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N_2O cycling at the core of the oxygen minimum zone off northern Chile

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ABSTRACT: Northern Chile's oxygen minimum zone (OMZ) is considered to be an important site of N_2O production and efflux into the atmosphere, with a potentially global impact. Seawater samples from the OMZ core were used to determine how different O_2 levels and electron donor/acceptor availability affect N_2O cycling. N_2O production by denitrification (N_2Opd; acetylene treatment) and nitrification (N_2Opn; allylthiourea [ATU] treatment), and N_2O consumption by denitrification (N_2Ocd), were determined with *in situ* O_2, anoxic (0 µM O_2), hypoxic (~22.3 µM O_2), and potential (added substrate) experimental conditions. Under *in situ* O_2 levels (~4.6 µM), total N_2O production (N_2Opd + N_2Opn) was ~2.62 µM d^{-1}. Denitrification was responsible for over 92% of the total N_2Opd and nitrification for less than 8%. Nearly 100% of the N_2O produced was, however, consumed by denitrification. NO_3^- was reduced twice as rapidly as NO_2^-. Under anoxia, N_2Opd and N_2Ocd rates decreased by over 90%. The NO_3^- reduction was similar to that observed with *in situ* O_2, whereas a high rate of NO_2^- accumulation was observed. Conversely, increasing O_2 levels (~22.3 µM) doubled N_2Opd. Consequently, N_2Opd or NO_2^- reduction seems to be the process most sensitive to O_2 fluctuations. Adding organic carbon and NO_3^- increased N_2Opd and N_2Ocd slightly, whereas additional N_2O increased N_2Ocd abruptly. The fate of reduced NO_3^- in the OMZ core was controlled mainly by O_2 concentrations and indirectly by available organic carbon. Both variables are susceptible to the changes experienced in the eastern South Pacific during the El Niño Southern Oscillation cycle.

KEY WORDS: Nitrous oxide cycling · Oxygen minimum zone · Denitrification · O_2 · Organic matter regulation

INTRODUCTION

The ocean is one of the principal natural sources of nitrous oxide (N_2O) in the atmosphere. The well-known climatological effects of N_2O on both the troposphere and the stratosphere make it an important greenhouse gas. The global flux of N_2O into the atmosphere (≈4 Tg yr^{-1}) is not spatially homogeneous, but is concentrated in regions influenced by upwelling, most of which are associated with oxygen minimum zones (OMZs) (Nevison et al. 2004). Although not widespread, OMZs, which are defined as intermediate waters having hypoxic (<22.5 µM) and sometimes anoxic dissolved O_2 concentrations (Kamykowski & Zentara 1990), can be found in the equatorial Pacific, eastern Pacific, and Indian oceans. Such OMZs could contribute 25 to 70% of the global N_2O coming from oceanic sources (Nevison et al. 2004) by way of N_2O cycling through nitrification and denitrification processes (Codispoti & Christensen 1985). Although both nitrification and denitrification produce N_2O under low oxygen concentrations, only denitrification consumes N_2O (Elkins et al. 1978). Denitrification takes place in suboxic or even anoxic waters (Codispoti & Christensen 1985), and involves several reduction steps from NO_3^- to N_2O or N_2 (Payne et al. 1971). Each reduction step is carried out by diverse bacteria. Whether the NO_3^- reduction is partial or complete depends on O_2 levels, nitrogen oxide acceptors, organic carbon availability, and the physiological state of the bacteria (Körner & Zumft 1989). N_2O reduction, at least in some denitrifying species, is believed to be

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more sensitive to O_2 than the other reduction steps (Betlach & Tiedje 1981); dissolved O_2 is the principal regulator of denitrifying reductases (Körner & Zumft 1989, Kester et al. 1997).

The way that environmental factors (e.g. O_2 and organic carbon) contribute to N_2O cycling is not yet clear, and few relevant studies have been carried out on OMZs (Law & Owens 1990, Naqvi & Noronha 1991, Naqvi et al. 2000) or suboxic waters (Elkins et al. 1978, Rönner & Sörensson 1985, Brettar & Rheinheimer 1992). High denitrification rates can lead to changes in the C and N exchanges between the ocean and the atmosphere, significantly influencing global climate change (Codispoti et al. 2001).

The eastern South Pacific's OMZ, particularly off northern Chile, impinges on the photic zone, and is one of the most shallow and severe OMZs in the world (Morales et al. 1999). Associated with the equatorial subsurface water mass between 50 and 400 m depth, northern Chile's OMZ is an appropriate place to study the effect of environmental changes on N_2O cycling in the water column. This study presents the results of controlled experiments with different O_2 levels and the addition of electron acceptors (NO_3^- and N_2O) and donors (dissolved organic matter, or DOM), assuming that these variables are the main controlling factors of N_2O production and consumption by the denitrifying community in the northern Chilean OMZ core.

MATERIALS AND METHODS

Study and sampling area. This study was carried out off Iquique (20° S) and Antofagasta (23° S), 2 centers of persistent coastal upwelling in the Humboldt Current System. The water samples were obtained during the December 2002 Dormido cruise (RV 'Purihaalar') off Antofagasta and the March 2003 Chups Cruise (RV 'Abate Molina') off Iquique. All stations were within 30 km of the coast except for one that was 200 km offshore (Chups cruise, Fig. 1). A vertical hydrocast was made using 5 l Niskin bottles attached to a CTDO (conductivity, temperature and dissolved oxygen) rosette sampler. Salinity, temperature, and dissolved O_2 were obtained from continuous CTDO records. Dissolved O_2 measurements were taken from discrete samples. The samples for N_2O were collected in vials (11 ml) with gas-tight screw caps; a clean tygon tube allowed each vial to be filled from the bottom, thereby eliminating all bubbles. After this, 50 µl of saturated $HgCl_2$ were added and the vials were stored upside down, in the dark, at 4°C until analysis. NO_2^- was measured onboard. Seawater samples for NO_3^- analysis were taken in 250 ml polyethylene vials, filtered and frozen until analysis.

