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Following the N_2O consumption at the Oxygen Minimum Zone in the eastern South Pacific

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M. Cornejo and L. Farías

Abstract

Oxygen deficient zones (OMZs), such as those found in the eastern South Pacific (ESP), are the most important N_2O sources in the world ocean relative to their volume. N_2O production is related to low O_2 concentrations and high primary productivity. However, when O_2 is sufficiently low, canonical denitrification takes place and N_2O consumption can be expected. N_2O distribution in the ESP was analyzed over a wide latitudinal range (from 5° to 30° S and 71°–76° to ~84° W) based on ~890 N_2O measurements. The intense consumption of N_2O appears to be related to secondary NO_2^- accumulation, the best indicator of very low O_2 levels. Using relationships that depend on threshold levels of O_2 (< 8µM) and nitrite (> 0.75 µM), we reproduced the apparent N_2O production (ΔN_2O) with high reliability ($r^2 = 0.73$ $p = 0.01$). Our results contribute to quantify the ratio of N_2O production/consumption that is being cycling in O_2 deficient water of N_2O and may improve the prediction of N_2O behavior under future scenarios of the OMZ expansion.

1 Introduction

Nitrous oxide (N_2O), a strong greenhouse gas and contributor to ozone depletion, is produced in the oceans mainly by nitrification (aerobic ammonium oxidation) and partial denitrification (dissimilative nitrate reduction to N_2O) under O_2 stress conditions (Codispoti and Christensen, 1985), contributing around 25% of the global atmospheric N_2O sources (Bange, 2006). However, when O_2 is near zero or anoxia is found, N_2O is consumed by denitrification, producing N_2. Denitrification is an anaerobic respiration process which uses NO_3^- as an electron acceptor instead of oxygen, mainly at very low oxygen levels or anoxia. It consists of several steps ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$), each one mediated by different enzymes (i.e., NO_3^- reductase, NO_2^- reductase, N_2O reductase) which show different sensitivities to O_2 levels (Bonin et al., 1989). Thus, in *Pseudomonas nautica* cultures, NO_3^- begins to be consumed at O_2 <125 µM, and

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N_2O at O_2 <~ 7.8 µM (Bonin et al., 1989). Under low O_2, or even anoxia, NO_2^- is accumulated in the first stages of denitrification (Bonin et al., 1987; Kester et al., 1997; Samuelsson, 1985) and N_2O production stops at high NO_2^- concentrations (Bonin et al., 1987). When NO_2^- decreases, N_2O production is reestablished and accumulation takes place.

There are few regions of the world ocean like the Oxygen Minimum Zones (OMZ) that possess marked oxygen gradients, which in turn trigger intense Nitrogen and particularly N_2O cycling. In these areas, it is common to observe a zone of high N_2O production surrounded by a N_2O consumption core (Codispoti and Christensen, 1985), making these areas, many of them associated with eastern boundary upwelling ecosystems, one of the main source of atmospheric N_2O in the world ocean (Seitzinger and Kroeze, 1998).

The OMZ of the eastern South Pacific (ESP), one of the most intense and shallow in the world ocean whom upper boundary can be as shallow as 50 m (Morales et al. 1999), is characterized by O_2 concentrations as low as 2 nM at its core (Revsbech et al., 2009), associated with intense denitrification and, where N_2O consumption exceeds its production (Codispoti et al., 1986). It drives to important fixed nitrogen losses with climatic implications. Despite this, the nitrogen cycle of the ESP's OMZ has so far not been the subject of systematic and intensive research. During the 70s and 80s, many studies were conducted in the OMZ of the eastern Pacific region to assess the role of denitrification in N loss along the secondary nitrite maximum (Carluchi and Schubert, 1969; Cline and Richard, 1972; Codispoti and Christensen, 1985). In recent years, the focus has been put on the anammox process as the main cause of nitrogen loss in the OMZs (Thamdrup et al., 2006; Lam et al., 2009). The latter has led to the dichotomy regarding the main process responsible for global N loss, i.e., denitrification or anammox (Kuyper et al., 2005; Ward et al., 2009), but the origin and cycling of N_2O in these areas has been ignored. In particular, no explanation has been provided for the high N_2O consumption, which occurs only by denitrification under very low O_2 conditions (Castro and Farias, 2004; Farias et al., 2007).

