On the Nitrous Oxide Accumulation in Intermediate Waters of the Eastern South Pacific Ocean

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Nitrous oxide (N₂O) is a powerful greenhouse gas principally produced by nitrification and denitrification in the marine environment. Observations were made in the eastern South Pacific (ESP), between 10° and 60°S, and ∼75°–88°W, from intermediate waters targeting Antarctic Intermediate Water (AAIW) at potential density of 27.0–27.1 kg m⁻³. Between 60° and 20°S, a gradual equatorward increase of N₂O from 8 to 26 nmol L⁻¹ was observed at density 27.0–27.1 kg m⁻³ where AAIW penetrates. Positive correlations were found between apparent N₂O production (∆N₂O) and O₂ utilization (AOU), and between ∆N₂O and NO⁻, which suggested that local N₂O production is predominantly produced by nitrification. Closer to the equator, between 20° and 10°S at AAIW core, a strong N₂O increase up to 75 nmol L⁻¹ was observed. Because negative correlations were found between ∆N₂O vs. NO⁻ and ∆N₂O vs. N* (a Nitrogen deficit index) and because ∆N₂O and AOU do not follow a linear trend, we suspect that, in addition to nitrification, denitrification also takes place in N₂O cycling. By making use of water mass mixing analyses, we show that an increase in N₂O occurs in the region where high oxygen from AAIW merges with low oxygen from Equatorial Subsurface Water (ESSW), creating favorable conditions for local N₂O production. We conclude that the non-linearity in the relationship between N₂O and O₂ is a result of mixing between two water masses with very different source characteristics, paired with the different time frames of nitrification and denitrification processes that impact water masses en route before they finally meet and mix in the ESP region.

Keywords: N₂O accumulation, nitrification, denitrification, eastern South Pacific Ocean, water mass analysis, intermediate waters

INTRODUCTION

Approximately 90% of the total marine nitrous oxide (N₂O) inventory occurs between depths just below the pycnocline down to ∼1000 m (Nevison et al., 1995, 2003; Suntharalingam and Sarmiento, 2000). Nitrification and denitrification are the principal processes involved in N₂O production. Both processes depend on organic matter availability and oxygen (O₂) levels, but whereas the former occurs under a wide range of oxygen conditions, the latter takes place only at suboxic and anoxic levels (Codispoti et al., 2001; Bange, 2008). Coastal upwelling systems with high primary production (PP) rates meet both these conditions for enhanced N₂O production (Nevison et al., 2004) and are closely linked to Oxygen Minimum Zones (OMZs). The formation/maintenance...
of OMZs is predominantly determined due to slow ventilation; while high aerobic respiration of particulate organic matter (POM) is probably of second importance (Karstensen et al., 2008).

$N_2O$ production by nitrification; i.e., the aerobic oxidation of ammonium ($NH_4^+$) to nitrite ($NO_2^-$), and then to nitrate ($NO_3^-$), is controlled by $O_2$ concentration. An increased $N_2O$ yield is triggered by lower concentrations of $O_2$ (Goreau et al., 1980) and previous studies have shown that significant quantities of $N_2O$ are produced by Bacteria and Archaea at $O_2$ concentrations below 5 $\mu$mol L$^{-1}$ (Frame and Casciotti, 2010; Santoro et al., 2011; Löscher et al., 2012). In addition, in a process known as nitrifier denitrification, autotrophic $NH_4^+$ oxidizing bacteria are able to produce $N_2O$ as a final product of $NO_2^-$ reduction (Poth and Focht, 1985). In contrast, denitrification, the process by which $NO_3^-$ is reduced to produce $N_2$ gas as final end product and $N_2O$ as an intermediate, only occurs at $O_2$ levels close to anoxia (Dalsgaard et al., 2014).

