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Anaerobic ammonium oxidation in the oxygen-deficient waters off northern Chile

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Abstract

We investigated the pathways of N_2 production in the oxygen-deficient water column of the eastern tropical South Pacific off Iquique, Chile, at 20°S, through short anoxic incubations with ^{15}N-labelled nitrogen compounds. The location was characterized by steep chemical gradients, with oxygen decreasing to below detection at ~50-m depth, while nitrite reached 6 μmol L^{-1} and ammonium was less than 50 nmol L^{-1}. Ammonium was oxidized to N_2 with no lag phase during the incubations, and when only NH_4^+ was ^{15}N-labeled, ^{15}N appeared in the form of $^{14}N^{15}N$, whereas $^{15}N^{15}N$ was not detected. Likewise, nitrite was reduced to N_2 at rates similar to the rates of ammonium oxidation, and when only NO_2^- was ^{15}N-labeled, ^{15}N appeared mainly as $^{14}N^{15}N$, whereas $^{15}N^{15}N$ appeared in only one incubation. These observations indicate that ammonium was oxidized and nitrite was reduced through the anammox reaction, whereas denitrification was generally not detected and, therefore, was a minor sink for nitrite. Anammox rates were highest, up to 0.7 nmol N_2 L^{-1} h^{-1}, just below the oxycline, whereas rates were undetectable, <0.2 nmol N_2 L^{-1} h^{-1}, deeper in the oxygen-deficient zone. Instead of complete denitrification to N_2, oxidation of organic matter during the incubations may have been coupled to reduction of nitrate to nitrite. This process was evident from strong increases in nitrite concentrations toward the end of the incubations. The results point to anammox as an active process in the major open-ocean oxygen-deficient zones, which are generally recognized as important sites of denitrification. Still, denitrification remains the simplest explanation for most of the nitrogen deficiency in these zones.

Anammox is the microbially catalyzed anaerobic oxidation of ammonium coupled to nitrite reduction, with production of nitrogen gas (Mulder 1989; van de Graaf et al. 1995), as indicated by the reaction

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$$

This process provides energy for growth for a specialized group of bacteria that belong to the clade of plantomycetes, and it has so far only been found in this group (Strous et al. 1999; Schmid et al. 2005). Although first discovered in a wastewater treatment system, the reaction has recently been added to the array of microbial processes that are important for nitrogen cycling in natural environments, including marine sediments (Thamdrup and Dalsgaard 2002; Dalsgaard and Thamdrup 2002) and anoxic water columns (Dalsgaard et al. 2003; Kuypers et al. 2003). In these environments, anammox is the only documented anaerobic pathway of ammonium oxidation, and it provides a second route from fixed nitrogen to N_2 next to microbial denitrification, which was previously thought to be the only important process that conveys such a conversion. (For recent reviews, see Hulth et al. [2005], Dalsgaard et al. [2005], and Kuypers et al. [2006].) As fixed nitrogen often limits marine primary production (Falkowski et al. 1998), investigations of the rates and regulation of this novel sink in the marine nitrogen cycle are of general importance for our understanding of oceanic biogeochemistry.

The anammox process is biochemically distinct from denitrification, as it involves hydrazine as an intermediate and forms N_2 by a one-to-one combination of nitrogen from the two sources (van de Graaf et al. 1995; Strous et al.

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1999; Jetten et al. 2005). In contrast, denitrification, used here in the strict or canonical sense, is a stepwise enzymatic reduction of nitrate via nitrite, nitric oxide, and nitrous oxide to N_2 (Zumft 1997; Codispoti et al. 2001). The reduction of nitrite to gaseous products is the defining part of this sequence.

The biochemical differences allow the separate quantification of the two processes in natural samples on the basis of the analysis of isotope-pairing patterns in N_2 formed after the addition of ^{15}N-labeled nitrate, nitrite, or ammonium (Dalsgaard and Thamdrup 2002; Thamdrup and Dalsgaard 2002). Thus, in anoxic incubations amended with $^{15}NH_4^+$, anammox is quantified through the accumulation of N_2 with mass 29, that is, $^{14}N^{15}N$ (van de Graaf et al. 1997; Thamdrup and Dalsgaard 2002). In incubations with $^{15}NO_3^-$ or $^{15}NO_2^-$, denitrification is distinguished by the formation of N_2 with mass 30, $^{15}N^{15}N$, whereas anammox produces $^{14}N^{15}N$. If $^{14}NO_3^-$ or $^{14}NO_2^-$ is naturally present, denitrification will also produce $^{14}N^{15}N$ and $^{14}N^{14}N$, and the relative contributions of the two processes are determined by assumption of random isotope pairing during denitrification (Nielsen 1992; Thamdrup and Dalsgaard 2002). The use of $^{15}NO_3^-$ relies on nitrate reduction to make nitrite available to the anammox process and on a relatively rapid turnover of the intermediate nitrite pool for ^{15}N to appear in N_2 at a constant rate. In systems with high nitrite concentrations, a lag phase will occur in the transfer of ^{15}N from nitrate to N_2 (Dalsgaard et al. 2003), and the use of $^{15}NO_2^-$ is preferable to $^{15}NO_3^-$ (Dalsgaard and Thamdrup 2002).

By use of the isotope approach, the relative contribution of anammox to N_2 production in marine sediments has been found to vary from below detection to ~70%, with typical contributions of 25–35% in deeper shelf sediments (Dalsgaard et al. 2005). The factors that govern this large variation is not clear, but higher contributions are generally found in sediments with lower total-mineralization rates, which suggests that competition with denitrifiers for nitrite could be an important factor (Thamdrup and Dalsgaard 2002; Engström et al. 2005; Trimmer et al. 2005).

