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Nitrous oxide distribution and its origin in the central and eastern South Pacific Subtropical Gyre

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Abstract

The biogeochemical mechanism of bacterial N_2O production in the ocean has been the subject of many discussions in recent years. New isotopomeric tools can help further knowledge on N_2O sources in natural environments. This research shows and compares hydrographic, nitrous oxide concentration, and N_2O isotopic and isotopomeric data from three stations across the South Pacific Ocean, from the center of the subtropical oligotrophic gyre (~26° S; 114° W) to the upwelling zone along the central Chilean coast (~34° S). Althought AOU/N_2O and NO_3^- trends support the idea that most of N_2O source (mainly from intermediate water (200–1000 m)) come from nitrification, N_2O isotopomeric composition (intramolecular distribution of ^{15}N isotopes in N_2O) reveals an abrupt change in the mechanism of nitrous oxide production, always observed through lower SP (site preference of ^{15}N), at a high – stability layer, where particles could act as microsites and N_2O would be produced by nitrifier denitrification (reduction of nitrite to nitrous oxide mediated by primary nitrifiers). There, nitrifier denitrification can account for 40% and 50% (center and east border of the gyre, respectively) of the nitrous oxide produced in this specific layer. This process could be associated with the deceleration of sinking organic particles in highly stable layers of the water column. In constrast, coastal upwelling system is characterized by oxygen deficient condition and some N deficit in a eutrophic system. Here, nitrous oxide accumulates up to 480% saturation, and isotopic and isotopomer signal show highly complex nitrous oxide production processes, which presumably reflect both the effect of nitrification and denitrification at low oxygen levels on N_2O production, but non N_2O consumption by denitrification was observed.

1 Introduction

Nitrous oxide exists in the atmosphere at trace levels. However, this “greenhouse gas” is of great environmental importance. It is 170 to 300 times more efficient (per

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molecule) than CO_2 (Manne and Richels 2001) and constitutes 5–6% of the greenhouse effect (Law and Ling 2001). The oceans are a net source of nitrous oxide toward the atmosphere, with an estimated average annual emission of 6 Tg N year^{-1}, corresponding to 20% of the global emissions (Nevison et al., 1995).

The relative importance of the biological processes producing nitrous oxide remains unclear. Nitrification is a chemotrophic process in which NH_4^+ and NO_2^- are aerobically oxidized to fix inorganic carbon. These reactions are carried out in two stages by different groups of microorganisms (Ward 2000). The first stage is ammonium oxidation to nitrite, carried out by organisms called ammonium-oxidizers or primary nitrifiers. In this reaction, hydroxylamine acts as an intermediate, and has been proposed as a precursor of nitrous oxide (Naqvi and Noronha, 1991; Ostrom et al., 2000). The second stage is nitrite oxidation to nitrate, with nitric oxide acting as an intermediary and possible precursor of nitrous oxide. This process is carried out by organisms called nitrite-oxidizers or secondary nitrifiers. In both cases, nitrous oxide is formed as a by-product; however, the biochemical mechanism by which it is formed is not clear (Stein and Yung, 2003; Wrage et al., 2001).

Denitrification is the reduction of oxidized inorganic nitrogen (NO_3^-) toward gaseous nitrogen forms (N_2, N_2O), and implies a loss of nitrogen from the system. This process is carried out by several organisms as a respiration process under suboxic (<0.1 ml/L O_2) or hypoxic (0.1–2 ml/L O_2) conditions, being nitrate the electron acceptor (Knowles, 1982). For this reason, denitrification is restricted to oxygen minimum areas (Codispoti et al., 2001; Gruber and Sarmiento, 1997) or to areas where an important accumulation of particulate organic matter takes place, such as the pycnocline (Alldredge and Cohen, 1987). Denitrification occurs in several stages, during which nitrous oxide is an intermediary, unlike nitrification Thus, nitrous oxide can be produced or consumed during nitrification (Elkins et al., 1978). In suboxic conditions (<0.1 ml/L), nitrous oxide produced by denitrification is almost entirely reduced to N_2, whereas at the oxycline, where the oxygen concentration is near 0.5 ml/L, the nitrous oxide production rate significantly increases and the reduction of nitrous oxide to nitrogen is inhibited (Castro

and Farías, 2004).

Moreover, certain species of nitrifying bacteria can produce nitrous oxide under oxygen stress conditions (<1 ml/L) by means of ammonium oxidation to nitrite, which in turn is reduced to nitrous oxide; this process is called "nitrifier denitrification" (Poth and Focht 1985) and is carried out by primary nitrifiers, like *Nitrosomona europaea*, a widely distributed nitrifying bacteria (Ritchie and Nicholas, 1972; Shaw et al., 2006). Kinetic and isotopic studies show that the second part of this process corresponds; indeed, to denitrification, and that the involved enzymes could be the same (Wrage et al., 2001). Figure 1 shows a general outline of these processes.

The typical vertical distribution of nitrous oxide in the open ocean shows that it is directly correlated with nitrate and inversely correlated with oxygen (Nevison et al., 2003; Oudot et al., 2002). Since nitrification is favored at low oxygen concentrations (Carlucci and McNally, 1969; Goreau et al., 1980), this distribution has usually been interpreted as incidental evidence that nitrification is the dominant process in oceanic nitrous oxide formation. Isotopic data, however, show that $\delta^{15}N(N_2O)$ values are higher than expected for nitrous oxide produced by nitrification in areas where the relationship between oxygen, nitrous oxide, and nitrate indicates that nitrification is the dominant process (Kim and Craig, 1990; Ostrom et al., 2000; Yoshida, 1988; Yoshida et al., 1989). Yoshida et al. (1989) proposed that denitrification occurs simultaneously with nitrification in anoxic micro sites in particulated organic matter.