Very limited information is available regarding $N_2O$ production in intermediate waters, approximately between 500 and 1200 m depth, (Suntharalingam and Sarmiento, 2000; Nevison et al., 2003; Freing et al., 2012), with several of these studies carried out in the eastern tropical North Pacific and the Arabian Sea (Naqvi and Noronha, 1991; Bange et al., 2005; Yamagishi et al., 2007; Fujii et al., 2013). The intermediate depths of the eastern South Pacific (ESP) are occupied by two water masses that move in opposite directions and mix as they converge, i.e., Equatorial Subsurface Water (ESSW) and Antarctic Intermediate Water (AAIW). The AAIW is formed along the front of the Antarctic Circumpolar Current (Sverdrup et al., 1942; McCartney, 1977; Talley, 1999) and spreads equatorward along the lower boundary of the thermocline, in a core layer with a potential density ($\sigma_\theta$) of about 27.0–27.1 kg m$^{-3}$ (AAIW$\sigma_\theta$). AAIW also has low outcrop temperatures and salinities, and therefore, a high capacity for storing dissolved gases (Georgi, 1979). Unlike AAIW, the ESSW is the end product of complex water mass transformations of different sources within the equatorial belt (Wyrtki, 1963, 1967; Tsuchiya and Talley, 1996; Fiedler and Talley, 2006). Being a product of mixing, the bulk of the contributing water masses that compose ESSW have a long residence times of at least several decades (Kessler, 2006), and ESSW is immediately identifiable by comparably very low $O_2$ levels, relative to AAIW.

Given the contrasting histories of these two water masses, taking into consideration their relative mixing properties is fundamental when interpreting biogeochemical fields in the ESP (Llanillo et al., 2013). Optimum Multiparameter (OMP) analysis (Tomczak and Large, 1989; Karstensen and Tomczak, 1998) is a tool that enables the separation between water mass mixing and bulk biogeochemical cycling. OMP analysis solves for linear mixing of source waters and time integrated modifications by biogeochemical cycling processes (Karstensen and Tomczak, 1998; Hupe and Kartsensen, 2000). For biogeochemical cycling, the analysis resolves the inverse co-variability between oxidation (reduction of oxygen) and remineralization (release of dissolved nutrients) according to a prescribed Redfield ratio. Water masses originating from different source regions are impacted by different biogeochemical modifications en route before they meet and mix. The mixing in turn can generate local apparent non-linearity in biogeochemical ratios, as shown by Schneider et al. (2005). These authors analyzed the output of a physical/biogeochemical model run with a constant Redfield ratio. Following this approach, we separated the mixing signal from the biogeochemical signal in the ESP, in order to estimate $N_2O$ production in intermediate waters. Several approaches have been used to estimate water age and the consequent accumulation of $N_2O$ over time, among them, Bange and Andreæ (1999) calculated an annual global $N_2O$ accumulation in deep waters using the age estimate provided by Broecker et al. (1988) with the radiocarbon methods. Freing et al. (2009, 2012) estimated $N_2O$ production rates by using the transient time distribution (TTD) approach, which provides age estimation using tracers, such as chlorofluorocarbon (CFC-12).

![Study area showing the station locations where physical and biogeochemical measurements were taken.](Image)
and sulfur hexafluoride (SF$_6$). Here, we estimated the water mass age using chlorofluorocarbon (CFC-11) data from the World Ocean Circulation Experiment (WOCE). We also analyzed relationships between biogeochemical parameters as apparent N$_2$O production ($\Delta$N$_2$O) with apparent oxygen utilization (AOU), NO$_3^-$, NO$_2^-$, and N$^+$ (a quasi-conservative tracer, defined as a linear combination of nitrate and phosphate, Gruber and Sarmiento, 1997) to estimate the origin of N$_2$O in AAIW.

### METHODS

#### Hydrographic Data
Observational data was used from a total of 14 cruises between 10° and 60°S, and off the coast of South America to 88°W (Figure 1, Table 1). Data were compiled from several cruises (Table 1): (1–4) the World Ocean Circulation Experiment (WOCE, United States) transects P19C, P19S, P21E, and P06E, with CFC measurements; (5) the Sonne 102 cruise; (6) the JAMSTEC cruise. The different Conductivity-Temperature-Depth (CTD) models used and the respective calibrations are described in subsequent reports to the respective cruises, and indicate that temperature can be considered accurate to 0.005 K and salinity to 0.005 PSS-78.