The first quantifications of anammox in anoxic water columns were reported from the anoxic basin of Golfo Dulce, Costa Rica (Dalsgaard et al. 2003). This anoxic basin opens to and exchanges water with the southern part of the eastern tropical North Pacific oxygen-minimum zone and is characterized by nitrate-rich, nonsulfidic conditions, except for sporadic occurrences of sulfide near the bottom (Richards et al. 1971; Thamdrup et al. 1996). Anammox contributed 32–58% of N_2 production at nonsulfidic depths (Dalsgaard et al. 2003). Because of the similarity in water chemistry, Dalsgaard and coworkers (2003) suggested that anammox could also be an important sink for fixed nitrogen in the oxygen-deficient cores of the large oceanic oxygen-minimum zones. Both anammox and denitrification were assumed to be fueled by the organic detritus in the water column. However, the relative contribution from anammox to N_2 production was somewhat higher than the 29% expected from a combination of organic-matter oxidation through denitrification with oxidation of the resulting ammonium by anammox, if the organic matter decomposed according to the Redfield ratio (Dalsgaard et al. 2003). This difference was suggested to result from preferential degradation of nitrogen-rich compounds.

While the anammox process in sediments and in Golfo Dulce was mainly identified through patterns of isotope pairing, anammox activity in the chemocline of the Black Sea (Kuypers et al. 2003) was linked to the presence of anammox bacteria through whole-cell fluorescent in situ hybridization (FISH) and through the occurrence of a unique type of lipids, ladderanes, which are only known from this group of organisms (Sinninghe-Damsté et al. 2002). Most recently, a combination of FISH, lipid analysis, and ^{15}N incubations also demonstrated anammox activity and biomass in the oxygen-deficient waters of the Benguela upwelling system over the Namibian Shelf (Kuypers et al. 2005). Surprisingly, no denitrification was detected there, so anammox was apparently the only source of N_2 in these waters. Denitrification was suggested to be inhibited by frequent incursions of oxygen into the oxygen-deficient depths of the dynamic shelf waters with denitrification activity recovering more slowly than anammox after such disturbances.

The studies summarized above not only indicate that anammox may play an important role in the marine nitrogen cycle as a sink for fixed nitrogen but also call for further investigations of the geographic distribution of the process and of its regulation. We have quantified anammox and denitrification in oxygen-deficient waters of the eastern tropical South Pacific (ETSP). This region is an important site for the loss of fixed nitrogen from the oceans, through what has so far been assumed to be denitrification (Fiadeiro and Strickland 1968; Codispoti and Packard 1980). Together with similar regions in the eastern tropical North Pacific (Cline and Richards 1972; Codispoti and Richards 1976), the Arabian Sea (Deuser et al. 1978; Naqvi 1994; Bange et al. 2005), and the Southeast Atlantic (Chapman and Shannon 1985; Tyrrell and Lucas 2002), N_2 formation in the oxygen-deficient water column is estimated to account for 25–50% of the total oceanic loss of fixed N (Gruber and Sarmiento 1997; Codispoti et al. 2001; Brandes and Devol 2002).

We here report the first combined quantification of anammox and denitrification activity in the ETSP. These measurements are also the first such measurements in oceanic oxygen-deficient waters that are not over a continental shelf.

Materials and methods

Study site—Samples were collected off Iquique in northern Chile at ~20°S in the southern part of the ETSP oxygen-deficient zone, which reaches from ~5°S to at least 30°S. Hydrographically, the waters off northern Chile are part of the Humboldt Current system. Oxygen-deficient equatorial subsurface water is transported poleward by the Peru-Chile Undercurrent, whereas surface water moves north with the Humboldt Current. A weak but persistent equatorward wind stress drives coastal upwelling from depths of ~100 m, which enhances surface-water productivity to ~40 km off the coast (Morales et al. 1996, 1999; Hormazabal et al. 2004). Off Iquique, oxygen concentrations

are typically <10 μmol L^{-1} (~0.2 mL L^{-1}) from depths of 40–50 m to 400 m, whereas nitrite concentrations reach >6 μmol L^{-1} at the same depths (Morales et al. 1996, 1999; Pantoja et al. 2004; Castro-González and Farías 2004), which indicates the activity of anaerobic mineralization processes (Cline and Richards 1972; Codispoti et al. 2001).

Water was collected from RV *Carlos Porter* during the DINAMO cruise in March 2004 on two closely spaced locations 22–26 km west of Iquique (Sta. 1: 70°19′W, 20°06′S and Sta. 2: 70°22′W, 20°10′S). Almost no continental shelf is present in this region, and the full water depth was 800–1,000 m. The positions were chosen from satellite-based chlorophyll distributions, which showed that coastal upwelling off Iquique sustained a relatively narrow (~20-km wide) band of chlorophyll-rich surface water (up to 10 μg L^{-1}) during the cruise, whereas chlorophyll-rich plumes stretched more than 50 km offshore, both north and south of Iquique (chlorophyll distribution map available as Web Appendix 1: http://www.aslo.org/lo/toc/vol_51/issue_5/2145a1.pdf). The stations were located just outside the high-chlorophyll region, where satellite-based chlorophyll concentrations were around 1 μg L^{-1}.

Samples for process studies were retrieved in a 30-liter Go-Flo bottle in three series of casts: Experiment 1 at Sta. 1, 21 March, from depths 30, 60, 100, 150, 250, and 350 m and Experiments 2 and 3 at Sta. 1 and 2, respectively, both sampled on 24 March, from depths 55 and 150 m. Supporting hydrographic data were obtained by use of an autonomous rosette system equipped with a Seabird 25 CTD, a Seabird 23B dissolved oxygen sensor, and 12 8-liter Niskin bottles.

For the process studies, the Go-Flo bottles were sampled in the ship's wet laboratory into 250-mL glass bottles with twofold to threefold overflow allowed and application of slight N_2 overpressure in the Go-Flo sampler to minimize oxygenation. Glass bottles were capped with butyl rubber stoppers that left neither headspace nor bubbles and stored in a refrigerator during transportation to the laboratory on land. Here, the samples were placed in a water bath at 13°C, and incubations were started within 12 h of sampling.

The ^{15}N tracer experiments were conducted as described by Dalsgaard et al. (2003). Swiftly, bottles were opened, 50 mL of water was removed for analysis of natural concentrations of nitrogen species, nitrogen compounds were added, and the bottles were closed again with butyl rubber stoppers. The stoppers were fitted with two glass tubes, one of which was connected to a bubbling stone, which permitted the subsequent purging with helium gas for 15 minutes. Nitrogen compounds were added from 5-mmol L^{-1} or 10-mmol L^{-1} stock solutions for the following treatments (final added concentration in μmol L^{-1} in parantheses): $^{15}NH_4^+$ (3), $^{15}NO_3^-$ (10), $^{15}NO_2^-$ (5), and $^{15}NO_2^- + {}^{14}NH_4^+$ (5 + 3). The resulting ^{15}N labeling of nitrogen pools was >98% with $^{15}NH_4^+$, 22–58% with $^{15}NO_3^-$, and 36–94% with $^{15}NO_2^-$.