Determination of N_2O cycling rates. N_2O cycling rates (by both nitrification and denitrification) were measured with 3 kinds of experiments: *in situ* O_2 levels (4.4 or 6.9 µM); anoxic (0 µM O_2) and hypoxic levels (22.3 µM O_2); and 'potential experiments', in which NO_3^-, N_2O, and DOM were added in order to measure the potential activity of enzymes present at the sampling time.

A combination of 15% (v/v) acetylene (Firestone & Tiedje 1979), 250 mM ATU (Ginestet et al. 1998), and 0.1g l^{-1} of chloramphenicol (Murray & Knowles 1999) were required for these assays. The acetylene, an inhibitor of N_2O reductase, and ammonium monooxygenase (AMO) avoided N_2O production by nitrification and its reduction by denitrification. In all experiments, the rate of N_2O production by denitrification (N_2Opd; acetylene treatment) corresponded to the rate of N_2O accumulated after inhibition with acetylene. ATU, a specific inhibitor of AMO, was used to evaluate net N_2O cycling of denitrification (net N_2Od), which equaled production minus consumption by denitrifiers only; it was also used to estimate N_2O production by nitrification (N_2Opn) as the difference between the control (net N_2O production by nitrification and denitrification) and the ATU experiment, below *in situ* O_2 levels. Likewise, the rate of N_2O consumed by denitrification (N_2Ocd) was estimated from the rates estimated in experiments with acetylene (N_2Opd) minus those estimates in the experiment without acetylene (below anoxia) or the ATU experiment (*in situ* O_2). Cloramphenicol was used to prevent novo synthesis only within potential experiments and their controls

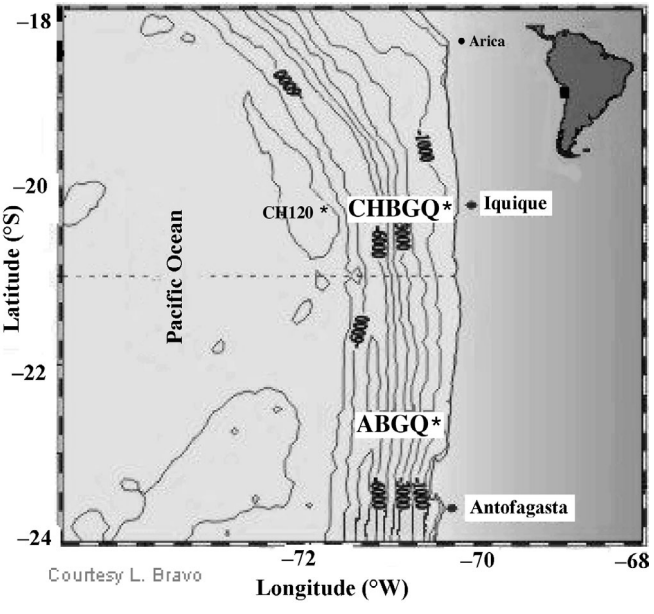

Fig. 1. Sampling sites in the oxygen minimum zone (OMZ) off northern Chile

(natural experiments), thereby assuring that the activity observed reflected the *in situ* metabolic rate with the enzymes present at the time of sampling.

Seawater samples were collected from the OMZ core (100 m depth for Antofagasta, 200 m depth for Iquique) with Niskin bottles and then dispensed — avoiding oxygenation — into 1 l bottles; these were taken to the laboratory for incubation assays less than 3 h later. A N_2 atmosphere was used for N_2O production and consumption experiments to avoid altering the *in situ* O_2 content. Each experiment was carried out in a 50 ml serum flask sealed with a rubber stopper. Flasks were placed in the dark, inside a thermoregulated water bath, at *in situ* temperature (11°C); 3 bottles were collected for analysis at 0, 3 or 6, and 12 h. After incubation, N_2 was used to displace 5 ml of water from each flask; the water was filtered and frozen for NO_2^- and NO_3^- analysis. The rest of the sample (45 ml) was treated with a $HgCl_2$ solution and the N_2O was measured from the headspace at the laboratory.

The same procedure was followed with the experiments under manipulated O_2 levels, with the difference that bubbling with N_2 was used to maintain O_2 levels at anoxia (0 µM), and bubbling with a O_2/N_2 (8% O_2, 92% N_2) mixture was used to maintain an 8% O_2 saturation (equivalent to ~22.3 µM O_2, in accordance with *in situ* temperature and salinity). The samples were left to re-equilibrate for 1 h under these O_2 concentrations before their subsequent distribution and incubation in 50 ml bottles. For the potential experiments, sodium acetate (1 mM), glucose (1 mM), KNO_3 (1 mM), and cloramphenicol (0.1 g l^{-1}) were added to 1 l bottles, mixing very well before dispensing in 50 ml bottles for incubation. These experiments were done only under *in situ* O_2 and anoxia.