#### Chemical Analysis

Measurements of dissolved N$_2$O were taken during eight cruises (Table 1). Here, we describe N$_2$O analysis from the Laboratory of Oceanographic Processes and Climate (PROFC-UdeC, Chile). N$_2$O samples were taken in triplicate in 20 mL vials and carefully sealed to avoid air bubbles. They were then preserved with 50 µL of saturated HgCl$_2$ and stored in darkness until analysis. N$_2$O was analyzed by creating a 5 mL headspace pure Helium and then equilibrated within the vial, and measured with a gas chromatogram (Shimadzu 17A) using an electron capture detector (ECD). The calibration curves were made previous to each measurement with five points using pure Helium, 0.1, 0.5, and 1 of N$_2$O standards and dry air. The ECD detector linearly responded to this concentration range and the analytical error for N$_2$O measurements was ~3%. The uncertainty of the measurements was calculated from the standard deviation of the triplicate measurements by
depth. Samples with a variation coefficient above 10% were not considered in the N₂O database. N₂O analyses from the M77-4 cruise are described in Kock et al. (2016). Oxygen and nutrient data, including descriptions of O₂ sensors used on CTD-O instruments (where applicable), are described in subsequent reports to the respective cruises. During the Galathea 3 cruise (Table 1), an ultrasensitive STOX O₂ sensor was tested, which allowed an O₂ detection limit of 1–10 nmol L⁻¹ (Revsbech et al., 2009).

**Data Analysis**

**Classical Approach**

N₂O accumulation rates throughout the AAIW core were estimated by determining the differences in concentration between two latitudes, and considering an estimated AAIW age. Age was derived using a standard procedure, which in brief involves converting observed CFC-11 into its atmospheric equivalent with the aid of the CFC-11 solubility function (Warner and Weiss, 1985), and this is then compared to the historical
atmospheric time series data (Walker et al., 2000), resulting in an estimate of the “year of last atmospheric contact.” The difference between the year of observation and the year of last atmospheric contact is interpreted as water age. This method has many uncertainties, including the CFC-11 saturation value in uptake regions (here assumed to be 100%), and particularly the impact of mixing with waters of different ages (Waugh et al., 2003). CFC-11 is a tracer that was introduced into the atmosphere in the 1950’s–1960’s, and thus cannot be used to determine water masses formed before that date, which is a potential problem in OMZ regions (Karstensen et al., 2008). Because age determination of the water mass in the OMZ has several associated anomalies due to slow circulation, we try to minimize uncertainty by only calculating ages for the lower thermocline, south of 16°S.

Due to the spatial distribution of data, and the relative paucity of observations, it is not possible to determine systematic temporal changes; however comparison of data located in similar positions feature the same trend (not shown). We are confident that despite some variability exists in the data due to time of analysis the main oceanic conditions prevailed among years.

On the other hand, because the data used belong to different years, data variability due to oscillation in the global ocean/atmosphere system as ENSO (El Niño/Southern Oscillation) may occur. ENSO is a periodic fluctuation in sea surface temperature (El Niño) and the air pressure of the overlying atmosphere (Southern Oscillation) across the equatorial Pacific Ocean. The fluctuations in sea surface temperature oscillate between two states: El Niño phase, with warmer than normal temperatures and La Niña phase with cooler than normal temperatures. Llanillo et al. (2013) examined the changes in the water mass structure and biogeochemical signals of two opposite phases of ENSO (El Niño and La Niña) in the eastern tropical South Pacific in 1993 and 2009. They found the largest ENSO impact in the water properties and water mass distribution in the upper 200 m north of 10°S with the result of the vertical motion of the oxygen minimum zone (OMZ). During El Niño event (warm phase), there was increased advection of relatively well-oxygenated Subtropical Surface Water (STW) which replaced the low-oxygen ESSW in the top 250 m of the water column. This input deepened the upper part of the OMZ. In contrast, during the 2009 La Niña conditions, the reinforced
trade winds drove enhanced upwelling, raising the upper part of the OMZ.

According to the Oceanic Niño Index (ONI), one of the primary indices used to monitor ENSO, conditions for El Niño (weak to moderate) predominated in the period analyzed (from 1992 to 2010, http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ensyears.shtml). We do not have data during the years 1996–1998, in which it was developed one of the most intense El Niño episodes.

Delta, or excess of \(N_2O\) (\(\Delta N_2O\)), is a measure of the apparent production of \(N_2O\) (Nevison et al., 1995), and was calculated as \(\Delta N_2O = [N_2O]_{\text{in situ}} - [N_2O]_{\text{eq}}\), where \([N_2O]_{\text{in situ}}\) is the measured concentration of \(N_2O\), and \([N_2O]_{\text{eq}}\) is the concentration of \(N_2O\) in saturation with the atmosphere at the time of the last atmospheric contact (provided by the CFC-11 age; Weiss and Price, 1980). Given its recent ventilation and uptake capacity (low temperatures), we expect AAIW to be affected by the anthropogenic increase in the \(N_2O\) atmospheric mixing ratio, and an atmospheric mole fraction was estimated for the time of the last atmospheric contact. The atmospheric concentration during the year of formation was derived from the slope of \(N_2O\) increase per year (Machida et al., 1995; http://www.esrl.noaa.gov/gmd/hats/combined/N2O, NOAA/ESRL program). Apparent Oxygen Utilization (AOU) was calculated as the difference between the equilibrium concentration (Garcia and Gordon, 1992) and observed \(O_2\).