After purging, the bottles were inverted, and from each bottle water was dispensed into 15 glass Exetainer vials (Labco) by means of a slight He overpressure and with overflow. The completely filled 12.6-mL vials were sealed with butyl rubber septa in screw caps, and incubation commenced in a water bath at 13°C. Incubations in the vials were terminated in triplicate five times during each experiment, including an initial sampling. For this procedure, 5 mL of water was withdrawn with syringe and needle through the septum, while the volume was replaced with He. Then, 100 μL of 50% $ZnCl_2$ solution was injected to halt biological activity. The vials were stored upside down for N_2 analysis, and the 5 mL of water withdrawn was distributed into three portions for analysis of NO_2^-, $NO_3^- + NO_2^-$, and NH_4^+.

Nitrogen isotopes in N_2 were analyzed by GC-IRMS, as described by Risgaard-Petersen and Rysgaard (1995). Water samples from the stations prepared similar to those from incubations, but without the addition of nitrogen compounds, were used as reference for the natural N_2 isotope ratios. Concentrations of N_2 were calibrated with standards prepared by injection of different amounts of N_2-saturated water into Exetainer vials that contained He-purged water. The excess of $^{14}N^{15}N$ and $^{15}N^{15}N$ in the samples were calculated as described by Thamdrup and Dalsgard (2000). The GC-IRMS system also detects ^{15}N-labeled N_2O after conversion to N_2. With our configuration of head-space sampling, the sensitivity toward N_2O is approximately half of the sensitivity toward N_2 because of the difference in the water-gas distribution coefficients for the two compounds.

Nitrite was analyzed spectrophotometrically just after sampling according to Grasshoff (1983). Samples for analysis of $NO_3^- + NO_2^-$ and NH_4^+ were stored frozen and analyzed by V^{3+} reduction, followed by chemiluminescense detection of NO (Braman and Hendrix 1989) and by spectrophotometry by application of the salicylate method (Bower and Holm-Hansen 1980), respectively. Because the natural NH_4^+ was typically below the detection limit of the salicylate method, NH_4^+ in the incubations with $^{15}NO_3^-$ was also measured fluorometrically by application of the orthophthaldialdehyde (OPA) method (Holmes et al. 1999) with a Turner Design AU-10 fluorometer. For this procedure, one Exetainer vial was opened at each sampling time, 2.6 mL of water was removed, and OPA reagent was added to the remaining 10 mL.

The mole fractions of ^{15}N label in NO_3^-, NO_2^-, and NH_4^+, F_x, were calculated from the natural concentrations of nitrogen species and the increase in concentration upon addition of ^{15}N label. Concentrations of N_2 produced by ammonium oxidation, $N_{2\,anammox}$, and denitrification, $N_{2\,denitrification}$, were calculated from the labeling fractions and the excess of ^{15}N-labeled N_2, $^{14}N^{15}N_{xs}$, and $^{15}N^{15}N_{xs}$, according to Thamdrup and Dalsgaard (2002). In incubations with $^{15}NH_4^+$,

$$N_{2\,anammox} = {}^{14}N^{15}N_{xs} \times F_{ammonium}^{-1} \tag{1}$$

In incubations with nitrite and, equivalently, in incubations with nitrate,

$$N_{2\,denitrification} = {}^{15}N^{15}N_{xs} \times F_{nitrite}^{-2} \text{ and} \tag{2}$$

$$N_{2\,anammox} = {}^{14}N^{15}N_{xs} \times F_{nitrite}^{-1} - N_{2\,denitrification} \times 2 \times [1 - F_{nitrite}]. \tag{3}$$

Rates were calculated from linear regression of ^{15}N-N_2 concentrations as a function of time.

Results

Hydrography—The physical and chemical parameters did not vary substantially during the 5 days of the DINAMO cruise, and profiles from the two stations and three casts were quite similar (Fig. 1). The water column was stabilized by a relatively steep density gradient, with σ_t increasing from 24.7 kg m^{-3} at the surface to 25.9 kg m^{-3} at 30 m and 26.3 kg m^{-3} at 100 m, which corresponds to a gradient of ~0.006 kg m^{-4} at 50 m and values of ~0.002 kg m^{-4} below 100 m. The oxygen-depleted zone was located close to the surface, with oxygen concentrations below 10 μmol L^{-1} from 30 m to 400 m and undetectable oxygen from 50 m to ~300 m (det. lim. 2 μmol L^{-1} [Fig. 1]). The oxygen-depleted zone contained a secondary nitrite maximum, with nitrite concentrations increasing steeply from <0.5 μmol L^{-1} at 30 m to 6–7 μmol L^{-1} at 100 m (Fig. 2). Similar high nitrite concentrations persisted to 250 m, but by 350 m, nitrite had returned below 0.5 μmol L^{-1}. The nitrite maximum coincided with a minimum in nitrate concentrations at ~15 μmol L^{-1}. Ammonium contributed little to dissolved inorganic nitrogen at concentrations generally below 0.1 μmol L^{-1} at oxic depths and typical concentrations of 0.02–0.03 μmol L^{-1} between 50 and 400 m. Phosphate concentrations (not shown) increased to ~2.7 μmol L^{-1} at 50-m depth and remained around this level through the oxygen-depleted zone. The waters were strongly deficient in dissolved inorganic nitrogen, DIN = $[NO_3^-]$ + $[NO_2^-]$ + $[NH_4^+]$, with deficiencies, estimated as N* (N* = (DIN − 16 × $[PO_4^{3-}]$ + 2.9 μmol L^{-1}) × 0.87; [Gruber and Sarmiento 1997]), peaking at ~20 μmol L^{-1} at 100 m (Fig. 2).