The determination of nitrous oxide isotopomers (i.e., the intramolecular distribution of ^{15}N in the linear NNO molecule) offers a useful and relatively new tool for elucidating nitrous oxide production mechanisms (Toyoda and Yoshida, 1999). $\delta^{15}N^{\alpha}$ is the relative isotopic abundance of ^{15}N for the central position and $\delta^{15}N^{\beta}$ for the terminal position. Site preference (SP) is defined as follows:

$$S.P. = \delta^{15}N^{\alpha} - \delta^{15}N^{\beta} \quad (1)$$

Considering that, nitrous oxide precursors contain only one nitrogen atom (NO_2^-, NO, NH_2OH) and excluding the possibility that different chemical species combine to form

nitrous oxide, the isotopomer distribution in nitrous oxide should be independent of the δ^{15}N of the precursors. It can therefore be determined by the biochemical reaction step in which equivalence between both nitrogen atoms is lost (Toyoda et al. 2002). This is the main advantage of the nitrous oxide isotopomer ratio over the conventional δ^{15}N ratio. Nevertheless, the lack of knowledge regarding the specific biochemical mechanisms involved in nitrous oxide production makes it difficult "to predict" which isotopomer signs correspond to which particular process (Schmidt et al., 2004). The few works that present isotopomer results in marine environments found positive SP values associated with nitrification (Popp et al., 2002; Toyoda et al., 2002) or denitrification (Yamagishi et al., 2005). The main problem in interpreting of these results is the context of nitrous oxide production processes and how these processes influence the isotopomer signal.

The South Pacific Central Gyre has been described as the most oligotrophic zone in the world ocean (Claustre and Maritorena, 2003), with an extreme nutrient (N and Fe) limitation (S. Bonnet, personal communication); it is also one of the least studied areas of the ocean (Daneri and Quiñones 2001). In contrast, the central Chile upwelling system is one of the most productive marine environments in the world, with high contributions of nitrate and other nutrients due to an upwelling regime and coastal contribution that supports a high rate of new production (Daneri et al., 2000). Otherwise, this area contains a intense oxygen minimum zone (OMZ), fed by the Peru Chile Undercurrent (PCUC), that have a significative influence in the entire community of water column (Morales et al., 1996). Thus, the transect stretching from the center of the gyre to the Chilean coast is an ideal area to study the variety of processes of nitrous oxide production along an extreme gradient.

The main objective of this work is to try to elucidate the changes in the nitrous oxide production processes related with different environmental conditions inside water column, and through the comparison of different environments, covered by the BIOSOPE cruise (Fig. 2), using isotopic and isotopomeric N_2O measurements, combined with hydrographic and biogeochemical data.

2 Methods

2.1 Sampling

All samples were collected during the BIOSOPE cruise (October-December 2004 or early austral spring) on board the R/V L'Atalante. The stations used in this work are GYR (260 655° S, 11 398 973° W), EGY (3 190 442° S, 9 140 665° W), and UPX (3 465 183° S, 7 247 625° W). These stations were chosen for their representatives of different zones along the trophic gradient: GYR is within the South Pacific Central Gyre, EGY is on the eastern border of the Gyre, and UPX is in the coastal upwelling zone, 33 kilometers from the shore (Fig. 2).

Temperature, salinity profiles were obtained from a Seabird SBE 911 + CTD measurements taken on board the R/V L'Atalante during the BIOSOPE cruise. Fluorescence (as Relative Fluorescence Unit, RFU) was measured in situ from a Chelsea AquaTracka fluorometer, attached to the same rosette as the sampling Niskin bottles and CTD instruments. Sampling was done one hour before sunrise, from twelve liter Niskin bottles attached to CTD-O rosette. The particle content was measured by a Underwater Video Profiler (UVP) system, as is described in (Gorsky et al., 2000), particle size is measured in mm as Equivalent Spherical Diameter (ESD) (Stemmann et al., 2000).

Seawater samples were collected for isotopic and nitrous oxide concentration analyses as well as for oxygen and nutrient analyses. The samples for isotopes, isotopomers and nitrous oxide analyses were transferred directly into 125 ml glass flasks, preserved with $HgCl_2$, and sealed with butyl rubber stoppers following the method described in Yamaghishi et al. (2001).

2.2 Nitrous oxide, oxygen, nitrate and phosphate determination

Nitrous oxide was determined in the seawater samples used for the isotopic analysis with gas chromatography and mass spectrometry detection. The method is well

described in Toyoda et al. (2005)

Oxygen was measured in situ from a Seabird SBE 43 oxygen meter. Oxygen sensor was calibrated through Winkcler volumetry. Nitrate was immediately analyzed on board according to the sensitive method of Raimbault et al. (1990). Phosphate was analyzed immediately on board by the method described in Tréguer and LeCorre (1975). The Apparent Oxygen Utilization (AOU) value was obtained subtracting the measured value of oxygen concentration from the saturation value computed at the temperature and salinity of seawater, whereas the difference between the N_2O saturation concentration and its measured concentration (ΔN_2O) in the seawater was used to infer its production (positive) or consumption (negative).

2.3 Isotopic and isotopomeric determinations

Isotopic and isotopomeric determinations were carried out at the Tokyo Institute of Technology using a Finnigan MAT 252 mass spectrometer following the method described in Toyoda and Yoshida (1999). For this, nitrous oxide was extracted from samples by sparging with He and introduced into a preconcentration-gas chromatography-isotopic ratio mass spectrometry system. $\delta^{15}N_{bulk}$ and the $\delta^{18}O(N_2O)$ are determined in relation to the atmospheric nitrogen and VSMOW, respectively. Nitrous oxide isotopomers are determined based on the analysis of ionic mass fragments (NO^+ and N_2O^+) formed by the electronic impact of nitrous oxide. This determination is possible since NO^+ fragments contain the central nitrogen (α), which allows the conversion of the fragment ratios into isotopic ratios of $^{14}N^{15}NO$ and $^{15}N^{14}NO$. Although there is a rearrangement reaction during the ionic fragmentation process, its magnitude can be known and corrected. Precision of the measurements is typically better than 0.5 for $\delta^{15}N_{bulk}$ and $\delta^{18}O$, and better than 1 for $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ (Toyoda et al., 2005). Isotopomeric ratios are calculated with the following expressions:

$$^{15}R^{\alpha} = \frac{\left[^{14}N^{15}N^{16}O\right]}{\left[^{14}N^{14}N^{16}O\right]} \quad (2)$$

$$^{15}R^{\beta} = \frac{\left[^{15}N^{14}N^{16}O\right]}{\left[^{14}N^{14}N^{16}O\right]} \quad (3)$$

$$\delta^{15}N^{\alpha} = \left\{ \frac{^{15}R^{\alpha}}{^{15}R^{\alpha}\,(\mathrm{std}) - 1} \right\} \times 1000 \quad (4)$$

$$\delta^{15}N^{\beta} = \left\{ \frac{^{15}R^{\beta}}{^{15}R^{\beta}\,(\mathrm{std}) - 1} \right\} \times 1000 \quad (5)$$

Furthermore, the definition of $\delta^{15}N_{bulk} = (\delta^{15}N^{\alpha} + \delta^{15}N^{\beta})/2$ allows us to compare the relative abundance of isotopomers α and β with the relative isotopic abundance of ^{15}N.