Finally, correlations of \(\Delta N_2O\) vs. AOU; \(NO^-; NO_2^-; \) and \(N^*\), along AAIW\(\theta\) were made through least square fit with Ocean Data View (ODV) program.

**Mixing Analysis Approach**

In order to distinguish inner ocean \(N_2O\) modifications due to the mixing of water masses with differences in source values, we applied the OMP analysis (http://omp.geomar.de). First, the source water characteristics for the ESP were defined on the basis of previous studies (De Pol-Holz et al., 2007; Silva et al., 2009; Llanillo et al., 2013), but also taking into consideration the data at hand. The source water characteristics used in this study are presented in Table 2. As seen from source water types, ESSW and AAIW are very different, the former being a mixture of multiple sources and low in \(O_2\) but high in \(N_2O\); while the latter is close to saturation in both \(O_2\) and \(N_2O\). For every single observational data point, a solution vector was derived using a non-negative least squares fit, and the outputs of the analysis were presented as water mass fractions and total amount of biogeochemical modifications. The fraction range was between 0 (no contributions of a specific water mass) and 1 (exclusive contribution of a specific water mass), and was then converted to 0–100%. The connection between nutrients and oxygen is established by using a fixed elementary ratio (Anderson and...
Varying this ratio did not significantly impact the results (Hupe and Kartsensen, 2000).

## RESULTS AND DISCUSSION

The distribution of physical (temperature and salinity) and biogeochemical (O$_2$ and N$_2$O) variables within ESP nicely depicts the water mass structure for the intermediate depth range of the ESP (Figure 2). The AAIW can be identified by its low temperature/high O$_2$ content, emanating as a tongue out of a core depth of about 600 m from about 60$^\circ$S toward the equator, along a potential density of 27.1 kg m$^{-3}$ (Figures 2B,C). The ESSW core spread along the 26.6 kg m$^{-3}$ isopycnal and is seen as a core of low O$_2$/high salinity water (Figures 2C,D). During its northward propagation, the AAIW core density gets warmer (3.0–8.0°C) and saltier (<34–34.6).

The presence of the OMZ is seen from depleted O$_2$ levels (<22 μmol L$^{-1}$, Figure 2C), between 100 and 400 m and north of 30°S. Lowest O$_2$-values, possibly reaching anoxia (<1 μmol L$^{-1}$), have been reported off Peru and northern Chile at shallow depths ~100–300 m (Canfield et al., 2010). At the AAIW core (27–27.1 kg m$^{-3}$; Figure 3), dissolved O$_2$ decreased from 325 to 58 μmol L$^{-1}$ (from 97 to 19% saturation), between 60° and 20°S (Figure 3A). In contrast, N$_2$O concentrations were lowest at high latitudes (close to the AAIW source region), with a value of 8 nmol L$^{-1}$ (68% saturation) at 58°S and gradually increasing to more than 26 nmol L$^{-1}$ (231% saturation) at 20°S (Figure 3B). Further north, O$_2$ levels fall drastically down to 0.12 μmol L$^{-1}$ or 4% saturation (minimum value recorded) at 12°S (Figure 3A); while N$_2$O at this O$_2$ concentration is 47 nmol L$^{-1}$ (412% saturation). Interestingly, this is not the maximum value of N$_2$O. The maximum found was 75 nmol L$^{-1}$ (657% saturation) at an O$_2$ concentration of 25 μmol L$^{-1}$ (8% saturation) at 17.6°S (Figure 3B). The greatest N$_2$O accumulation was found between the core waters of ESSW and AAIW at the 26.8 kg m$^{-3}$ isopycnal, with concentrations of up to 87 nmol L$^{-1}$ at 13°S (Figure 4). This indicates favorable conditions for local N$_2$O production.