Incubations—Generally, no significant changes occurred in the concentrations of ammonium or nitrate over the first four rounds of sampling (24–36 h of incubation). Only between the next to last and the last sampling, at 46–55 h, some incubations exhibited marked concentration changes, which signaled an abrupt acceleration of rates (Fig. 3). Thus, the ammonium concentrations, as measured with the sensitive OPA method in incubations with $^{15}NO_3^-$, remained at 0.03–0.05 μmol L^{-1} through the first four samplings, similar to the concentrations in situ. Excepted from this result were the samples from the hypoxic zone at 30 m and initial samples from 100 m at Sta. 1, 21 March, all of which had relatively high concentrations of 0.15–0.22 μmol L^{-1}. The ammonium concentration tended to decrease between the two last samplings. Linear regression of ammonium concentrations over time for the first four samplings constrained the rate of change in the $^{15}NO_3^-$ incubations to between −0.0009 and 0.0018 μmol L^{-1} h^{-1}, with standard errors (SE) of 0.0004–0.0016 μmol L^{-1} h^{-1}. In incubations with added ammonium, no detectable change occurred in ammonium concentrations during the first samplings, whereas concentrations decreased during the last part of the incubation (data not shown).

Nitrite and nitrate concentrations also remained stable through the fourth sampling, with concentrations similar to the in situ level in incubations without addition of these species (Fig. 3). In several vials from different stations, depths, and treatments, the nitrite concentration increased abruptly by up to several micromolar before the fifth and final sampling, which corresponded to production rates of up to 0.4 μmol L^{-1} h^{-1}. Often, only one or two of the three vials sampled terminally exhibited such elevated concentrations. The rapid and variable production of nitrite always mirrored a decrease in nitrate (i.e., the combined concentration of nitrite and nitrate did not change substantially), which indicated that nitrite formed through nitrate reduction and that this process occurred much faster than the further reduction of nitrite (data not shown). The rate of change in nitrite concentrations, excluding the final increases, was constrained to between −0.012 and 0.018 μmol L^{-1} h^{-1}, with SE of 0.001–0.015 μmol L^{-1} h^{-1}, whereas changes in nitrate + nitrate were within the range of −0.04 to 0.08 μmol L^{-1} h^{-1}, SE 0.003–0.05 μmol L^{-1} h^{-1}.

Whereas nitrogen transformations were not detectable through changes in bulk concentrations, anaerobic ammonium oxidation to N_2 was detected in $^{15}NH_4^+$ incubations of the upper part of the oxygen-deficient zone in all three experiments (Figs. 4, 5 [all rates listed in Web Appendix 2: http://www.aslo.org/lo/toc/vol_51/issue_5/2145a2.pdf]). Thus, a significant production of $^{14}N^{15}N$ occurred at 55/60, 100, and 150 m at Sta. 1 on both 21 and 24 March and at 55 m at Sta. 2 (slopes significantly greater than 0, $p < 0.05$, in linear regressions of $^{14}N^{15}N$ over time). Conversely, $^{14}N^{15}N$ production was not detected at 30, 250, and 350 m at Sta. 1 or at 150 m at Sta. 2, and no detectable production of $^{15}N^{15}N$ occurred in any of the $^{15}NH_4^+$ incubations. The average standard error for the slope of regressions of $^{14}N^{15}N$ or $^{15}N^{15}N$ versus time corresponded to detection limits of 0.13 nmol L^{-1} h^{-1} and 0.035 nmol L^{-1} h^{-1} for the two species, respectively (i.e., the rate at which the slope would be greater than 0 at the $p < 0.05$ level).

With the nearly complete labeling of the ammonium pool ($F_{ammonium} \geq 0.96$ [Web Appendix 2]), the exclusive production of $^{14}N^{15}N$ indicated anammox as the active process, and the rate of $^{14}N^{15}N$ production essentially corresponded to the anammox rate (Eq. 1) (van de Graaf et al. 1995; Thamdrup and Dalsgaard 2002; Dalsgaard et al. 2003). In all experiments, the highest anammox rate was attained at 55/60 m, where it ranged from 0.2 to 0.7 nmol L^{-1} h^{-1} (Fig. 5). The rates of ammonium oxidation observed with $^{15}NH_4^+$ would not lead to detectable changes in the ammonium concentration, which is consistent with our observations.

The incubations with $^{15}NO_2^-$ further supported the occurrence of anammox, whereas denitrification was generally not detected. Thus, production of $^{14}N^{15}N$ was observed with no lag phase in all six samples from 55 or 60 m incubated with either $^{15}NO_2^-$ or $^{15}NO_2^-$ + $^{14}NH_4^+$ (example in Fig. 4), whereas $^{15}N^{15}N$ was only produced without lag in a single incubation, which was with $^{15}NO_2^-$ + $^{14}NH_4^+$ at Sta. 1, 24 March (see Web Appendix 2). Accumulation of ^{15}N-labeled N_2O was not detected in

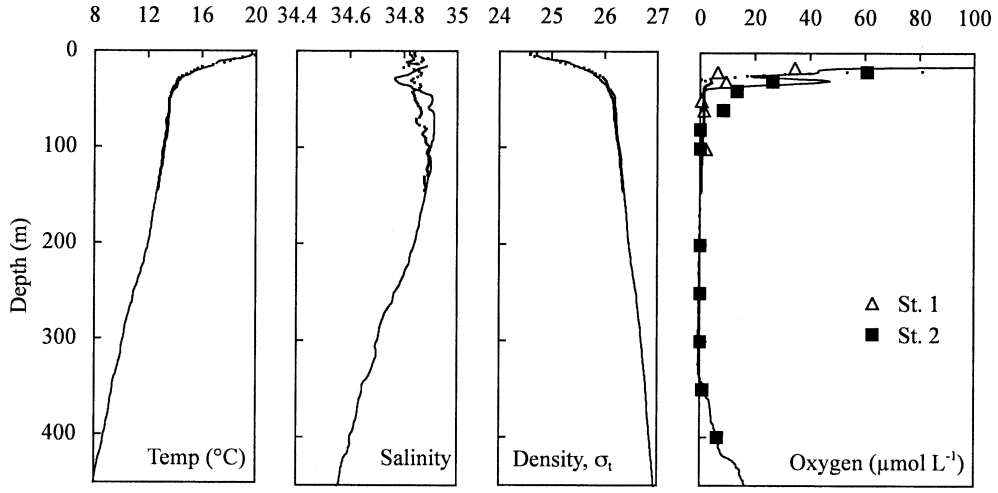

Fig. 1. Temperature, salinity, density (kg m^{-3}), and oxygen concentrations (left to right) measured off Iquique during the cruise. CTD-data from Sta. 1, 22 March, are shown as dots that represent individual data points to 145 m. CTD data from Sta. 2, 24 March, are shown as a continuous thin line to 450 m. Winkler-oxygen data from the same casts are shown as triangles (Sta. 1) and squares (Sta. 2).