Chemical analysis. Dissolved O_2 measurements were made using a semiautomatic version of the Winkler method (Williams & Jenkinson 1982) based on a photometric endpoint detector, a Dosimat 665 (Metrohom) and a chart recorder (<0.1% coefficient of variation). The N_2O analysis for both seawater samples and experiments was achieved by He equilibration in the vial (McAullife 1971) followed by headspace quantification with a gas chromatograph (Varian model 3380) outfitted with a ^{63}Ni electron-capture detector maintained at 350°C. N_2O separation was achieved on a 3 m molecular column kept at 60°C, using an ultra-high purity 5% CH_4+Ar mixture as a carrier. The measurements were calibrated with 0.1 and 1 ppm standard N_2O mixtures (Scotty II) supplied by Alltech. The dissolved N_2O concentration was calculated from the headspace gas concentration using the temperature and salinity-dependent partitioning coefficient (Weiss & Price 1980). The coefficient variation of dissolved N_2O measurements between replicates was <3%, and the average precision was always better than 3% (at the σ level). NO_2^- was measured by manual colorimetric analyses (Bendschneider & Robinson 1952). NO_3^- was reduced by a copper-cadmium column to NO_2^- and determined as NO_2^- as outlined by Grasshoff & Koroleff (1983).

Data and statistical analysis. The production/consumption rates of N_2O, NO_3^-, and NO_2^- were calculated by a lineal regression analysis from the concentration change measured in 3 replicates at each sampling time (0, 3, 6, and 12 h). The N_2O concentrations in the samples were plotted against time and fitted to the linear model ($At = A_0 \pm mt$) using the method of least squares, where t is the incubation time; A_0 is the N_2O concentration at $t = 0$; and m is the linear slope. The rates were calculated from the slope and expressed in µM d^{-1}. Rate uncertainties (±) were calculated from the errors in the linear regression estimation. Positive values represented N_2O or nutrient accumulation through time, whereas negative values indicated its consumption. The significant differences between the slopes were used in conjunction with Student's *t*-test to evaluate the differences between the rates.

RESULTS

Dissolved O_2, N_2O, and nutrient distributions off Iquique and Antofagasta

Vertical distributions of dissolved O_2, N_2O, and nutrients are shown in Fig. 2. At the Antofagasta station (ABGQ), the O_2 concentration dropped from nearly 200 µM (mixed layer) to ca. 10.25 µM (50 m depth), generating a sharp oxycline, and reached ca. 4.46 µM at 200 m depth. The O_2 distribution at the coastal station off Iquique (CHBGQ) was similar to that of ABGQ, but lower O_2 values (<1.8 µM) were found at 200 m depth. The oceanic station (CH120) showed a deeper oxycline between 40 and 70 m depth and the O_2 levels remained ~5 µM between 100 and 300 m depth.

At ABGQ, the vertical N_2O distribution had a narrow maximum located at 60 m depth (0.18 µM) and a consumption zone between 75 and 200 m depth (<0.12 µM). A marked peak in the N_2O concentrations (>0.30 µM) could be seen at 40 (CH120) and 50 m (CHBGQ) depth, followed by a pronounced N_2O depletion (<0.05 µM) between 100 and 300 m depth. All maximum values were confined to the lower half of the oxycline and the upper part of the OMZ.

Nutrients profiles are only shown for ABGQ and CHBGQ. The vertical NO_2^- distribution presented a secondary maximum (SNM) with concentrations up to 8 µM, which was always immersed in the OMZ

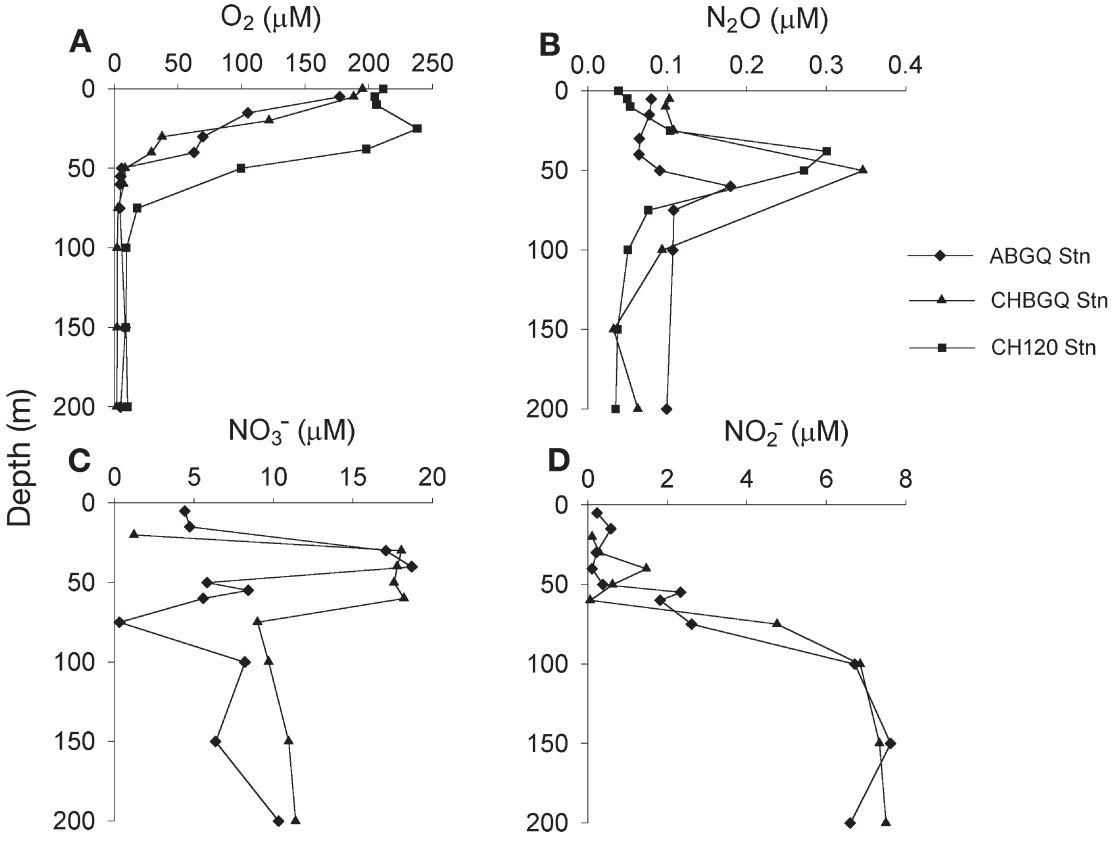

Fig. 2. (A) Oxygen, (B) nitrous oxide, (C) nitrate, and (D) nitrite distributions in the OMZ water column off Antofagasta (ABGQ) and Iquique at coastal (CHBGQ) and offshore (CH120) stations

between 55 and 200 m depth and associated with low NO_3^- levels (<10 μM) at both sites. Maximum NO_3^- concentrations (~20 μM) were found between 30 and 60 m depth and within the OMZ core, the concentrations varied between 5 and 10 μM.