N$_2$O$_3$ distribution displayed a relatively linear increase within the core of AAIW from 60° to 10°S, with a local maximum of 45.6 μmol L$^{-1}$ at 15°S (Figure 3C); whereas NO$_2^-$ distribution was almost constant (<0.1 μmol L$^{-1}$) over most of the southern part of the study area (Figure 3D). Similar to N$_2$O, there was a large increase in NO$_3^-$ concentration north of 20°S, with a maximum value of 1.5 μmol L$^{-1}$ north of 15°S (Figure 3D).

In order to quantify the contribution of AAIW and ESSW within the ESP, we applied the OMP analysis (Tomczak and Large, 1989; Karstensen and Tomczak, 1998). Between 55° and 30°S, the field is dominated by AAIW (>80%, Figure 5A), while further north at about 20°S and where the strongest increase in N$_2$O was found, both water masses occupied about 50% (Figure 5). Even further north at ~15°S, only about 30% of AAIW remained (Figure 5A). ESSW contribution, by definition, inversely mirrors AAIW (both adding up to 100%, Figure 5B). Making use of the water mass fractions obtained for the meridional transect at ~80°W from the OMP analysis (Figure 6), and the N$_2$O concentrations in source regions, we calculated the distributions of salinity and N$_2$O that we expected to find if only mixing were to alter parameter fields. Salinity, being a conservative parameter, serves as a control variable to validate the reconstruction. Reconstructed salinity (Figure 6C) differed only by ~0.03 psu (Figure 6D) from observed salinity (Figure 6B), and supported the validity of estimated mixing. For N$_2$O, in contrast, we found a difference between “mixing only” (Figure 6F) and the observed field (Figure 6E) of up to 32.5 nmol L$^{-1}$ (Figure 6G), which can be interpreted as the signature of biogeochemical N$_2$O cycling.

At mid-latitudes (~20°–30°S), where mixing becomes more important, a change in N$_2$O distribution is clearly discernable, which is sensitive to the effects of O$_2$(Figures 2, 3, 5). Combining the age of AAIW with the AOU and N$_2$O distribution throughout the study area, it was possible to estimate the mean rates of O$_2$ consumption (apparent oxygen utilization rate, AOUR) and N$_2$O accumulation within the core of AAIW (Table 3). Between 54° and 30°S, we obtained an AOUR of 26.38 nmol L$^{-1}$ d$^{-1}$ and a N$_2$O accumulation rate of 0.61 pmol L$^{-1}$ d$^{-1}$; while between 22°S and 18°S, we observed an almost twice as high AOUR and a disproportionately high (~29 times) increase in N$_2$O accumulation (Table 3). Mixing and biogeochemical cycling operate on different time scales and therefore the dilution of a water mass does not necessarily correlate with its age (i.e., the time spent by the water mass in the inner ocean). The sluggish flow underneath the OMZ generates very long residence times.

<table>
<thead>
<tr>
<th>Latitude</th>
<th>54°S</th>
<th>30°S</th>
<th>22°S</th>
<th>18°S</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>10</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD O$_2$ levels (μmol L$^{-1}$)</td>
<td>288.11 ± 16.3 (n = 10)</td>
<td>191.79 ± 26.23 (n = 8)</td>
<td>85.38 ± 45.18 (n = 4)</td>
<td>14.26 ± 8.95 (n = 5)</td>
</tr>
<tr>
<td>O$_2$ consumption rates (nmol L$^{-1}$ d$^{-1}$)</td>
<td>26.38 (n = 10)</td>
<td>14.86 ± 4.88 (n = 8)</td>
<td>22.90 ± 5.77 (n = 4)</td>
<td>55.30 ± 14.35 (n = 5)</td>
</tr>
<tr>
<td>Mean ± SD N$_2$O (nmol L$^{-1}$)</td>
<td>12.62 ± 1.36 (n = 10)</td>
<td>14.86 ± 4.88 (n = 8)</td>
<td>22.90 ± 5.77 (n = 4)</td>
<td>55.30 ± 14.35 (n = 5)</td>
</tr>
<tr>
<td>N$_2$O accumulation rates (pmol L$^{-1}$ d$^{-1}$)</td>
<td>0.61 (n = 10)</td>
<td>17.75 (n = 5)</td>
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FIGURE 7 | Vertical profiles of O$_2$ (µmol L$^{-1}$, black line) and N$_2$O (nmol L$^{-1}$, red line) shown at two representative stations (north and south of 20°S) in the eastern South Pacific. (A) OMZ region and (B) ventilated region.

which in turn may allow for intense N$_2$O production. CFC derived water ages may not reflect the true age of the water in this region, where significant mixing with the ESSW is present, because a large volume of the original source water may be free of CFC (formed prior to 1950 when CFC was released into the atmosphere), and as such, CFC age cannot represent the age of water masses that contribute to the bulk of the water parcels (Waugh et al., 2003).