any of the incubations. As denitrification is identified through the production of $^{15}N^{15}N$ (or $^{15}N^{15}NO$) from $^{15}NO_2^-$ or $^{15}NO_3^-$ (Eq. 2), this incubation represented the only detection of denitrification from nitrite and yielded a denitrification rate of 0.69 ± 0.15 nmol L^{-1} h^{-1}. Although denitrification was not significant in the parallel incubation with $^{15}NO_2^-$ alone (0.06 ± 0.33 nmol L^{-1} h^{-1}), no significant difference was seen between the rates in these

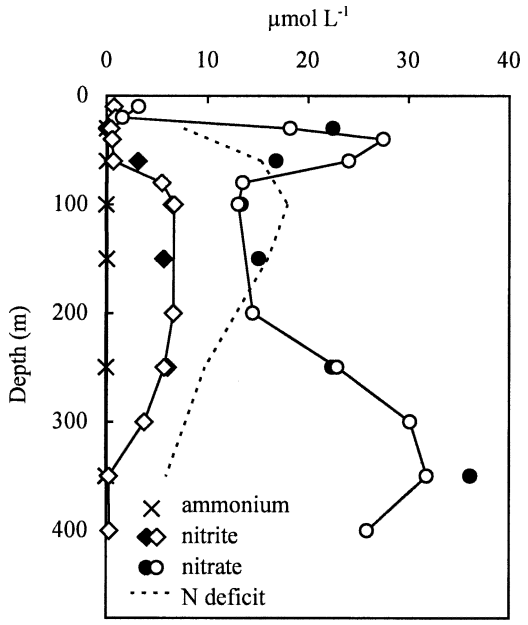

Fig. 2. Ammonium, nitrite, nitrate, and DIN deficiency as N*. Filled symbols: Sta. 1, 21 March. Open symbols: Sta. 2. Ammonium and N* (dashed line) are only shown for Sta. 1.

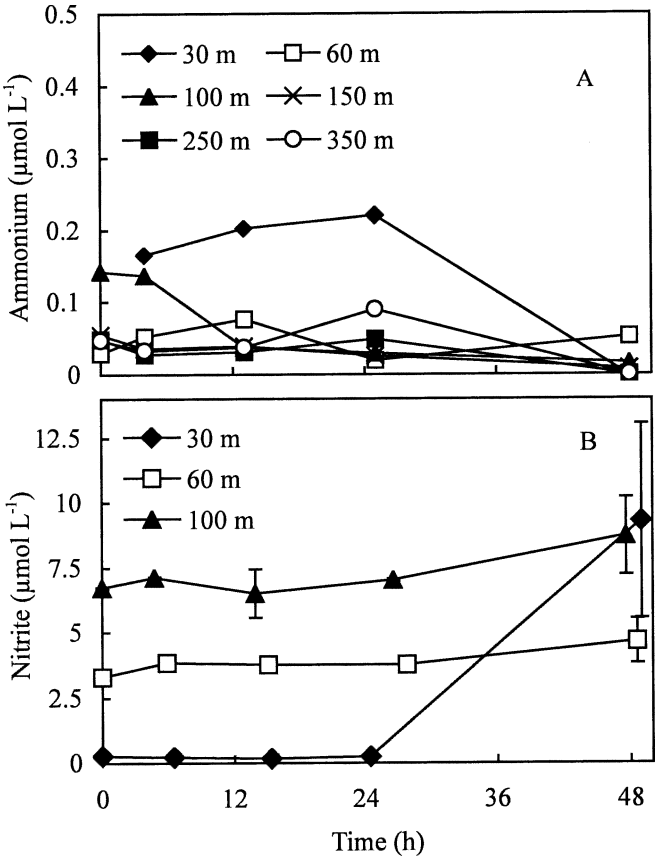

Fig. 3. Examples of concentration changes during incubations from Sta. 1, 21 March. (A) Ammonium concentrations in $^{15}NO_3^-$-amended incubations. (B) Nitrite concentrations in $^{15}NH_4^+$-amended incubations from 30, 60, and 100 m. Ammonium data represent single incubations, whereas nitrite concentrations represent mean ± SD of triplicate incubations.

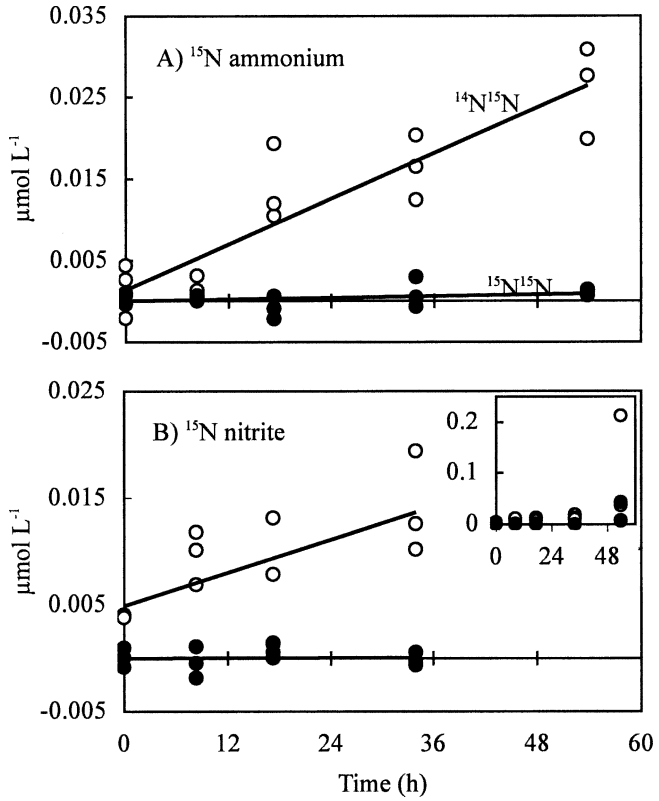

Fig. 4. Examples of the production of ^{15}N-labeled N_2 during incubations. Data from Sta. 2, 55 m. (A) Incubations with $^{15}NH_4^+$. (B) Incubations with $^{15}NO_2^-$. Lines are linear regressions. The high concentrations measured for the last sampling with $^{15}NO_2^-$ were not included in the regression and are seen only on the larger scale in the insert.

two treatments. The detection limit for denitrification at the average standard error was 0.23 nmol L^{-1} h^{-1}.