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The importance of the OMZ in nitrogen loss and N_2O production, under predicted sceneries of expansion and intensification of the OMZ (Stramma et al., 2000), make a better understanding of current and future N_2O behavior in the region necessary, as well as the variables associated with N_2O consumption and the sensitivity of the N_2O cycle to O_2 levels.

Models of N_2O in the OMZ are based on the premise that N_2O is produced by nitrification and denitrification, according to O_2 concentrations observed in the ocean (Nevinson et al., 1995; Suntharalingan et al., 2000). These models are supported by both, experiments of N_2O production by nitrification (Goreau et al., 1980) and estimations of in situ N_2O production by denitrification, resulting in increasing N_2O production as O_2 decreases (Kester et al., 1997). But the models do not include consumption by denitrification at low O_2 concentrations (<8 µM) (Nevinson et al., 2003). For this reason, the results of these model outputs are poorly fitted in areas such as the OMZ cores of the Arabian Sea and the eastern tropical North Pacific.

Here we show the analysis of the behavior of N_2O in the intense OMZ of the ESP, examining the factors that drive its consumption. Then we assess an approach for determining N_2O distribution when O_2 concentrations fall below 8 µM, observed most of the time in the coastal OMZ of the ESP. We examine two correlations: one dependent on O_2 concentrations and the other dependent on NO_2^- concentrations. Finally, we combine our results with previously reported equations for N_2O production in the OMZ, when O_2 concentrations are above 8 µM.

1.1 Methods

1.1.1 Hydrographic, biogeochemical and N_2O variables

Data from 10 cruises carried out between 5° S and 30° S and from the coast to 81° W, were analyzed, including CTD data, O_2, NO_3^-, NO_2^- and N_2O concentrations collected between 2000 and 2010 (Table 1; Fig. 1a). Oxygen concentrations were obtained by two methods: Winkler analysis and STOX sensor, as indicated in Table 1.

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N_2O concentrations were obtained by discrete sampling of seawater at different depths using 20 ml-vials and poisoned with $HgCl_2$ (50,µL of 50% saturated $HgCl_2$). The determination of N_2O concentrations was done using the headspace technique (McAullife, 1971) in a Gas Chromatograph (Varian 3380) equipped with a Poropack-Q column and an electron capture detector (ECD). The calibration curve was made with 5 points (He, 0.1 ppm, air, 0.5 ppm and 1 ppm). A total of 890 N_2O measurements were analyzed. Filtered water was collected for nutrients analyses in clean plastic flasks (30 mL) and frozen until analysis in the laboratory. Nutrient concentrations were obtained by standard colorimetric methods (Grasshoff et al., 1983).

1.1.2 Data analysis

The data analysis included the calculation of apparent oxygen utilization (AOU) (Murray and Riley 1969), which was estimated by subtracting in situ O_2 concentrations from the oxygen saturation value (as a function of temperature, salinity and depth), while apparent N_2O production (ΔN_2O) (Yoshinari, 1976), computed by subtracting the N_2O saturation concentration (Weiss and Price 1980) as a function of depth and temperature) from the in situ N_2O concentration. Negative (positive) AOU values indicate production (consumption) of O_2, while the reverse is true for ΔN_2O.