N_2O cycling under *in situ* O_2

Table 1 summarizes the N_2O cycling rates obtained for *in situ* O_2 experiments at both sampling sites, and Fig. 3 shows examples of typical regressions for the control, ATU, and acetylene experiments. The N_2O, NO_3^-, and NO_2^- concentrations at the initial incubation time of each experiment (both stations) are also detailed in Table 1. At ABGQ, the net N_2O cycling (control) measured at *in situ* O_2 was significantly different from zero. Total N_2O production reached 2.62 μM d^{-1}; 92% was produced by denitrification and 8% by nitrification. Almost 100% of this N_2O production was consumed by denitrification. NO_3^- was reduced twice as rapidly as NO_2^- during the experiment. A significantly negative net N_2O rate was found for *in situ* O_2 at CHBGQ and N_2Opd and N_2Opn equaled 96 and 4% of total N_2O production (2.72 μM d^{-1}). Here, the estimated N_2Ocd rate was significantly ($t_{\alpha(2)}$: 0.01) higher off Antofagasta, although the NO_3^- and NO_2^- consumption rates were lower.

N_2O cycling under manipulated O_2

Table 2 summarizes the N_2O cycling rates obtained under simulated O_2 levels. At ABGQ, net N_2Od cycling did not differ significantly from zero with anoxic conditions, but the N_2Opd and N_2Ocd decreased significantly (92%, $t_{\alpha(2)}$: 0.002) with respect to *in situ* O_2. The NO_3^- consumption rate was similar to that seen with *in situ* O_2, although the NO_2^- rate presented a strong accumulation. The anoxia at the CHBGQ station also produced a significant ($t_{\alpha(2)}$: 0.05) decrease in N_2Opd (93%) and in N_2Ocd (74%) in relation to *in situ* O_2; nevertheless, the N_2Opd rate was similar to that observed at ABGQ. The N_2Ocd was 3 times higher at CHBGQ than at ABGQ under anoxia, resulting in a significantly negative net N_2Od

rate. NO_2^- accumulation was also observed at CHBGQ, although at a lower rate than at ABGQ. On the other hand, increasing O_2 levels (~22.3 µM) significantly ($t_{\alpha(2)}$: 0.05) increased the N_2Opd and NO_3^- reduction with respect to the control or the *in situ* O_2 experiment.

Table 1. N_2O, NO_3^-, and NO_2^- recycling rates (mean ± error) obtained through the incubations, subject to the *in situ* O_2 level of the water column samples taken from the OMZ core off Antofagasta (ABGQ) and Iquique (CHBGQ). N_2Opd: N_2O production by denitrification; N_2Opn: N_2O production by nitrification; N_2Ocd: N_2O consumption by denitrification; net N_2Od: net N_2O by denitrification; net N_2O: corresponds to $N_2Opd + N_2Opn + N_2Ocd$. ATU: allylthiourea. ND: not determined

Stn	Initial condition (µM)	Process	Treatment	N_2O (µM d^{-1})	NO_3^- (µM d^{-1})	NO_2^- (µM d^{-1})
ABGQ	O_2: 6.9 N_2O: 0.29 NO_3^-: 20.5 NO_2^-: 5.5	Net N_2O	Control	0.10 ± 0.03 ($p < 0.01$, $r^2 = 0.6$)	ND	ND
		Net N_2Od	ATU	−0.12 ± 0.10 ($p < 0.30$, $r^2 = 0.1$)	−9.8 ± 6.5	3.6 ± 0.0
		N_2Opd	Acetylene	2.40 ± 0.41 ($p < 0.00$, $r^2 = 0.8$)	−8.6 ± 6.2	−3.8 ± 3.2
		N_2Opn	Estimated	0.22 ± 0.08	ND	ND
		N_2Ocd	Estimated	−2.52 ± 0.31	ND	ND
CHBGQ	O_2: 4.4 N_2O: 0.21 NO_3^-: 24.2 NO_2^-: 3.2	Net N_2O	Control	−0.49 ± 0.19 ($p < 0.02$, $r^2 = 0.5$)	ND	ND
		Net N_2Od	ATU	−0.60 ± 0.34 ($p < 0.13$, $r^2 = 0.2$)	−9.1 ± 4.6	1.2 ± 0.5
		N_2Opd	Acetylene	2.61 ± 1.05 ($p < 0.00$, $r^2 = 0.5$)	−6.4 ± 5.0	−1.6 ± 1.4
		N_2Opn	Estimated	0.11 ± 0.15	ND	ND
		N_2Ocd	Estimated	−3.21 ± 0.71	ND	ND

Table 2. N_2O, NO_3^-, and NO_2^- recycling rates (mean ± error) obtained from the incubation subject to anoxia and ≈8% O_2 saturation of water column samples taken from the OMZ core off Antofagasta (ABGQ) and Iquique (CHBGQ). N_2Opd: N_2O production by denitrification; N_2Ocd: N_2O consumption by denitrification; Net N_2Od: net N_2O by denitrification; net N_2O: corresponds to $N_2Opd + N_2Opn + N_2Ocd$. ND: not determined