Anammox rates in the $^{15}NO_2^-$ experiments at 55/60-m depth, obtained according to Eq. 3, ranged from 0.1 to 1.1 nmol L^{-1} h^{-1} and were significantly greater than 0, except for the incubation in which denitrification was active (Fig. 5) ($p < 0.05$). The rates were statistically similar for parallel incubations with and without added $^{14}NH_4^+$ and also similar to the rates in the parallel $^{15}NH_4^+$ incubation. The typical detection limit for anammox in our $^{15}NO_2^-$ incubations was 0.46 nmol L^{-1} h^{-1}. This high value results from error propagation through the combination of $^{14}N^{15}N$ and $^{15}N^{15}N$ results, even when $^{15}N^{15}N$ production is not significant (Eq. 3), and the relatively low ^{15}N labeling of the NO_2^- pool also decreased the sensitivity compared with incubations with $^{15}NH_4^+$. Thus, the anammox rates obtained with $^{15}NH_4^+$ at 100-m and 150-m depths were too low to be detected with $^{15}NO_2^-$.

Between the forth and fifth sampling rounds, a strong acceleration in the production of ^{15}N -labeled N_2 from nitrite took place in some, but not all, of those vials in which an abrupt increase in nitrite was also observed (example in Fig. 4). The accelerated N_2 production included both $^{14}N^{15}N$ and $^{15}N^{15}N$, which indicated active denitrification, and occurred in the $^{15}NO_2^-$ treatments from Sta. 1, 21 March, 30 m and 24 March, 55 m and from Sta. 2, 55 and 150 m, as well as in the $^{15}NO_2^-$ + $^{14}NH_4^+$ treatment from Sta. 1, 60 m. Rates of denitrification during this terminal phase could not be determined precisely, because the concomitant acceleration in the reduction of nitrate to nitrite (Fig. 3) led to large decreases in the mole fraction of $^{15}NO_2^-$ in nitrite, $F_{nitrite}$ (Eq. 2), during the time between the last two samplings. Minimum estimates of denitrification rates, obtained by use of the higher initial $F_{nitrite}$, which should be representative of the incubation until the second to last sampling (before $^{15}NO_2^-$ was further diluted by the reduction of $^{14}NO_3^-$), ranged from 2 to 38 nmol L^{-1} h^{-1} and were at least one order of magnitude higher than the anammox rates during the preceding part of the incubation.

Reduction of $^{15}NO_3^-$ to N_2 was only detected in the two samples from 55 m at Sta. 1 and 2, 24 March (Web Appendix 2). At Sta. 2, only $^{14}N^{15}N$ was produced, at a rate of 0.25 ± 0.08 nmol L^{-1} h^{-1}, whereas at Sta. 1, only $^{15}N^{15}N$ accumulated at 0.08 ± 0.03 nmol L^{-1} h^{-1}. The detection of N_2 production from $^{15}NO_3^-$ implies that any $^{15}NO_2^-$, which formed as an intermediate, was not mixed with the large ambient nitrite pool. Any ^{15}N lost from the cells as nitrite would be diluted so much by the high ambient nitrite concentration that its further reduction to N_2 would not be detectable at the observed rates of nitrite reduction. The production of $^{15}N^{15}N$ at Sta. 1, 55 m, corresponds to a rate of denitrification from nitrate to N_2 of 0.24 ± 0.08 nmol N_2 L^{-1} h^{-1}. This cast was the same cast in which denitrification was detected with $^{15}NO_2^-$ + $^{14}NH_4^+$. The production of only $^{14}N^{15}N$ at Sta. 2 could indicate nitrate utilization by anammox bacteria, although this hypothesis requires much further substantiation. A terminal increase in N_2 production was observed in one $^{15}NO_3^-$ replicate each from Sta. 1 and 2, where nitrite concentrations also increased abruptly (data not shown).

Discussion

Anammox—Our results provide the first direct evidence of anaerobic ammonium oxidation in the oxygen-deficient zone of the eastern tropical South Pacific. The linear production of $^{14}N^{15}N$ without delay from $^{15}NH_4^+$ was consistent with the anammox reaction (Fig. 4) (van de Graaf et al. 1997; Thamdrup and Dalsgaard 2002; Dalsgaard et al. 2003). Any direct oxidation of ammonium to N_2 with a nonnitrogenous oxidant (e.g., Mn oxide [Murray et al. 1995; Luther et al. 1997]) would produce mainly $^{15}N^{15}N$. Another alternative mechanism for N_2 formation, the coupling of a nitrification-like oxidation to nitrite or nitrate with denitrification, would expectedly result in a lag phase for the accumulation of ^{15}N in N_2 because the label would first need to build up in the large, unlabeled nitrate or nitrite pools. In addition, this mechanism would also lead to the production of $^{15}N^{15}N$, which was not seen. Anammox activity was further supported by the incubations with $^{15}NO_2^-$, where the observed dominance of $^{14}N^{15}N$ production over $^{15}N^{15}N$ deviated strongly from the ratio expected from denitrification with random isotope pairing but was consistent with