2.4 Data analysis

The Brunt-Vaisälä frequency (BVF) was determined using temperature and salinity data. For better data interpretation, profiles of BVF were visually fitted to an eight-term Gaussian model included in MATLAB software. Mixed layer was determined averaging depth of four criteria, as is described in the official data source of Biosope cruise (http://www.obs-vlfr.fr/proof/php/bio_log_basicfiles.php). The photic layer is defined as the area in which the light intensity is reduced to 1% its surface value. This determination was made by averaging readings from four different sensors during the BIOSOPE cruise.

3 Results and discussion

3.1 General water column characteristics

The three studied stations are representative of three characteristic environments and allow us to compare the nitrous oxide sources for these different oceanographic

regimes. The GYR station is located at the center of South Pacific Gyre, with severe oligotrophy (Claustre and Maritorena, 2003). The chlorophyll fluorescence peak (0.23 RFU) at this station is at ~180 m, just below the bottom of the photic layer (164 m). The shallower pycnocline is located at 10 m. A second, thicker pycnocline is observed between 100 and 500 m, as shown by the Brunt-Vaisälä frequency plot in Fig. 3a.

The EGY station is located on the eastern border of the South Pacific Gyre, in a less oligotrophic environment. The chlorophyll maximum is shallower and higher than at the previous station, with a fluorescence peak (0.34 RFU) at 52 m, well above the depth the photic layer (92 m). The shallower pycnocline is located at 26 m and the Brunt-Vaisälä frequency plot shows a second, thicker pycnocline between 120 and 400 m (Fig. 3b).

The UPX station is located off the coast of central Chile, in a high productivity environment. The chlorophyll fluorescence peak (0.81 RFU) at this station is found at 32 m, but fluorescence is still high at the surface (0.70 RFU). The peak is close to the base of the photic layer (37 m) and the end of mixed layer (36 m). The Brunt-Vaisälä frequency plot showed a high stability zone from 20 m to 60 m (Fig. 3c).

Changes in the values and shapes of the chlorophyll profiles from west to east clearly shows the expected differences in productivity due to the enhanced nutrient supply, associated with vertical advection of preformed nitrate from equatorial subsurface water (ESSW), which has a pre-existing very low O_2 and rich NO_3^- levels (Silva 1987).

3.2 Nitrous oxide, oxygen, and nutrient behavior

Vertical gases distributions are shown in Fig. 4. Nitrous oxide is slightly oversaturated at GYR and EGY from the surface to ≈200 m (≈114% and ≈105%, respectively), and is highly oversaturated below this depth (≈230%, at both stations) (Figs. 4a, b). N_2O profiles at GYR and EGY are almost mirrored with oxygen profiles; indicating that the amount of nitrous oxide produced in the water column is strongly driven by the O_2 concentration. Otherwise, at the UPX station, the whole water column is highly N_2O-oversaturated (from 230% at the surface to 480% at the nitrous oxide maximum at 350 m). In this station, O_2 concentration has a strong minimum (≈10 μmol/Kg) between

150 m to 300 m, and do not show a clear relationship with N_2O profile.

Nitrate and phosphate are shown in Fig. 5. In GYR and EGY stations both ions have a very similar profile, with low concentrations at first 200 m. Below this depth, the nitrate concentration gradually increase to 40 μmol/Kg (1000 m) at GYR and 35 μmol/Kg (400 m) at EGY. In both (GYR and EGY) station, an abrupt increase in nitrate and phosphate concentration close to 400 m coincides with maximums in N_2O concentration, suggesting an increase in the rate of nitrification at this depth. In UPX station, nitrate is highly concentrated at the surface (Fig. 5c) due to the upwelling of subsurface nutrient-rich water (Daneri et al., 2000). Although nitrate and phosphate shows a similar trend, the profile appears more complicated than oceanic station. N_2O and nitrate show very similar profiles in this station, suggesting that the nitrous oxide production-consumption processes are the same for nitrate.

The PO_4^{-3} v/s NO_3^- plots (Figs. 5a, b, small plots) show that at stations GYR and EGY both nutrients behave according to the Redfield ratio, agreeing with nitrification as the main source of nitrous oxide in these stations. At UPX, nitrogen clearly tends to be lost from the system (phosphate excess in relation to nitrate), probably due to denitrification (Fig. 5c, small plot).

In order to obtain information about the processes involved in N_2O cycling along the water column, AOU vs ΔN_2O and AOU vs NO_3^- from below the photic layer (200 m) and to 1000 m at GYR and EGY were estimated (Figs. 6a and b). Both ΔN_2O and NO_3^- correlated positively with AOU, supporting the view that primary N_2O production arises from nitrification. The ratio of N_2O production to O_2 consumption (on a molar basis), referred to as the N_2O oxidative ratio, was 0.88×10^{-4} μM^{-1}. Since NO_3^- regeneration was also linearly related to AOU, it was possible to calculate the ratio of N_2O production to NO_3^- production as 0.36 % (expressed as a percentage on the μmol-N basis). The N_2O oxidative ratio (i.e., produced N_2O per mol of respired O_2) and the reaction yielding N_2O (i.e., produced N_2O per mol of produced NO_3^-) coincide with the oceanic values reported by Cohen and Gordon (1979) for the North East Pacific. The good correlation between nitrate and observed AOU indicates that the occurrence of nitrification is the

source of nitrate and discards the occurrence of denitrification (i.e., reduction of nitrate to nitrous oxide or nitrogen) inside particles, as proposed by Yoshida et al. (1989).