2 Results and discussions

2.1 Observing the ESP's OMZ (0–30° S)

The meridional distributions of O_2, NO_3^-, NO_2^- and N_2O are shown in Fig. 1. Oxygen deficient waters are clearly observed off Peru and northern Chile, delimiting an OMZ that has become one of the shallowest and most intense in the world ocean (Paulmier and Ruiz-Pino, 2009). Vertically, the depth of the upper boundary of the OMZ, considered here as O_2 concentrations ~45 µM, fluctuated between 22 and 80 m. This location

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depends on the distance from the coast. Below the upper boundary, O_2 concentrations decreased abruptly until they approached zero, creating an anoxic environment. In fact, our data shows a nucleus of O_2 concentrations under 5 µM that occupy most of the OMZ (58 % of the data from the OMZ). The lower boundary of the OMZ was observed between 450 and 730 m depth. As the OMZ spreads south with the Peru-Chile undercurrent (Strub et al., 1998), associated with equatorial subsurface water (ESSW), its structure becomes modified with maximum thickness between 5° and 17° S, after which its thickness decreases; at southern latitudes (26° S), the ventilation of the OMZ by the intrusion of the minimum salinity waters results in increasing O_2 concentrations to above 45 µM.

Thus, the OMZ core is an isolated environment surrounded by two sharp oxyclines and also haloclines, where most processes take place under very low O_2 conditions (microaerofilic or even anaerobic processes), with several consequences for the nitrogen cycle. Nitrate reduction and denitrification is thermodynamically favorable, driving, along with anammox, intense N loss and N-species recycling (Codispoti and Richards, 1976; Farias et al., 2009; Lam et al., 2009; Ward et al., 2009). Both processes can produce N_2, but only denitrification consumes NO_3^-, leading to strong NO_2^- and sometime N_2O accumulation (Fig. 1c and d). On the other hand, meridional and vertical N_2O structure reveals the sensitivity of the N_2O cycle to O_2 levels and reflect the intensity and extension of denitrification (Fig. 1e), characterized by two maxima located at both boundaries (up to 275 nM, note that the scale of the plot only extends up to 100 nM N_2O), and a strong minimum at its core, where N_2O undersaturation is as low as 40% with values lower than the previous reported (Farias et al., 2007). The N_2O minimum is located between 11 and 21° S, centered at ~26.4σ_t typically related to the nitrite maximum (up to 23,µM reported by Codispoti et al., 1986), the secondary nitrite maxima (SNM), not only in the ESP but also in the OMZ of the Arabian Sea (Patra et al., 1999; Nicholls et al., 2007), which is a clear signal of active dissimilative nitrate reduction and denitrification, followed clearly by observed nitrate minimum and nitrate deficit (2–20 µM; Fig. 1c). Since N_2O reduction to N_2 by denitrification is the only known process

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able to consume N_2O, the subsaturations indicate that this process is effectively acting within the region, contrary to recent reports that show denitrification to be unimportant in the OMZ of the ESP (Lam et al., 2009; Ward et al., 2009). Nevertheless, the question of why NO_2^- accumulation and N_2O consumption occurs in the OMZ core remains unresolved.

2.2 Existing N_2O models of the OMZ

Due to the climatic and ecological importance of N_2O, and given its extreme sensitivity to threshold O_2 concentrations, there has been interest in modeling N_2O in the ocean for several decades (Nevinson et al., 1995, 2003; Butler et al., 1989). The first attempt was based on empirical relationships with temperature and the strong correlation with apparent oxygen utilization (AOU) and NO_3^-, considering nitrification as the main process that generates N_2O in the ocean given the ubiquitous presence of O_2 (Yoshinari, 1976) . Recently, a depth relationship and experimental results have been incorporated, which have improved models and determined with relative reliability N_2O concentrations and its exchange with the atmosphere (Suntharalingam et al., 2000; Nevinson et al., 2003; Butler et al., 1989). However, N_2O consumption by denitrification in the OMZ has not been included in these models, leaving the N_2O cycle unresolved (Nevinson et al., 2003).

We implemented Nevison's model (henceforth referred to as NM) toN_2O distribution in the ESP OMZ, illustrated in Fig. 2. The vertical distribution of the ΔN_2O/AOU ratio along the coast of the ESP (Fig. 2a), an estimation of N_2O production based on O_2 consumption, is similar to those previously reported for the area (Nevinson et al., 2003). High ratios surround the OMZ because suboxic and hypoxic conditions favor N_2O production (up to 0.9 nM μM^{-1}), while lower and even negative values in the OMZ core are mainly due to high N_2O consumption (meaning negative ΔN_2O, from -0.07 nM μM^{-1}).