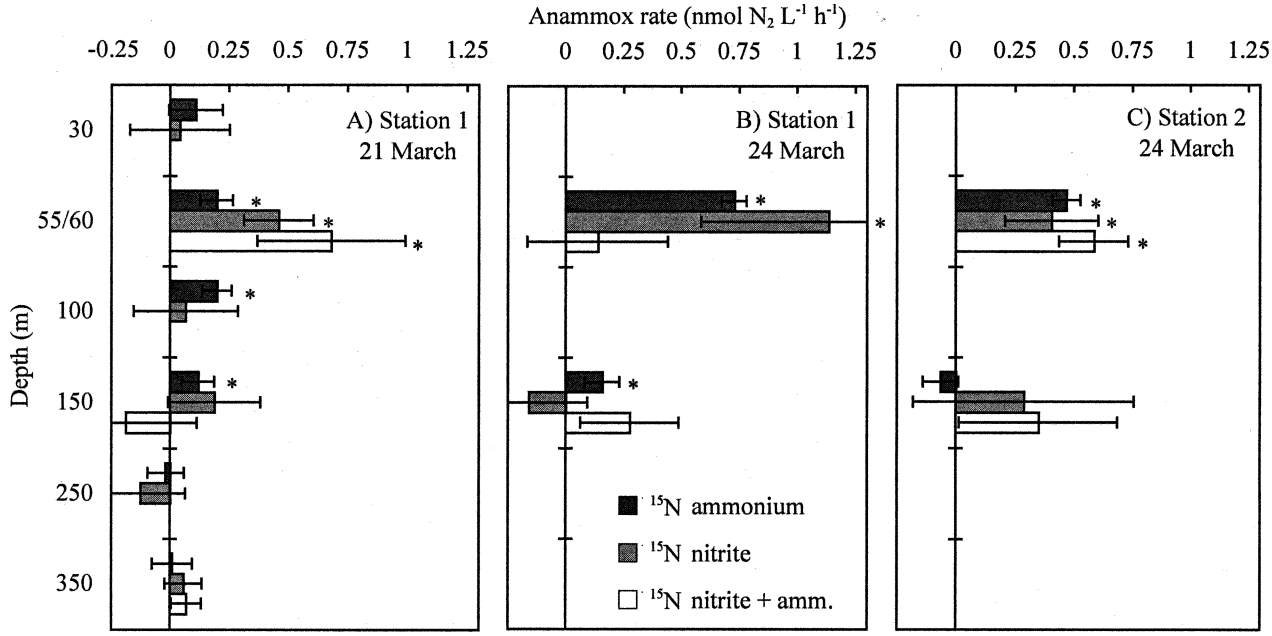

Fig. 5. Rates ± SE of anammox in the water column off Iquique as based on incubations with $^{15}NH_4^+$ (black), $^{15}NO_2^-$ (gray), and $^{15}NO_2^- + {}^{14}NH_4^+$ (white). (A) Sta. 1, 21 March. (B) Sta. 1, 24 March. (C) Sta. 2, 24 March. Note that rates from 60 m at Sta. 1, 21 March, are aligned with those from 55 m at Sta. 1 and 2, 24 March. An asterisk indicates that the rate is significantly greater than 0 ($p < 0.05$).

anammox at rates similar to those determined with $^{15}NH_4^+$ (Figs. 4, 5). Thus, our results document the significance of the anammox process in the Chilean waters. Molecular biological analysis of samples collected off Iquique shows the presence of anammox bacteria, consistent with our results (A. Galán pers. comm.).

The hydrographic conditions during the DINAMO cruise closely resembled those previously reported from the area in the absence of El Niño (e.g., Morales et al. 1996; Pantoja et al. 2004; Castro-González and Farias 2004). Very similar depth distributions of oxygen, nitrite, and the deficit of nitrate + nitrite have also frequently been found farther north off Peru (e.g., Fiadeiro and Strickland 1968; Codispoti and Packard 1980; Lipschultz et al. 1990), although conditions along the Peruvian margin are quite variable in space and time (e.g., Dugdale et al. 1977; Codispoti et al. 1986; Copin-Montégut and Raimbault 1994). Thus, our findings may be representative of a substantial part of the oxygen-deficient water in the eastern tropical South Pacific. Together with the previous demonstrations of anammox in Golfo Dulce, Costa Rica (Dalsgaard et al. 2003), and in the oxygen-minimum zone off Namibia (Kuypers et al. 2005), our results substantiate the importance of anammox in nitrogen cycling within oxygen-deficient oceanic water columns.

Although previous studies have demonstrated anammox through incubations with $^{15}NH_4^+$ and $^{15}NO_3^-$ (Dalsgaard et al. 2003; Kuypers et al. 2003, 2005), this study is the first demonstration of anammox in anoxic waters by means of incubations with $^{15}NO_2^-$. In agreement with the current understanding of the anammox process, we found that $^{15}NO_2^-$ was immediately converted to N_2, and the derived anammox rates were similar to those determined with $^{15}NH_4^+$ (Figs. 4, 5). In contrast, production of ^{15}N-labeled N_2 was generally not observed in the $^{15}NO_3^-$ incubations. These results are consistent with the current understanding of the anammox process as a coproportionation of nitrite and ammonium (van de Graaf et al. 1995, 1997) and with anammox bacteria primarily acquiring nitrite from the extracellular nitrite pool. If instead, the anammox bacteria reduced nitrate to nitrite coupled to the oxidation of organics, as observed in a culture from wastewater (Güven et al. 2005), and primarily shunted this authigenic nitrite to the anammox process, then we should observe the production of $^{14}N^{15}N$ in incubations with $^{15}NO_3^-$. Such a production was only observed in one incubation. Thus, in systems such as the present, with slow turnover of the nitrite pool, $^{15}NO_2^-$ incubations provide a more precise quantification of anammox than do $^{15}NO_3^-$ incubations, as well as important additional information on the pathways of nitrogen cycling.

Earlier studies with sediments have indicated that nitrite saturation of natural anammox communities occurs at or below 3 μmol L^{-1} (Dalsgaard and Thamdrup 2002; Trimmer et al. 2003, 2005), which suggests that anammox off Iquique should be limited by ammonium rather than nitrite. In Golfo Dulce, an increase of ammonium concentrations from natural levels of <0.3 μmol L^{-1} to 10 μmol L^{-1} increased the anammox rate twofold to fourfold (Dalsgaard et al. 2003). Off Iquique, however, no significant difference was seen between rates obtained in $^{15}NO_2^-$ incubations at the extremely low natural ammonium concentrations and with 3 μmol L^{-1} of ammonium added (Fig. 5). The $^{15}NO_2^-$-based rates were also statistically similar to those obtained with $^{15}NH_4^+$, although with $^{15}NO_2^-$, anammox could only be detected at 55/60-m depth because of the higher detection limit in this type of incubation (Fig. 5). In analogy to our results, Kuypers

and coworkers (2005) also reported overall similar rates of anammox with or without the addition of ammonium to $^{15}NO_3^-$ incubations with ammonium-depleted waters off Namibia. The different ammonium sensitivities observed in Golfo Dulce and at the open-ocean sites could be related to the difference in added ammonium concentrations but may also reflect real differences in the kinetics of ammonium utilization of the anammox communities. Highly efficient ammonium consumption by the prokaryotic community in general has previously been demonstrated with samples from the upper boundary of the oxygen-deficient zone off Chile (Molina et al. 2005). Our results call for further studies of the functional response of the natural anammox communities to substrate concentrations.