3.3 $\delta^{15}\mathbf{N}_{bulk}$, δ^{18}O, and isotopomers of nitrous oxide

$\delta^{15}N_{bulk}$ does not vary significantly throughout the water column at the three stations. Although $\delta^{15}N_{bulk}$ has been defined as equivalent to conventional $\delta^{15}N$ (Toyoda and Yoshida, 1999), our results show that this equivalence is still not clear. Instead, SP signal imprint in the water column clear differences among studied stations. At the GYR and EGY stations, the SP values in the mixed layer are close to the expected atmospheric N_2O value (19±2‰) (Yoshida and Toyoda, 2000), indicating that surface N_2O originates mainly from ocean-atmosphere interactions (Figs. 7a, b). At UPX, however, the SP value in the mixed layer (≈14‰) is lower than in the air, indicating the influence of N_2O upwelled with subsurface waters (Fig. 7c). Although the accepted value for the SP of atmospheric N_2O was determined in air samples from the North West Pacific, the high mixing rates of tropospheric gases and the high residence times of atmospheric nitrous oxide over 120 years (IPCC, 1996) make the value given by Yoshida and Toyoda (2000) a good approximation.

Site preference between both pycnoclines (shallower and deeper) at the oceanic stations shows high vertical variability. Due to nitrous oxide production in this zone is very low and nitrification should be absent in the euphotic layer due to inhibition by sunlight (Guerrero and Jones, 1996; Olson, 1981) the high variability of SP is probably associated with non-bacterial nitrous oxide production (Delwich, 1981). At UPX, where the mixed layer coincides with the photic layer, SP and $\delta^{18}O$ (N_2O) values over the pycnocline are quite stable.

Site preferences at GYR and EGY show conspicuous SP minima (11.5‰ and 8.5‰) in the secondary pycnoclines (represented in Fig. 3 by high Brunt-Vaisälä frequency values) at 350 m and 250 m, followed by a gradual increase up to 22‰ in deeper waters. These minima are also observed at UPX (40 m) within the primary pycnocline. Below

the pycnocline, SP values do not show clear depth trend.

Since SP is independent from $\delta^{15}N$ of its precursors, or the extent of the reaction, and is only dependant on the reaction mechanism, the observed changes in SP in the water column must be associated with changes in the mechanistic sources of nitrous oxide (Schmidt et al., 2004). The SP minima at oceanic and coastal stations must be influenced by a particular process that produces low SP nitrous oxide.

Toyoda et al. (2002) proposed that nitrification should yield a $\delta^{15}N^{\alpha}$ enriched nitrous oxide due to the existence of a hyponitrite (-ONNO-) intermediary followed by the selective breakage of the NO bond where the lightest isotopes are located, based on the ZPE (zero point energy) for nitrous oxide (Zuñiga et al., 2003). This mechanism has been described for some nitric oxide reductases (Hendriks et al., 2000; Wasser et al., 2002). Culture experiments carried out by Sutka et al. (2006) show that ammonium oxidation carried out by primary nitrifiers (i.e. *Nitrosomona europaea* and *Nitrosospira multiformis*) produces nitrous oxide enriched in $\delta^{15}N^{\alpha}$, with average SP values of 33‰. Despite the lack of conclusive evidence about this specific mechanism in nitrification, if we assume that nitrification is the main process of nitrous oxide production, at least at the oceanic stations, positive SP values below the photic layer can be attributed to nitrification.

These results, as well as other culture experiments carried out by Sutka et al. (2003, 2006) show that, under oxygen stress, nitrite reduction to nitrous oxide mediated by primary nitrifiers (i.e. *Nitrosomona europaea* and *Nitrosospira multiformis*), called nitrifier denitrification (Poth and Focht, 1985), produces N_2O with an average SP value of zero. This means that there is no selectivity of nitrogen atoms during the N_2O production. Evidence of sequential mechanisms of nitrous oxide production by nitrite reductive processes in bacteria has been reported several times in the biochemical literature (Aerssens et al., 1986; Averill and Tiedje, 1982; Weeg-Aerssens et al., 1988; Zafiriou et al., 1989). Such mechanisms involve the successive addition of one molecule of precursor (NO_2^- or NO) to the enzyme. The resulting ^{15}N distribution must be statistically determined by the entrance of the precursor, contrary to the selective

effect of simultaneous mechanisms proposed by Toyoda et al. (2002). Although it has been pointed out that this process (nitrifier denitrification) occurs under oxygen stress, Shaw et al. (2006) has demonstrated that bacteria of the genera *Nitrosospira* and *Nitrosomona*, both widely distributed primary nitrifiers, are capable of producing nitrous oxide via nitrifier denitrification in the presence of nitrite, even in aerobic environments. Despite nitrifier denitrification has not been studied in marine environments, and its possible ecological role is far from being established, several hypotheses have been proposed. Wrage et al. (2001) proposed thermodynamic reasons for the occurrence of nitrifier denitrification due to the negative Gibbs free energy for the $NH_4^+ \rightarrow NO_2^- \rightarrow N_2O$ processes, which are enhanced at low pH. However is unlikely in marine environments due to the stability of pH and because it has been demonstrated that nitrite is toxic for primary nitrifiers, since it reduces ammonium monooxygenase activity (Stein and Arp 1998). Nitrite accumulation around sinking particles due to the sudden loss of speed in the pycnocline could activate nitrifier denitrification enzymes on primary nitrifiers as a detoxification mechanism, as pointed out by Poth and Focht (1985).

It is important to emphasize that $\delta^{15}N^{\alpha}$ enrichment due to denitrifying nitrous oxide consumption in anoxic microsites of particles in oceanic environments, as previously pointed out (Popp et al., 2002; Toyoda et al., 2002), is not expected because nitrous oxide reduction only occurs at very low oxygen concentrations (Castro and Farías, 2004; Elkins et al., 1978). Very large aggregates are necessary for the occurrence of highly anoxic microsites in particles and this is unlikely in open oligotrophic or even mesotrophic environments. If this process occurs, its effect must be negligible.