Two typical profiles of O$_2$ and N$_2$O for north and south of the 20°S (i.e., 16° and 34°S) are shown in Figure 7. These include the OMZ (northern) and ventilated (southern) regions of the ESP. North of 20°S, two N$_2$O maxima are observed (Figure 7A); one is located in the upper layer (30–80 m depth) and coincides with the sharp upper oxycline, where N$_2$O production is associated with nitrification and partial denitrification (Farias et al., 2009a; Codispoti, 2010). Another peak is found at greater depths within the lower oxycline, at the boundary between AAIW and ESSW. Despite the formation of a weaker oxycline at this location, enhanced N$_2$O accumulation continues to be triggered. The layer between these maxima (upper and lower oxycline) is the core of the OMZ, where intense N$_2$O consumption by denitrification is observed (Farias et al., 2009b) and is mainly composed of ESSW. This double-peak structure is consistent with that found by Kock et al. (2016) in offshore waters of the OMZ off Peru (south of 5°S). In the more oxygenated southern section at 34°S, a typical N$_2$O profile shows a gradual increase of N$_2$O with depth (Figure 7B), within the low-O$_2$ layer, contrasting with profiles from the northern region (Figure 7A).

If N$_2$O is a product of nitrification, the regional correlation of $\Delta$N$_2$O and AOU, and $\Delta$N$_2$O and NO$_3^-$ (Figure 8), can be used to estimate $\Delta$N$_2$O associated with a certain level of O$_2$ consumption caused by the remineralization of POM (Yoshinari, 1976; Nevison et al., 2003). In addition, correlations between $\Delta$N$_2$O and NO$_3^-$ (a good indicator of suboxia/anoxia in OMZs, given that its accumulation is associated with dissimilative NO$_3^-$ reduction; Codispoti et al., 1986; Cornejo and Farias, 2012), and between $\Delta$N$_2$O and N* (indicating the balance between Nitrogen sources and sinks, Gruber and Sarmiento, 1997) suggest the occurrence of denitrification. Between 60° and 20°S, at AAIW core (27–27.1 kg m$^{-3}$), positive correlations were found between $\Delta$N$_2$O and AOU (Figure 8A, red line), and $\Delta$N$_2$O and NO$_3^-$ (Figure 8B, red line) indicating that N$_2$O and NO$_3^-$ are produced simultaneously to the consumption of O$_2$ during the northward movement of AAIW, and hence nitrification dominates the nitrogen cycling. We also observed a lack of correlation between $\Delta$N$_2$O and NO$_3^-$ (Figure 8C), and $\Delta$N$_2$O and N* (Figure 8D), which may indicate that neither in situ denitrification nor signals of denitrification from isopycnal and diapycnal mixing with...
surrounding water masses are expected. However, between 20° and 10°S, at the same density range, the situation was different. The abrupt increase in ΔN₂O was not accompanied by an AOU increase (Figure 8A). We also observed negative correlations between ΔN₂O and NO₃⁻ (Figure 8B, black dashed line) and ΔN₂O and N* (Figure 8D, black dashed line) and a positive correlation between ΔN₂O and NO₂⁻ (Figure 8C, black dashed line). These trends imply that denitrification produces N₂O (at high AOU/low O₂ concentrations), but also that lateral and vertical mixing may enrich the AAIW core with NO₃⁻ and NO₂⁻ that originates from ESSW (which has relatively high NO₃⁻ and NO₂⁻).

From the ΔN₂O and AOU correlation (Figure 8) it is clear that the low O₂ (25 μmol L⁻¹) and high N₂O (75 nmol L⁻¹; 600 m) concentrations in the core of AAIW at 17.6°S cannot be explained by nitrification only. Moreover, high N₂O concentrations in the ESSW core (26.8 kg m⁻³) at 16°S (76.8 nmol L⁻¹; 350 m) and at 13.3°S (87 nmol L⁻¹; 400 m) are observed and interpreted as a clear sign for denitrifying processes. The OMP analysis (Figure 5) revealed mixing between AAIW, a water mass from the south dominated by nitrification, and ESSW, a water mass under the impact of denitrification processes from the north, in the transition area underneath the OMZ. As such it is primarily the mixing that creates high N₂O-values, even in the core of the AAIW.