However, the NM is only well fitted to the ESP OMZ results in the upper and lower oxyclines, while a poor fit was obtained within the OMZ. In the OMZ core, NM predicts an extreme increase in N_2O production, while the real data show important N_2O consumption (Fig. 2a). The same poor fit was observed by authors studying the intense OMZ of the Arabian Sea. NM considers 4 µM as the critical oxygen level, where N_2O production by nitrification and denitrification is enhanced at lower O_2concentrations, but dismisses any N_2O consumption by denitrification, resulting in marked N_2O accumulation at low O_2 concentrations. Due to highly sensitive to O_2 concentrations, and taking into consideration the possible biases in O_2 measurements, (e.g., detection limit of the Winkler method; CTD response; contamination during the sample collection, etc.), and that about 60 % of our N_2O measurements were taken from waters with O_2 levels under 4 µM, the NM criterion is not reasonable for our study area when modeling vertical and meridional N_2O evolution. However, even when taking into account oxygen levels above 8 µM, outputs were not correlated with in situ data (r^2 N_2O modeled vs. N_2O in situ = 0.19, n = 252; Fig. 2c).

The NM is also a function of depth, which may change according to the analyzed region. The regional dependence of the ΔN_2O/AOU ratio on depth was demonstrated with measurements below 1000 m depth. A good fit is observed, but with different coefficients than for the NM. A modified NM with new coefficients still produces a bad fit between outputs and in situ N_2O (data not shown).

Given that most of the poorly fitted data in the NM coincides with high nitrite concentrations (note the color of the points in the Fig. 2b–d), where low N_2O concentrations are observed even at O_2 as high as 15.5 µM, we re-assessed the NM using NO_2^- concentrations under 0.75 µM and O_2 above 8 µM, i.e. for the region without denitrification. The ΔN_2Os obtained agreed with in situ ΔN_2O ($r^2_{N_2O \text{ modeled vs. } N_2O \text{ in situ}} = 0.76$ n = 228; Fig. 2c).

2.3 Factors related to the N_2O dynamic in the OMZ of the ESP

The relationship between O_2 concentration and N_2O evolution by denitrification is poorly understood in terms of threshold O_2 levels. Experiments with *P. nautica* show the evolution of every step of denitrification (i.e., NO_3^- reduction; NO_2^- reduction and

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N_2O reduction), as a function of O_2 levels (Bonin et al., 1989), and it relationship was modeled (Fig. 3a) to obtain the rate of N_2O consumption as an exponential function of O_2, as follows:

$$\Delta N_2O = -123 \times \exp(-0.35 \times [O_2]) + 70.7 \qquad (1)$$

Because the reduced range of O_2 is taken into account (0–8 µM) and given the high sensitivity of the N_2O cycle at the core of the OMZ to O_2 levels, equation (1) was tested in waters below 75 m depth and with O_2 concentrations lower than 8 µM during cruises collecting high quality O_2 data (three cruises which used STOX sensors: Galathea 3 (2007), MOOMZ II (2009) and MOOMZ III (2010)).The application of equation (1) to our results produced a good fit ($r^2 = 0.66$; Fig. 3a). However, high quality O_2 data in the ESP are scarce and the model result could have a better fit, because we explored a second approximation.