$\delta^{18}O$ (N_2O) is always hard to elucidate due to its dependence on the isotopic signature of precursors. The $\delta^{18}O$ (N_2O) profile below the mixed layer at GYR and EGY and below 500 m at UPX are similar in shape to the SP, as previously observed by Toyoda et al., (2002) and Popp et al. (2002), indicating that $\delta^{18}O$ (N_2O) is also driven by nitrous oxide production mechanisms. The primary source of O in the N_2O produced by primary nitrification is dissolved O_2 (Ostrom et al., 2000), since it is expected that $\delta^{18}O$ (N_2O) will be greater than 40‰. Furthermore, the preferential breakage of bonds of the

lightest isotopes during the intermediate hyponitrile step should drive the $\delta^{18}O$ (N_2O), as occurs with $^{15}N^{\alpha}$ enrichment (Schmidt et al., 2004). The source of the oxygen atom in nitrous oxide produced by nitrite reduction is the nitrite (Aerssens et al. 1986; Averill 1996). Therefore, if this (nitrite) is produced in situ by primary nitrifiers, it must be isotopically depleted compared to ammonium and oxygen. Thus, the resulting N_2O must be depleted in ^{18}O. This explains why $\delta^{18}O$ (N_2O) is lighter in the secondary pycnocline, even when dissolved O_2 should be isotopically enriched due to the respiration of organic matter accumulated in this pycnocline.

The behavior of SP and $\delta^{18}O$ (N_2O) below the pycnocline at UPX is more complicated than at the oceanic stations. Maximum values of $\delta^{18}O$ (N_2O) between 56‰ and 60‰ coincide with the oxygen minimum zone (OMZ). This can be interpreted as the influence of the isotopic enrichment of O_2 due to respiration. From this, it can be concluded that the main source of N_2O is nitrification. Otherwise, SP in the OMZ (≈17‰) is diminished related to the nitrous oxide released by nitrification at the oceanic station (≈22‰), probably due to the influence of nitrifier denitrification. In any case, these two processes could be running simultaneously. The SP maximum is observed at 400 m, coinciding with the nitrate maximum and the rising oxygen concentration at the OMZ, and slightly deeper than the nitrous oxide maximum (50.29 nmol Kg^{-1}). This suggests an enhancement of the extend of nitrification at this depth. Minima of $\delta^{18}O$ (N_2O), SP, and N_2O are found at UPX (600 m), as is an oxygen maximum. As this parameter seems to be related to the core of a water mass identified at this depth (S≈34.3 and T≈5.5°C), the oxygen maximum is seemingly influenced by a process occurring at the origin of this water.

Studies have demonstrated that temporal oxygen-depleted microsites are possible in marine snow in light absence (Alldredge and Cohen, 1987; Ploug, 2001; Ploug et al., 1997). The oxygen consumption around sinking particles strongly depends on the advection-diffusion balance driven by sinking velocities (Csanady, 1986; Kiørboe et al., 2001), with a higher oxygen depletion accompanying slower-falling particles. Although the stations GYR and EGY are located in a very oligotrophic area, the accumulation of

sinking particles at the steepest density gradient layer inside the pycnocline (see Brunt-Vaisälä frequency plots, Fig. 3) should allow the formation of aggregates suitable for nitrogen reductive processes, as proposed by Yoshida (Yoshida et al., 1989; Yoshida and Toyoda, 2000). Figure 8 show the particle count between 0.052 mm to 8.438 mm of ESD (a wide range of marine snow size) at three sampled stations (C. Gorsky and M. Picheral, personal communication). In GYR and EGY station an small particle accumulation is observed at 300 m (≈35 part/lt) and 50 m (≈45 part/lt) respectively, roughly coincident with high stability zones. The particle content in EGY station below the photic zone shows no significative accumulation, the mean particle content between 200 m and 400 m is ≈22 part/lt. Otherwise, the high particle content in UPX station is closely related to stability profile. Those results indicate that is possible to find particles suitable to form oxygen depleted microsites even in oligotrophic marine environment.

3.4 Contribution of nitrifier denitrification

At GYR and EGY, it is possible to identify at least 3 sources of N_2O, according to the shape of the SP profiles. A first source can be attributed to mixing with the air in the shallow mixed layer, where the average SP at both stations is about 17‰ slightly below the value expected for the atmosphere (19±2‰) (Yoshida and Toyoda, 2000). A second source is represented by the SP signal below 1000 m, which averaged 22‰ and a third source is located at the SP minimum located at 320 m and 250 m at GYR and EGY stations respectively.

Assuming that there is no significant contribution from air-sea transfer below the pycnocline, and that SP values below 1000 m are representative only of ammonium oxidation, it is possible to build a simple two-box model to elucidate the contribution of ammonium oxidation and nitrite reduction to the minimum SP zone at GYR and EGY using SP values. The fraction of N_2O produced by nitrifier denitrification (F) can be determined as follows:

$$F = \frac{SP_{IM} - SP_{ao}}{SP_{nr} - SP_{ao}} \quad (6)$$

where SP_{IM} is the SP value at the site preference minimum, SP_{ao} is the SP value attributable to ammonium oxidation (equal to the SP value below 1000 m), and SP_{nr} is the SP value attributable to nitrite reduction, equal to zero, regarding the biochemical literature discussed above, and the values obtained in cultures with induced nitrifier denitrification (Sutka et al., 2004; Sutka et al., 2006). The assumption that SP values below 1000 m are representative of nitrification is supported by the $\delta^{15}N^{\alpha}$ enrichment predicted for this process and the agreement of the nitrate-phosphate ratio with the Redfield ratio for those stations shown in Fig. 3. Calculated F values of 0.4 and 0.5 (GYR and EGY respectively) demonstrate that nitrifier denitrification pathways can be an important source of nitrous oxide, even in oligotrophic and well-oxygenated waters.

At UPX, the nitrous oxide production processes seems to be more complicated than at the oceanic station. Coastal environments are commonly highly dynamic, with a high influence of horizontal advection. Because of this, the model applied here to the oceanic station should not be valid for the coastal station. Nevertheless, nitrifier denitrification could be an important source of nitrous oxide to the atmosphere, regarding the SP-depleted nitrous oxide present in the mixed layer (related to the expected atmospheric SP values).

4 Conclusions and perspectives

Our results show that nitrous oxide sources in the ocean are far from being identified. Even in very stable oligotrophic environments, different mechanism could coexist. In this sense, new tools like nitrous oxide isotopomer determination open the doors to fresh information about nitrogen cycling processes.

SP signal supporting the evidence of the occurrence of nitrifier denitrification in highly oligotrophic environments is one of the most striking contributions of this work. Particular chemical dynamics of this process under such conditions, as well the role of particles, are subject of further challenging studies.

The importance of the occurrence of nitrifier denitrification in oceanic environments

for the whole nitrogen cycle may be enormous, considering that the Central South Pacific Gyre represents the major part of the South Pacific. Moreover, such conditions are found in the other oceans, thus rendering of this process a potentially determinant contribution to the nitrous oxide budget.

