 10.1111/j.1469-8137.2006.01649.x
- Varese, G. C., Portinaro, S., Trotta, A., Scannerini, S., LuppiMosca, A. M., and Martinotti, M. G. (1996). Bacteria associated with *Suillus grevillei* sporocarps and ectomycorrhizae and their effects on *in vitro* growth of the mycobiont. *Symbiosis* 21, 129–147.
- Verbruggen, E., Kiers, E. T., Bakelaar, P. N. C., Roling, W. F. M., and van der Heijden, M. G. A. (2012). Provision of contrasting ecosystem services by soil communities from different agricultural fields. *Plant Soil* 350, 43–55. doi: 10.1007/s11104-011-0828-5
- Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* 255, 571–586. doi: 10.1023/A:1026037216893
- Vesterdal, L., Elberling, B., Christiansen, J. R., Callesen, I., and Schmidt, I. K. (2012). Soil respiration and rates of soil carbon turnover differ among six common European tree species. *For. Ecol. Mang.* 264, 185–196. doi: 10.1016/j.foreco.2011.10.009
- Vogt, K. A., Grier, C. C., Edmonds, R. L., and Meier, C. E. (1982). Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis* (Dougl) Forbes ecosystems in western Washington. *Ecol.* 63, 370–380. doi: 10.2307/1938955
- Wallander, H. (2006). “Mineral dissolution by ectomycorrhizal fungi,” in *Fungi in Biogeochemical Cycles*, ed G. M. Gadd ed (Cambridge: Cambridge University Press), 28–50. doi: 10.1017/CBO9780511550522.015
- Wallander, H., Göransson, H., and Rosengren, U. (2004). Production, standing biomass and natural abundance of ^{15}N and ^{13}C in ectomycorrhizal mycelia collected at different soil depths in two forest types. *Oecologia* 139, 89–97. doi: 10.1007/s00442-003-1477-z
- Wang, B., and Qui, Y. L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16, 299–363. doi: 10.1007/s00572-005-0033-6
- Warren, J. M., Iverson, C. M., Garten, C. T. Jr., Norby, R. J., Childs, J., Brice, D., et al. (2012). Timing and magnitude of C partitioning through a young loblolly pine (*Pinus taeda* L.) stand using C-13 labeling and shade treatments. *Tree Physiol.* 32, 799–813. doi: 10.1093/treephys/tp129
- Weintraub, M., Scott-Denton, L., Schmidt, S., and Monson, R. (2007). The effect of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutrient availability in subalpine forest ecosystem. *Oecologia* 154, 327. doi: 10.1007/s00442-007-0804-1
- Welc, M., Bunemann, E. K., Fliessbach, A., Frossard, E., and Jansa, J. (2012). Soil bacterial and fungal communities along a soil chronosequence assessed by fatty acid profiling. *Soil Biol. Biochem.* 49, 184–192. doi: 10.1016/j.soilbio.2012.01.032
- Whiteside, M. D., Digman, M. A., Gratton, E., and Treseder, K. K. (2012). Organic nitrogen uptake by arbuscular mycorrhizal fungi in a boreal forest. *Soil Biol. Biochem.* 55, 7–13. doi: 10.1016/j.soilbio.2012.06.001
- Wright, S. F., and Upadhyaya, A. (1998). A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil* 198, 97–107. doi: 10.1023/A:1004347701584
- Wu, B., Nara, K., and Hogetsu, T. (2002). Spatiotemporal transfer of carbon-14-labelled photosynthate from ectomycorrhizal *Pinus densiflora* seedlings to extraradical mycelia. *Mycorrhiza* 12, 83–88. doi: 10.1007/s00572-001-0157-2
- Wu, Q. S., Li, G. H., and Zou, Y. N. (2011). Improvement of root system architecture in peach (*Prunus persicalucosa*) seedlings by arbuscular mycorrhizal fungi, related to allocation of glucose/sucrose to root. *Not. Bot. Hort. Agrobot. CLUJ.* 39, 232–236.
- Xavier, J. J. C., and Germida, J. J. (2003). Bacteria associated with *Glomus clarum* spores influence mycorrhizal activity. *Soil Biol. Biochem.* 35, 471–478. doi: 10.1016/S0038-0717(03)00003-8
- Yang, J., Kloepper, J. W., and Ryu, C. (2009). Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci.* 14, 1–4. doi: 10.1016/j.tplants.2008.10.004
- Yarwood, S., Myrold, D. D., and Höglberg, M. N. (2009). Termination of below-ground C allocation by trees alters soil fungal and bacterial communities in a boreal forest. *FEMS Microb. Ecol.* 70, 151–162. doi: 10.1111/j.1574-6941.2009.00733.x
- Zak, D. R., Pregitzer, K. S., Curtis, P. S., Teeri, J. A., Fogel, R., and Randlett, D. L. (1993). Elevated atmospheric CO₂ and feedback between carbon and nitrogen cycles. *Plant Soil* 151, 105–117. doi: 10.1007/BF00010791

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 22 May 2013; accepted: 13 May 2014; published online: 03 June 2014.
 Citation: Churchland C and Grayston SJ (2014) Specificity of plant-microbe interactions in the tree mycorrhizosphere biome and consequences for soil C cycling. *Front. Microbiol.* 5:261. doi: 10.3389/fmicb.2014.00261
 This article was submitted to Terrestrial Microbiology, a section of the journal *Frontiers in Microbiology*.
 Copyright © 2014 Churchland and Grayston. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.