The second biogeochemical variable studied, associated with N_2O consumption at the core of the OMZ, is the secondary NO_2^- maximum. Considering ΔN_2O measurements in waters with O_2 concentrations under 8 µM, and NO_2^- concentrations above 0.75 µM, the relationship between NO_2^- and ΔN_2O fits an exponential function (Fig. 3b), where higher ΔN_2O at lower NO_2^-. In order to obtain a linear fit, ΔN_2O was plotted as a function of inverse NO_2^- (Fig. 3b). Considering this association between both variables, the following equation was obtained:

$$\Delta N_2O = 39.145 \pm 8 \times [NO]^{-1} - 9.2744 \qquad (2)$$

The modeled ΔN_2O values obtained from Eq. (2) are reasonably fitted to the real data for the secondary NO_2^- maximum, which covers a wide range of N_2O concentrations, from undersaturation to oversaturation in the core of the OMZ, based on NO_2^- concentrations (a highly confident measurement).

Equation (2), which is the better approximation for waters with O_2 below 8 µM, was combined with the NM for waters with O_2 above 8 µM and NO_2^- concentrations lower

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than 0.75 µM, to obtain the best fit forvertical N_2O distribution in the OMZ (Fig. 3c). A significant ($r^2 = 0.71$; $p = 0.01$) fit was obtained between the new equation and the in situ data, producing a representative ΔN_2O profile. Using a combination of the two equations, the poor fit previously obtained for the OMZ core data from Nevison's work appears now to be well resolved, and a complete profile can be despicted from the O_2 and NO_2^-concentrations.

2.4 Implications of modeling N_2O consumption in the OMZ core

As most of the ocean has higher O_2 concentrations than those required by denitrification, the assumption that the N_2O cycle is driven mostly by nitrification production and air-sea exchange is a good approximation. However, the OMZ is a complex system within the N_2O cycle, where different processes involved in N_2O production and consumption can coexist, both microaerophilic and anaerobic processes. Taking into account our data and the WOCE data, the OMZ core between 5° and 30° S, with O_2 concentrations below 8 µM, occupies a volume of 85×10^4 km^3. Currently, N_2O consumption within OMZ cores has not been included in ocean N_2O models. Therefore, this study represents an important contribution to future models of OMZ expansion. Undoubtedly, more experimental work on the N_2O cycle within the OMZ, with precision methods for O_2 and metal availability measurements, are necessary in order to better understand the cycling of this significant greenhouse gas.

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Table 1. Number of N_2O profiles and locations of cruises included in the analysis.

Cruise	Date	N° of profiles	Latitudinal range of sampling
MINOX	Mar 2000	4	20.8° S–21.2° S
Iquique 2000	Sep 2000	2	21.1° S
Iquique 2001	May 2001	7	21.1° S
Iquique 2002	Apr 2002	1	21.1° S
Dinamo	Mar 2004	1	20.1° S
Prodeploy	Jul 2004	1	20.3° S
Knorr	Nov 2005	26	3.6° S–17.7° S
Galathea	Feb 2007	16	5.3° S–29.3° S
MOOMZ II	Aug 2009	7	20.1° S
MOOMZ III	Jan 2010	5	20.1° S

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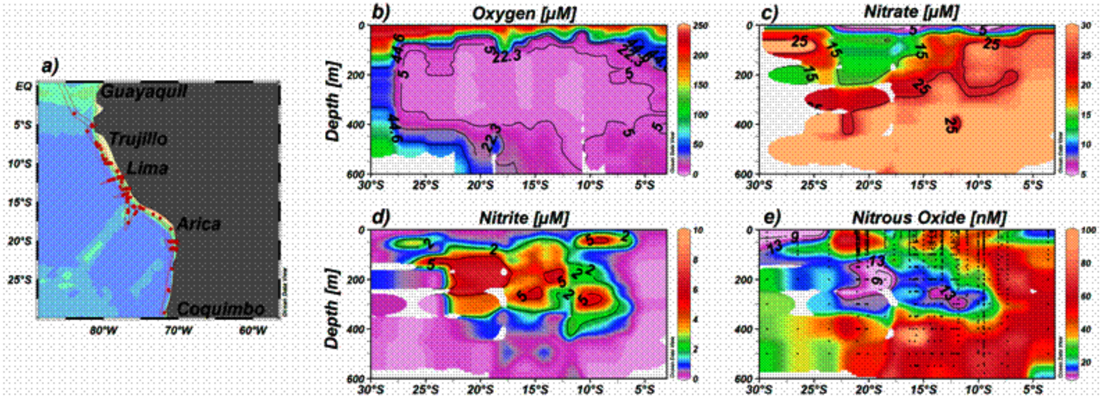

Fig. 1. (a) Study Area. Red points indicate the stations included in meridional vertical distributions of: **(b)** Oxygen [µM]; **(c)** nitrate [µM]; Nitrite [µM]; and Nitrous oxide [nM].