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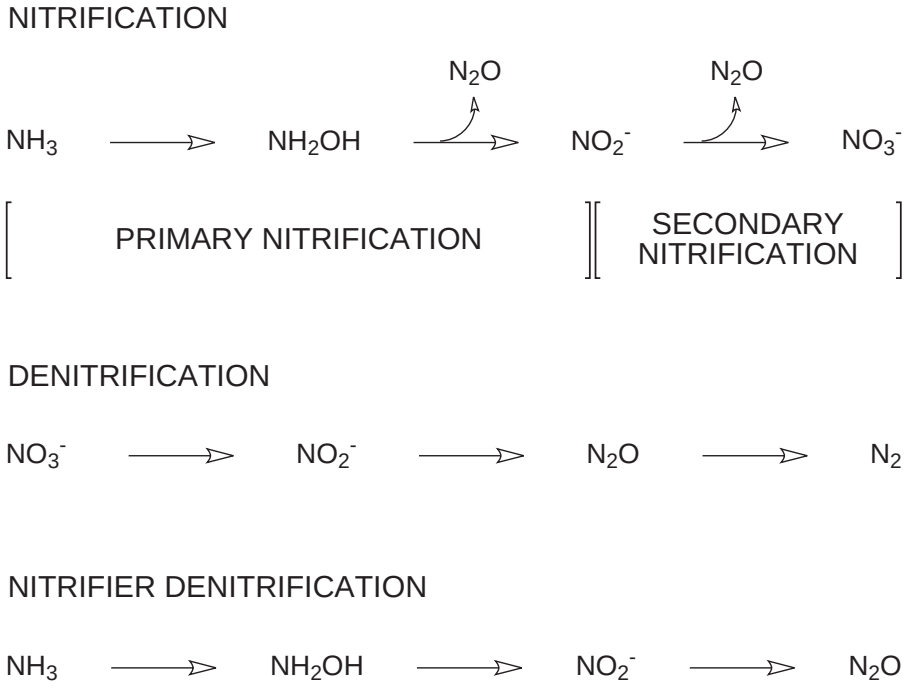

Fig. 1. Outline of different pathways of nitrous oxide production. Adapted from Wrage et al. (2001).

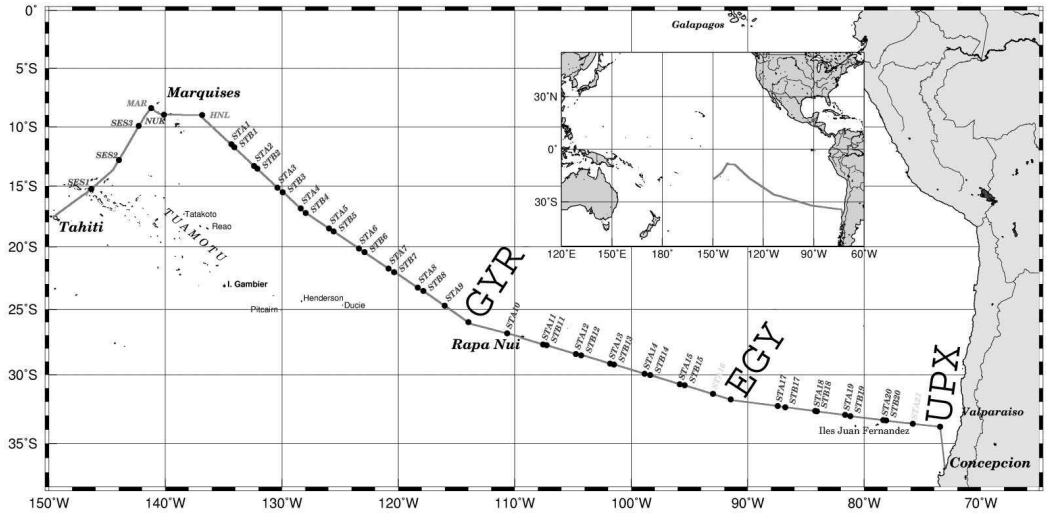

Fig. 2. Sampled stations during Biosope Cruise, GYR, EGY and UPX are indicated in bigger letters.

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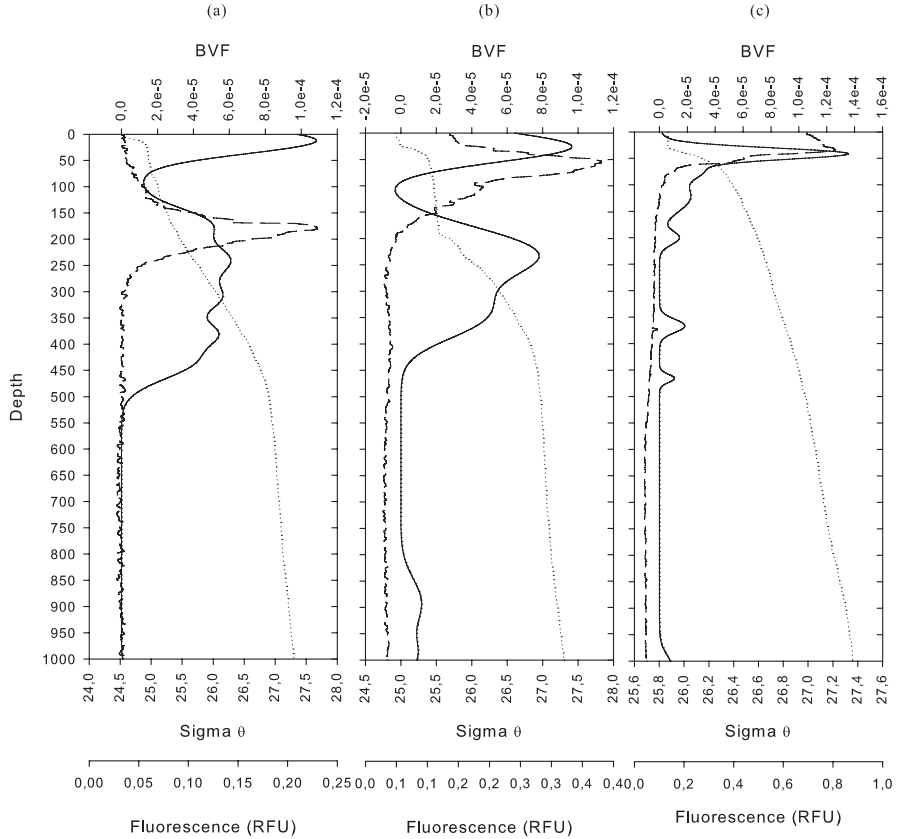

Fig. 3. Vertical profiles at stations **(a)**: GYR, **(b)**: EGY and **(c)**: UPX. The parameters shown are: Potential density (dotted line), Brunt-Vaisälä frecuency (continuous line), and Fluorescence (dashed line).