Stn	Initial condition (µM)	Process	Treatment	N_2O (µM d^{-1})	NO_3^- (µM d^{-1})	NO_2^- (µM d^{-1})
ABGQ	O_2: 0.0 N_2O: 0.09 NO_3^-: 16.4 NO_2^-: 4.1	Net N_2Od	Control	−0.02 ± 0.02 ($p < 0.27$, $r^2 = 0.1$)	−7.0 ± 5.6	3.6 ± 0.3
		N_2Opd	Acetylene	0.20 ± 0.15 ($p < 0.34$, $r^2 = 0.3$)	−7.2 ± 5.8	9.4 ± 4.3
		N_2Ocd	Estimated	−0.22 ± 0.13	ND	ND
CHBGQ	O_2: 0.0 N_2O: 0.15 NO_3^-: 25.0 NO_2^-: 3.4	Net N_2Od	Control	−0.62 ± 0.09 ($p < 0.00$, $r^2 = 0.8$)	−7.2 ± 2.6	−2.4 ± 2.0
		N_2Opd	Acetylene	0.17 ± 0.18 ($p < 0.27$, $r^2 = 0.2$)	−8.4 ± 5.8	1.1 ± 1.0
		N_2Ocd	Estimated	−0.79 ± 0.09	ND	ND
CHBGQ	O_2: ≈22.3					
		Net N_2O	Control	0.84 ± 0.70 ($p < 0.28$, $r^2 = 0.3$)	−45.6 ± 33.4	−1.7 ± 0.4
		N_2Opd	O_2 + Acetylene	5.16 ± 1.29 ($p < 0.01$, $r^2 = 0.8$)	−48.2 ± 43.3	−1.0 ± 0.7

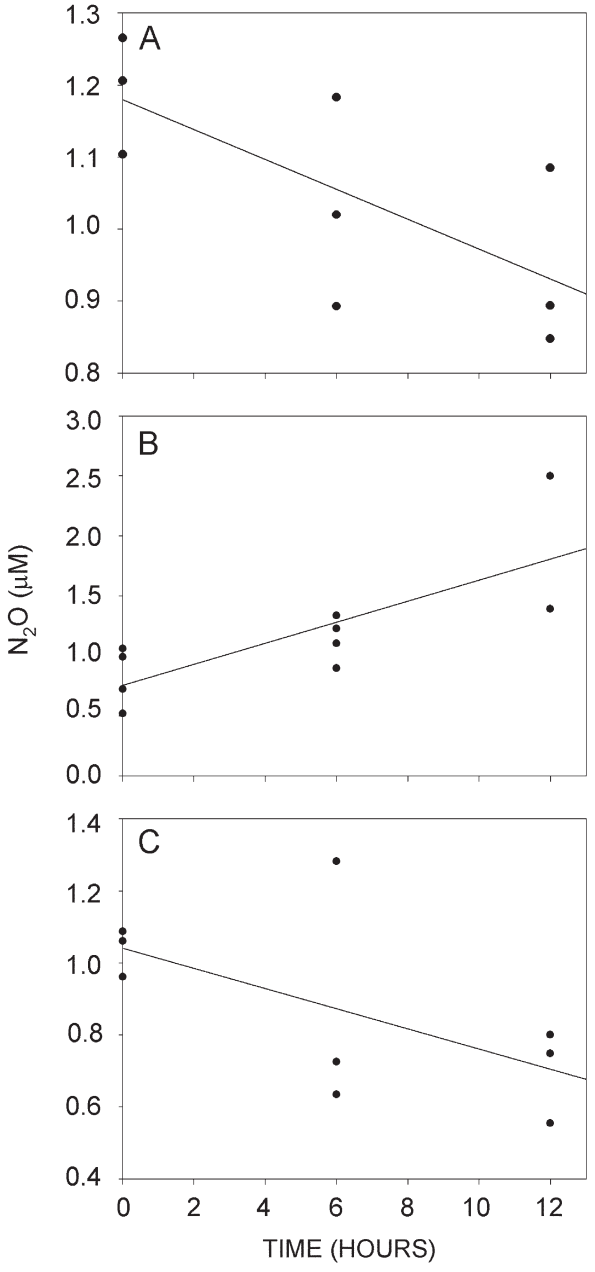

Fig. 3. N_2O evolution with time in (A) control, (B) acetylene, and (C) ATU experiments incubated below *in situ* O_2 levels at the CHBGQ station

Natural and potential N_2O cycling

Fig. 4 presents the N_2O cycling rates of the natural (control) and potential experiments under *in situ* O_2 and anoxia in Antofagasta and anoxia in Iquique. With *in situ* O_2 at ABGQ, the natural rates of N_2Opd and N_2Ocd were 1.29 ± 0.50 and 0.67 ± 0.09 µM d^{-1}. These rates were significantly ($t_{\alpha(2)}$: 0.05) lower than those in experiments without cloramphenicol (*in situ* O_2 levels; Table 1), favoring the proportion of N_2O gas of the total product (46%) in the natural experiment with a net rate of 0.62 ± 0.58 µM d^{-1}. Although the potential rates of N_2Opd (1.61 ± 0.48 µM d^{-1}) and N_2Ocd (0.96 ± 0.03 µM d^{-1}) increased by 25 ($t_{\alpha(2)}$: 0.5) and 43% ($t_{\alpha(2)}$: 0.2) with the addition of NO_3^- and DOM with respect to the natural rates at *in situ* O_2, the net N_2Od rates were similar in both experiments.