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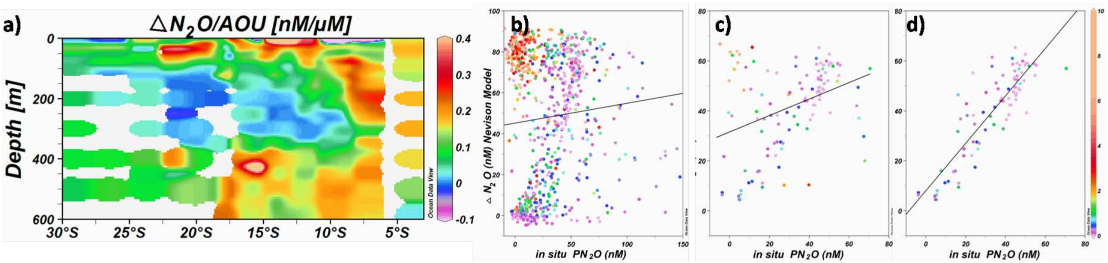

Fig. 2. **(a)** Meridional distribution of ΔN_2O/AOU ratio along the ESP. **(b–d)** In situ ΔN_2O [nM] versus ΔN_2O modeled by the Nevison et al equation: **(b)** including the entire eastern South Pacific; **(c)** including only measurements from water with oxygen levels above 8 µM; and **(d)** ΔN_2O from waters with oxygen levels above 8 µM and nitrite below 0.75 µM. The color indicates the nitrite concentration of each datum [µM].

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Interactive Discussion

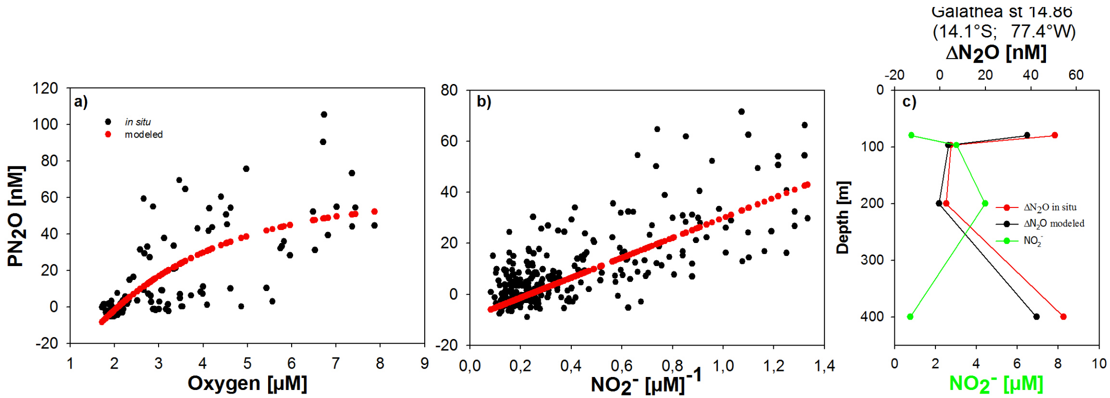

Fig. 3. (a) ΔN_2O in situ (black circles) and modeled according to Bonin experiments (red circles) varying with the oxygen; **(b)** .ΔN_2O in situ (black circles) and modeled (red circles) as a function of inverse NO_2^- concentrations; **(c)** Profile of ΔN_2O in situ (red points) and modeled as a function of NO_2^- (black points) and NO_2^- concentrations (green points) from Galathea expedition station 14.86.

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