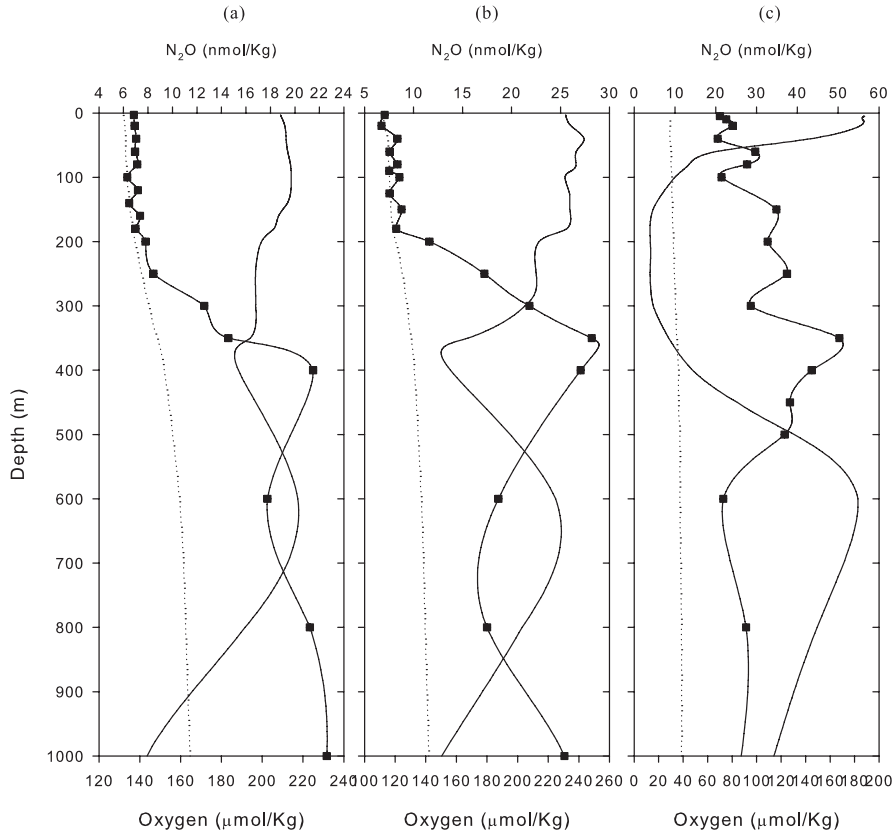

Fig. 4. Vertical profiles at stations **(a)**: GYR, **(b)**: EGY **(c)**: UPX. The parameters shown are: N_2O saturation (dotted line), N_2O (squares) and O_2 (continuous line).

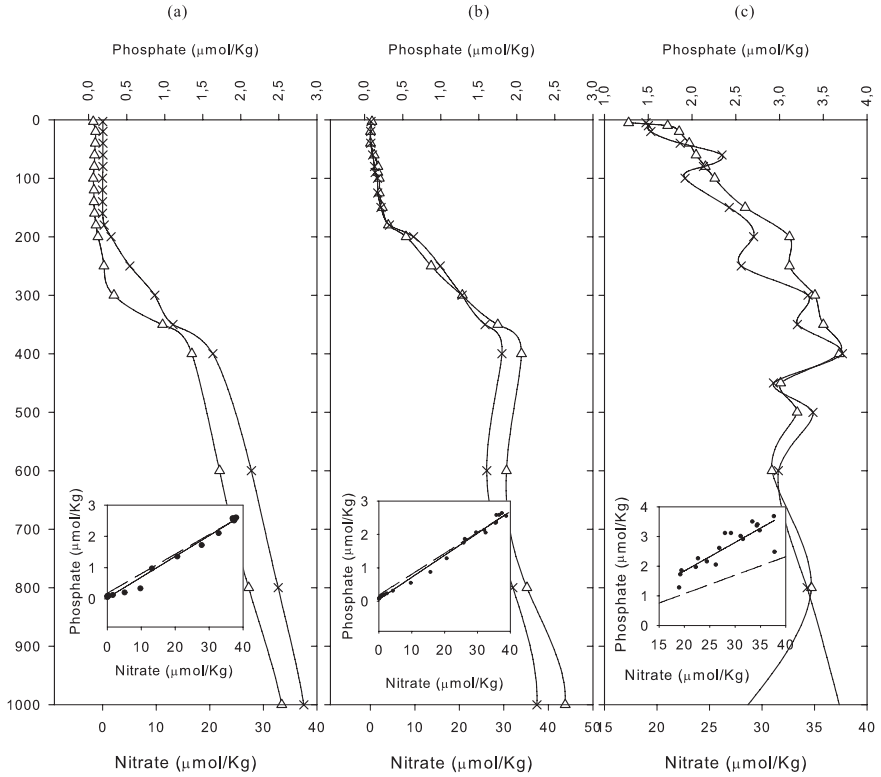

Fig. 5. Vertical profiles at stations **(a)**: GYR, **(b)**: EGY **(c)**: UPX. The parameters shown are: Nitrate (cross) and phosphate (open triangle). Small plots shows nitrate v/s phosphate ratio. Solid line shows linear regression of data, dashed line shows the equation deduced from Redfield N:P ratio (16:1) (Gruber and Sarmiento, 1997).