At ABGQ, although anoxia alone produced a strong decrease in N_2O production and consumption (Table 2), the natural experiments under anoxia showed a stronger decrease in N_2Opd (75%, $t_{\alpha(2)}$: 0.05) than in N_2Ocd (32%, $t_{\alpha(2)}$: 0.2) with rates of 0.05 ± 0.00 and 0.15 ± 0.09 µM d^{-1}. The N_2Opd also presented a significant ($t_{\alpha(2)}$: 0.05) decrease with respect to the *in situ* natural experiments, suggesting that the N_2Opd was actively regulated by the synthesis of new NO_2^- reductases and O_2. Equally, the N_2Opd and N_2Ocd at ABGQ were enhanced by more than 200% in the anoxic potential experiments to rates of 0.28 ± 0.09 and 0.33 ± 0.06 µM d^{-1}; the net N_2Od, however, was very low and not significantly different from zero. At CHBGQ, under anoxic conditions, N_2Opd and N_2Ocd rates were high (1.20 ± 0.58 and 1.56 ± 0.40 µM d^{-1}) and significantly higher than those obtained off Antofagasta, also under anoxic conditions. The availability of carbon plus NO_3^- also favored a slightly greater NO_2^- reduction, resulting in a 30% accruement ($t_{\alpha(2)}$: 0.5) in N_2Opd to a rate of 1.56 ± 0.25 µM d^{-1}, whereas 65% ($t_{a(2)}$: 0.05) of the total N_2O was reduced to N_2, favoring the proportion of N_2O as a total gas product (34%) in these experiments. The addition of N_2O (~13 µM) plus NO_3^- and DOM under anoxic conditions significantly enhanced reduction activity, producing the highest N_2Ocd rate ($t_{\alpha(2)}$: 0.05) observed in these experiments: 7.47 ± 2.38 µM d^{-1}.

At 200 m depth in the CH120 station, the N_2Opd rates in natural experiments averaged 0.96 ± 0.33 µM d^{-1}, as did those of the coastal station at the same depth. Nevertheless, a low N_2O reduction rate held the proportion of N_2O in the total gas product at 54% for the oceanic station, unlike the coastal station, where the N_2O was totally consumed. On the other hand, the addition of electron acceptors and donors significantly ($t_{\alpha(2)}$: 0.005) enhanced the reduction of N_2O to N_2 with a net N_2Od rate of −0.28 ± 0.15 µM d^{-1}, implying that these substrates are limiting the last step of denitrification within the OMZ core to 200 km from the coast.

DISCUSSION

The OMZ in the eastern South Pacific

The OMZ core was considered herein as a zone where O_2 concentrations could reach values under the suboxic level (≤4.4 µM). The core itself starts at

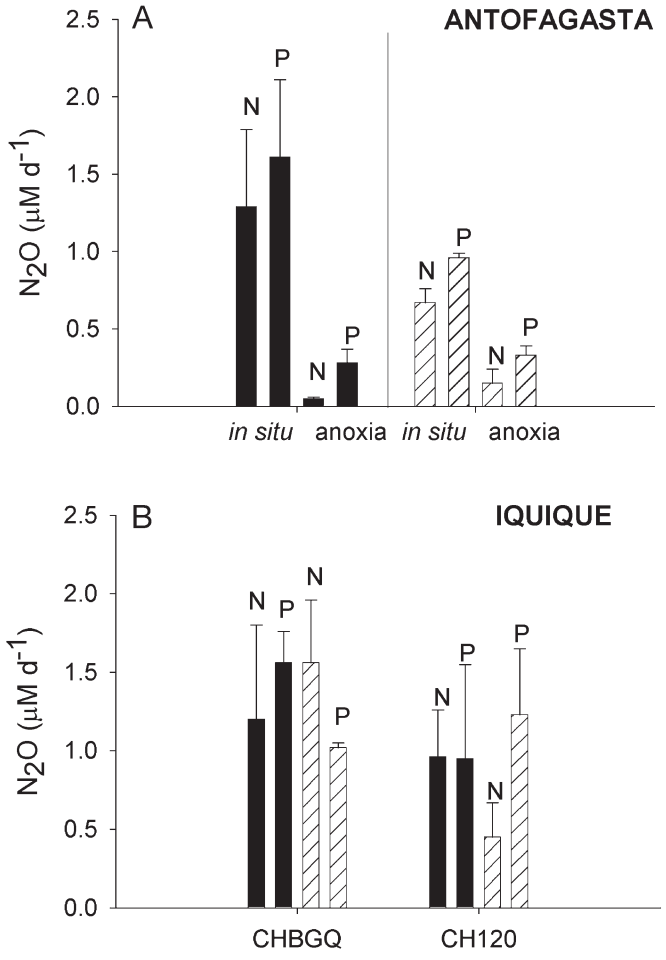

Fig. 4. N_2O production (black bars) and consumption (hatched bars) rates by denitrification in experiments without (N) and with nitrate, sodium acetate, and glucose (P) at (A) Antofagasta (*in situ* O_2 and anoxia) and (B) Iquique (anoxia) at coastal (CHBGQ) and offshore (CH120) stations

the oxycline base, where it coincides with a conspicuous NO_2^- peak (SNM) and NO_3^- deficit (≤10 µM), both features that help delineate OMZs (Morrison et al. 1999). In the OMZ core, the denitrification process consumes the greatest NO_3^- fraction, explaining the NO_2^- accumulation and the presence of a broad SNM between 70 and 400 m depth, with values up to 9 µM. Furthermore, anomalous N:P Redfield ratios (Davies & Morales 1998), as well as an apparent N_2O depletion at the OMZ core with respect to the upper and lower oxycline (L. Farías et al. unpubl. data), have been attributed to the denitrification process in this OMZ. The NO_2^- and N_2O distributions are biogeochemical indicators of real suboxic conditions, typical of denitrifying environments. Such distributions have been described earlier in the South Pacific Ocean by Elkins et al. (1978) and Codispoti & Christensen (1985).