Studies have shown that significant quantities of N₂O are produced by Bacteria and Archaea at O₂ concentrations below 5 μmol L⁻¹ (Frame and Casciotti, 2010; Santoro et al., 2011; Löschler et al., 2012). On the other hand, Kalvelage et al. (2011) found that aerobic and anaerobic N-cycling pathways in the OMZ can co-occur over a larger range of O₂ concentrations than previously thought. They have found that anammox, which is the anaerobic oxidation of ammonium resulting in the production of dinitrogen gas and nitrogen loss, could take place under O₂ levels up to about 20 μmol L⁻¹, while anaerobic NO₃⁻ reduction was fully active up to at least 25 μmol L⁻¹. Thus, apparently 25 μmol L⁻¹ of O₂ is very close to where both aerobic and anaerobic processes can occur simultaneously. Hence, part of the signal may point toward anammox processes in the lower part of the OMZ, impacting the AAIW density range.

Another possible explanation for the increase in N₂O north of 20°S is the higher bacterial activity product of the increase...
in primary productivity (PP) and the subsequent export of POM to the studied depth range (Suess, 1980). We have no direct estimates of PP during sampling, but considering the climatological distribution of surface chlorophyll from satellite data (Thomas et al., 2001; Yuras et al., 2005), no enhanced PP is found between 20° and 10°S, and none in comparison with the region further south (Farias et al., 2009b; Fernandez et al., 2009; Aracena et al., 2011). The most widespread and biologically productive areas are found over the continental shelf between 42°–50°S, 32°–37°S, and 0°–10°S (Kellog and Mohriak, 2001). As such, we exclude regionally enhanced POM export as a cause of the N₂O increase between 20° and 10°S.

It has been shown that changes in the ventilation rates of AAIW have immediate consequences on the oceanic inventories of O₂ and N-species (Galbraith et al., 2004; Meissner et al., 2005). Under reported scenarios of reduced AAIW formation (Downes et al., 2009), the AAIW O₂ inventory will also decrease and, according to our data, this would result in an increase in the N₂O inventory. In a previous study using an oceanic biogeochemical model to predict the impact of climate change on O₂ cycling, Matear et al. (2000) found a potential O₂ reduction of up to 70 μmol L⁻¹ in the Southern Ocean. In general, ocean models predict a global decline in the O₂ inventory of up to 7% over the next century, and this scenario will continue for at least a thousand years into the future (Keeling et al., 2010). Variability in intermediate water masses is tightly linked to global changes given their relatively close connection to the surface ocean (Banks et al., 2000; Matear et al., 2000). Based on our observations, we expect that a decrease in O₂ in source waters will result in an even larger accumulation of N₂O between ESSW and AAIW.

CONCLUSION AND IMPLICATIONS

This study reports N₂O accumulation in intermediate waters, with a focus on Antarctic Intermediate Water (AAIW, 27–27.1 kg m⁻³) in the eastern South Pacific Ocean. Here, we found two distinct N₂O cycling regimes: first, a regime of slow accumulation of N₂O in waters that are well-oxygenated and located between 20° and 60°S. ΔN₂O to AOU correlation suggest that this accumulation is the result of nitrification. The second regime is of an abrupt increase in N₂O north of 20°S. Different correlations suggest that either nitrification (in low O₂ waters) or denitrification may be responsible for the observed increase.

A water mass mixing analysis was used to investigate the ΔN₂O with respect to the dominant water masses: the high-O₂ AAIW that originates in the south; and the low-O₂ ESSW that originates at the equator. Notably, the N₂O maximum found at the core of AAIW was associated with water relatively oxygenated (O₂ concentration of 25 μmol L⁻¹). Our findings did not support the association between increased productivity/organic matter supply in this region and high N₂O accumulation. We conclude that the sluggish flow of water in the region underneath the OMZ, and the subsequent extended residence times water masses, facilitate intense N₂O production. Therefore, N₂O production at intermediate water depths should not be disregarded in ocean N₂O budgets.

AUTHOR CONTRIBUTIONS

All authors contributed extensively to the work presented in this paper: CC analyzed data and wrote the manuscript. JK supervised OMP analysis and edited the manuscript. LF supervised the project, and edited the manuscript. All authors discussed the results and commented on the manuscript at all stages.

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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.

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