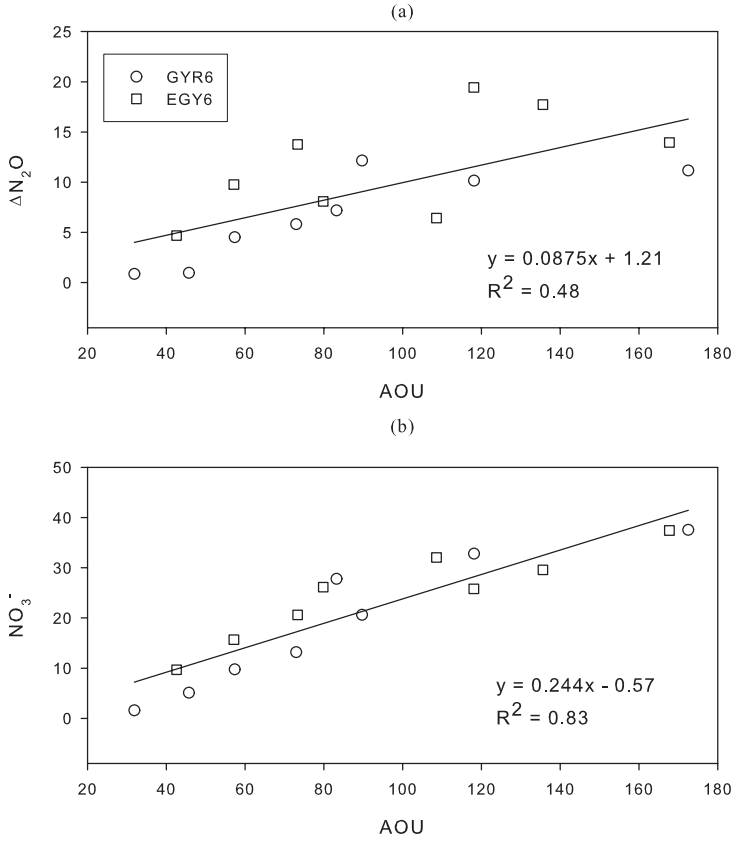

Fig. 6. (a) AUO v/s N_2O and **(b)** AUO v/s NO_3^-. Continuous lines are the linear regressions.

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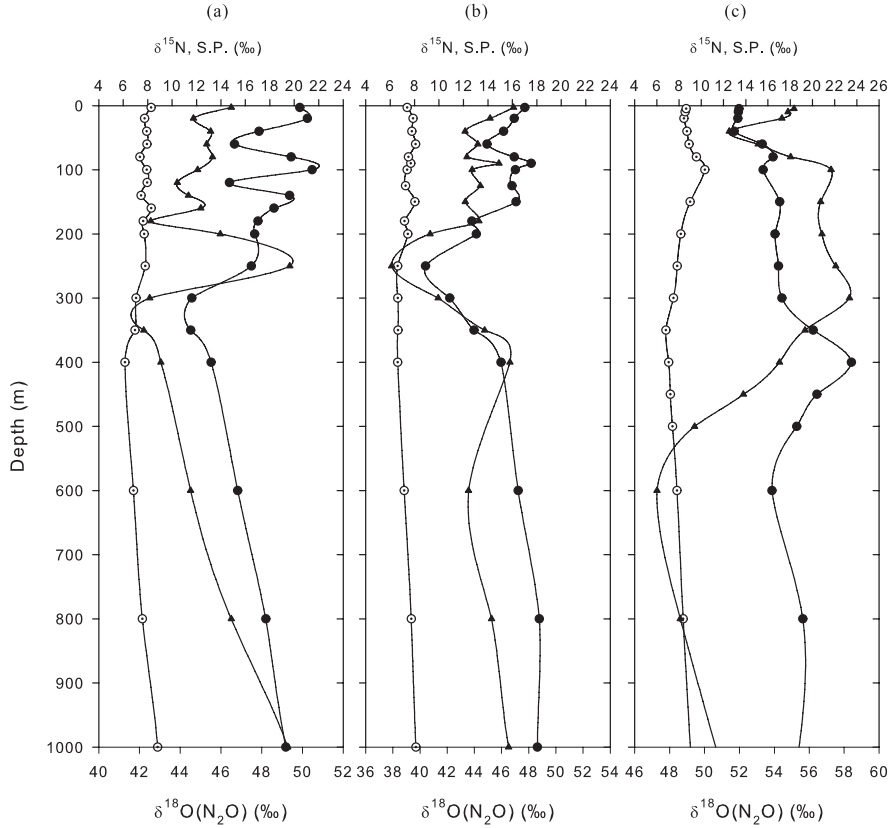

Fig. 7. Vertical profiles at stations **(a)**: GYR, **(b)**: EGY **(c)**: UPX. The parameters shown are: $\delta^{15}N_{bulk}$, (open circle): Site preference (solid circle), $\delta^{18}O(N_2O)$ (solid triangle).

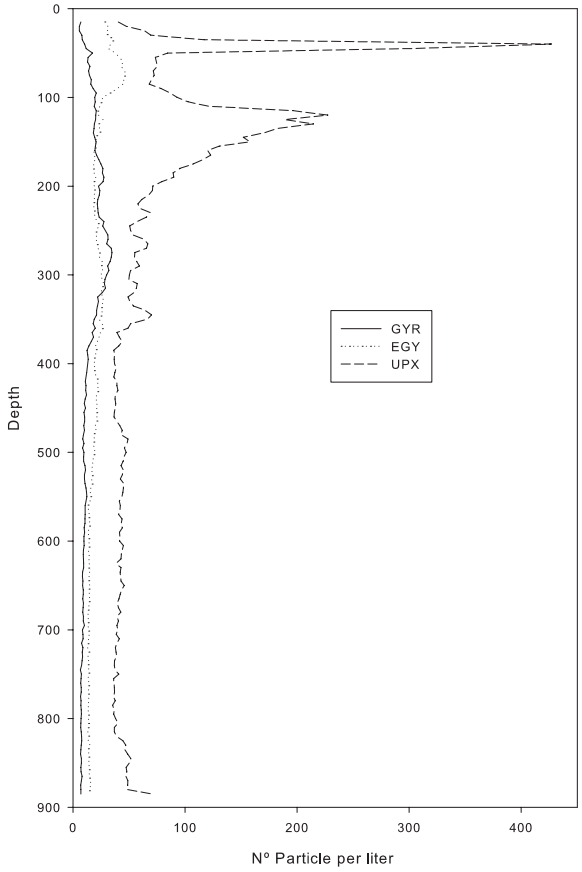

Fig. 8. Particle content at the stations : GYR (continuous line), EGY (dotted line) and UPX (dashed line).

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