The O_2 levels during the sampling time permitted high NO_3^- reduction rates (near to 8 µM d^{-1}) in both study areas. These rates were faster than those of NO_2^- reduction (3.8 µM d^{-1}), producing a concomitant NO_2^- accumulation. At *in situ* O_2 levels, the NO_2^- reduction, herein considered as a denitrification process, contributed between 92 (ABGQ) and 96% (CHBGQ) of the N_2O production; N_2O, however, was rapidly consumed (reduced to N_2) in the OMZ. This study's denitrification rates (1.6 to 3.7 µM d^{-1}), obtained by means of N_2O production under *in situ* O_2, are the first ones reported for the eastern South Pacific between 20 and 23° S. Although these rates were estimated at only 2 stations, the estimates come from 2 of the most important upwelling areas off Chile during the summer, making them the most representative for the area to date. The values are similar to those reported by Omnes et al. (1996) and Bianchi et al. (1994) for the Mediterranean Sea (1.0 to 4.3 µM d^{-1}), but are higher than those reported for other suboxic areas, such as the Baltic Sea (Rönner & Sörensson 1985, Brettar & Rheinheimer 1992), the Arabian Sea (0.09 to 0.11 µM d^{-1}) (Naqvi et al. 1993), the Indian Shelf (0.3 µM d^{-1}) (Naqvi et al. 2000), and the Peruvian OMZ (0.0032 µM d^{-1}) (Elkins et al. 1978), also measured through laboratory experiments.

Our denitrification rates (13 g N m^3 yr^{-1}) are 2 orders of magnitude higher than those estimated by Codispoti et al. (1989) between 10 and 25°S (0.17g N m^3 yr^{-1}). Such differences in denitrification rates could be due to changes in oxygenation of the southward Peru-Chile undercurrent. The variation of O_2 concentrations can differentially affect each denitrification step depending on the O_2 threshold for each reductase. Nitrifying activity could also be enhanced, contributing to N_2O, NO_3^-, and NO_2^- production, which are used, in turn, as denitrification acceptors (Bonin et al. 1989, Kester et al. 1997).

So far, estimates of water column denitrification, including our own study, cover a small spatial and temporal scale. The open water off Peru, which is certainly the most comparable area, was studied intently during the 1980s and denitrification rates were determined through values of integrated N deficit in the water column. However, this estimation method appears to underestimate the nitrate removal due to denitrification by a factor of ≈2 (Codispoti et al. 2001). Moreover, the same authors mentioned the possible exclusion of reactions and interactions in the N cycle.

Separate measurements of N_2O cycling by nitrification and denitrification could represent an oversimplification of the real pathways of N loss that are occurring in the area. Our measured N_2O cycling rates may be the product of nitrifier denitrification (Wrage et al. 2001) and/or coupling between nitri-

fiers and denitrifiers, as suggested by Codispoti & Christensen (1985), if some dominant nitrifiers were not affected by acetylene. In this sense, although acetylene and ATU appear to be specific inhibitors, their inhibitory effect can be reversible and, in the case of acetylene, ineffective for some ammonia oxidizers (Wrage et al. 2004) even when considering that the sample's storage with $HgCl_2$ was a good procedure with liable N_2O concentrations and rate determinations.

In fact, nitrification contributed <8% of the N_2O production in the sampling area. Our results show that below suboxic conditions, this process takes place — as deduced from global models (Nevison et al. 2003) or through direct measurements on waters of the Indian Shelf (Naqvi et al. 2000), the Baltic Sea (Brettar & Rheinheimer 1992), suboxic estuaries (De Bie et al. 2002), and marine bacteria cultures (Goreau et al. 1980, Bonin et al. 1989, De Bie et al. 2002) — where the N_2O produced from NH_4^+ oxidation increased within microenvironments having low O_2 and NH_4^+ availability (Avrahami et al. 2002). In addition to this, potential nitrification rates (0.091 to 0.62 µM d^{-1}) have been measurable in the OMZ core off Iquique (V. Molina et al. unpubl. data) and Peru (Ward et al. 1989) in spite of low NH_4^+ values and high pelagic NO_3^- respiration rates, supporting the idea of a tight coupling between nitrifying and denitrifying communities participating in N_2O production at O_2 levels ≤ 11 µM (Law & Owens 1990, Naqvi & Noronha 1991). However, it is necessary to keep in mind that the N_2O contribution can also come from bacteria capable of nitrifying denitrification (Wrage et al. 2001) and even NO_3^- ammonification (Omnes et al. 1996), since these groups could also be present in this area.

O_2 regulation of N_2O cycling off Northern Chile

Dissolved O_2 was a key factor in the regulation of N_2O cycling in the OMZ core. Anoxia strongly reduced both N_2O production and consumption in the experiments carried out off Iquique and Antofagasta. Denitrification rates, measured as N_2Opd under anoxia (0.05 to 0.35 µM d^{-1}), were in the same range as those previously reported by Rönner & Sörensson (1985) and Brettar & Rheinheimer (1992) for the Baltic Sea (0.015 to 0.15 µM d^{-1}), Naqvi et al. (1993) for the Arabian Sea (0.09 to 0.11 µM d^{-1}), Naqvi et al. (2000) in the Indian Shelf (0.3 BM d^{-1}), and Bonin et al. (2002) in an estuarine area (0.01 to 0.13 µM d^{-1}). These drastic differences in NO_2^- reduction under anoxia led to higher NO_2^- accumulations off Antofagasta than off Iquique. Similar observations were made in pure culture studies (Firestone & Tiedje 1979, Bonin et al. 1989, Körner & Zumft 1989, Otte et al. 1996). Therefore, in each environment, denitrifying communities appear to respond to anoxia with different enzymatic expression levels or with a sequential synthesis of enzymes. This response also depends on the bacteria's physiological status, abundance, and substrate availability (Firestone & Tiedje 1979).

Although anoxic events rarely occur in this OMZ, anaerobic microenvironments within macro-particles are favored in upwelling areas. Thus, changes in O_2 levels inside these microenvironments would affect the composition of gaseous and ionic denitrification products (Michotey & Bonin 1997), as observed in our anoxic assays, where NO_2^- accumulated at rates up to 9 µM d^{-1}.

NO_2^- reduction was enhanced when O_2 levels increased to ≈22.3 µM. The incomplete denitrification process generated active N_2O production by denitrifiers. Our experiments corroborate the fact that denitrifiers are able to produce more N_2O with high O_2 concentrations. Numerous laboratory experiments with denitrifying bacteria resulted in N_2Opd being enhanced between ≈22 and 111 µM O_2 (Lloyd et al. 1987, Otte et al. 1996, Takaya et al. 2003). Accordingly, denitrification can operate at even higher O_2 levels than assumed for natural conditions, presently not higher than ≈9.0 µM O_2 (Rönner & Sörensson 1985). The high activity of denitrifying bacteria at ≈22.3 µM O_2 in natural waters corroborates that most denitrifiers, and perhaps autotrophic nitrifiers with a denitrifying capacity, might produce higher N_2O:N_2 ratios as the O_2 tension increases to oxic conditions, since the N_2O reductase is strongly inhibited (Bonin et al. 1989, Wrage et al. 2001, Takaya et al. 2003). The observed increase in the N_2O:N_2 ratio shows that changes in O_2 levels are strong regulators of N_2O cycling in a habitat of fluctuating O_2 tensions, as observed in this OMZ, particularly at the oxyclines. These increments in net N_2O production in the OMZ core can generate a major efflux of N_2O into the atmosphere. Such situations could occur during El Niño events, when oxygenation in the upper water column off northern Chile can double (Ulloa et al. 2001). These events produce strong physical and biological changes in the OMZ off northern Chile, affecting O_2 levels, NO_3^- and organic carbon supply, and gas fluxes in the water column (González et al. 1998, Ulloa et al. 2001).

Natural and potential N_2O cycling in the OMZ off northern Chile

DOM and NO_3^- availability are generally accepted as important factors controlling the occurrence, rate,

and composition of denitrification's gaseous products (Brettar & Rheinheimer 1992). Differences in N_2Opd and N_2Ocd between natural and potential experiments were only statistically significant ($t_{\alpha(2)}$: 0.05) in anoxia, suggesting that a community capable of reducing NO_2^- and N_2O was present and active during sampling and that its activity was enhanced below anoxia and slightly limited by substrates such as DOM and NO_3^-. However, the low N_2O levels observed in Iquique's OMZ core could seriously limit N_2O reduction to N_2, as was observed from our potential anoxic experiments; this was also observed for bacterial cultures (Körner & Zumft 1989). The concentration of electron acceptors such as N_2O in the water column would be restrained by preexisting N_2O levels coming from horizontal advection, by those coming from oxyclines via molecular or turbulent diffusion, or by those produced by NO_2^- reduction.

Now, the similar N_2Opd rates observed between natural and potential experiments in the offshore station could be explained by potential incubation biases such as the inhibition of N_2O reduction by NO_3^- addition, as was reported in sediments and bacterial cultures (Körner & Zumft 1989, Baumgartner & Conrad 1992). Otherwise, some DOM limitations in the offshore station could explain the increased N_2Ocd after enrichment with substrates. This suggests that, although there are high fluxes of particulate organic carbon to the deep ocean in this coastal upwelling area, its offshore transport can be scarce.

An important fraction of the organic matter from the Humboldt Current System off Chile is being channeled through bacteria (63 to 96% off Antofagasta), but substrate availability seems to be very important in limiting bacterial activity and its abundance in this OMZ (Troncoso et al. 2003). The differences observed in N_2Opd and N_2Ocd — between natural and potential experiments as well as between stations — can imply differences in the relative abundance of denitrifying bacteria. Sometimes the available substrate is not used effectively by denitrifiers, which prefer N-rich amino acids (Van Mooy et al. 2002); however, no such preferential degradation of amino acids took place in northern Chile's OMZ (Pantoja et al. 2003).

Other non-environmental factors such as physiological behavior, interactions with other organisms, adaptations to each environment, and diversity within denitrifying guilds must be considered. Consequently, the next step in understanding these processes should be the study of denitrifier diversity in this OMZ through functional genes, which are likely to be important for a better understanding of the function of N cycling in the eastern South Pacific Ocean.

CONCLUSIONS

The OMZ core off northern Chile has a very active denitrifying community that is able to reduce between 30 and 40% of the NO_3^- to N_2. The area's N_2O cycling rate is faster than previously estimated for the eastern South Pacific and other areas with similar suboxic conditions, thereby enhancing the area's significance in the global N_2O and N budget. The results also indicate that seasonal upwelling probably influenced higher rates of N_2O cycling and denitrification measured during this study, which could have been subestimated previously.

N_2O cycling was mainly influenced by O_2 levels and, to a lesser degree, by electron donor and acceptor availability. Therefore, changes in the O_2 levels of the water mass, produced by different oceanographic process (e.g. El Niño-Southern Oscillation), could affect N_2O production in the OMZ directly through denitrification or indirectly through the NO_3^- or N_2O available through nitrification.

According to our results, O_2 changes — not nutrient supply changes — are the most important factors in the global N_2O budget, since increasing and decreasing O_2 directly affect the N_2O cycling that might generate more N_2O efflux or influx from the OMZ during El Niño or La Niña events.

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