Anammox activity peaked in the shallowest part of the oxygen-deficient zone, and in the $^{15}NH_4^+$ incubations, which had the highest precision and lowest detection limit, the process was observed at decreasing rates to 150-m depth. This relative depth distribution is consistent with sinking organic matter as the ultimate source of ammonium, and it supports previous findings that the zone around the base of the oxycline is the site of particularly high microbial activity (Codispoti and Packard 1980; Lipschultz et al. 1990; Molina et al. 2005). The variation in anammox rates between casts (from 0.2 to 0.7 nmol L^{-1} h^{-1} with $^{15}NH_4^+$ at 55/60 m) may in part reflect slight displacements of the activity peak relative to the coarse spatial resolution of sampling, although both temporal and horizontal variability of rates most likely also contributed. The two casts at Sta. 1 sampled different water masses because of the relatively strong currents in the Humboldt Current system. Satellite images recorded during the cruise show occasional patches of chlorophyll-rich water drifting in the region of our stations (Web Appendix 1). Sinking of detritus from such patches and from upwelling plumes, such as those located north and south of Iquique, may result in fluctuating microbial activity levels below the oxycline.

At $\leq$0.7 nmol N_2 L^{-1} h^{-1}, anammox rates off Iquique were lower than in Golfo Dulce (1–18 nmol L^{-1} h^{-1}, $^{15}NO_3^-$ incubations [Dalsgaard et al. 2003]), whereas the range just overlapped with those measured off Namibia (0.4–8 nmol L^{-1} h^{-1}, $^{15}NH_4^+$ incubations [Kuypers at al. 2005]; the maximum rate from the present study is taken from $^{15}NH_4^+$ incubations, as these incubations have the lowest standard error). These differences likely reflect variations in microbial activity in general, although no other measures of activity are available to confirm this hypothesis.

Our anammox rates were more comparable to previous incubation-based estimates of denitrification off Peru. There, as inferred from electron transport system activity assays at several locations, denitrification attained highest rates of 0.4–1.9 nmol L^{-1} N_2 h^{-1} in the shallowest part of the oxygen-deficient zone, and rates decreased steeply with depth (Codispoti and Packard 1980). Similar rate maxima (0.4–1.7 nmol L^{-1} N_2 h^{-1}) and depth distributions were estimated from rates of nitrate reduction to nitrite in incubations with $^{15}NO_3^-$, $^{15}NO_2^-$, and $^{15}NH_4^+$ under the assumption that 30% of the nitrite produced was reduced further to N_2 (Lipschultz et al. 1990). These indirectly determined denitrification rates were further supported by loss rates of fixed nitrogen derived by reaction-transport modeling (Codispoti and Packard 1980; Anderson et al. 1982). Although large regional variations in rates are likely, these comparisons support the importance of anammox as a sink for fixed nitrogen in the eastern tropical South Pacific.

Denitrification—Next to the demonstration of anammox, the most striking finding of this study was the general absence of detectable denitrification, except for the sample from Sta. 1, 55 m, 24 March, and the ultimate part of a few other incubations (Figs. 4, 5). At Sta. 1, 55 m, 24 March, the rate of denitrification in the $^{15}NO_2^- + {}^{14}NH_4^+$ incubation was similar to the rate of anammox measured with $^{15}NH_4^+$ (0.69 $\pm$ 0.15 vs. 0.73 $\pm$ 0.05 nmol L^{-1} h^{-1}), which corresponds to equal contributions of the two processes to N_2 production. The contribution of denitrification in all other water samples in which anammox was detected can be constrained more tightly than possible from the detection limit in individual incubations, by averaging all denitrification rates from the incubations of these samples with $^{15}NO_2^-$ and $^{15}NO_2^- + {}^{14}NH_4^+$. This averaging yields a denitrification estimate of 0.016 $\pm$ 0.025 nmol N_2 L^{-1} h^{-1} (mean $\pm$ SE, $n = 9$), with an upper 95% confidence limit of 0.063 nmol L^{-1} h^{-1}. The combined anammox rate for the same samples was 0.23 $\pm$ 0.05 nmol L^{-1} h^{-1}. Thus, the contribution of denitrification is constrained to $<$26% of N_2 production.

When denitrification appeared near the end of incubations, nitrite concentrations also increased abruptly. At rates up to 0.4 μmol L^{-1} h^{-1}, the nitrite production was too high to realistically reflect rates in situ. We, therefore, consider these late changes in both nitrate reduction and N_2 production as a reflection of an incubation artifact or "bottle effect," similar to the abrupt increase in activity generally observed during longer incubations of water samples for quantification of oxygen respiration (e.g., ZoBell and Anderson 1936; Robinson and Williams 2005). Nevertheless, the occasional appearance of denitrification in our experiments indicated the presence of denitrifiers at all stations. The presence of denitrifying microorganisms in the water column off Iquique was previously indicated by observations of both anaerobic production and consumption of N_2O (Castro-González and Farías 2004) and by isolation of cytochrome cd_1 nitrite-reductase genes from the bacterioplankton (Castro-González et al. 2005).

Denitrification is thought to be the most important mechanism for nitrogen removal and carbon oxidation in oxygen-deficient zones. In Golfo Dulce, denitrification accounted for 42–68% of N_2 production at nonsulfidic depths (Dalsgaard et al. 2003). These values are somewhat lower than the 71% expected during a complete mineralization of Redfield-type organic matter through denitrification in combination with oxidation of all the released ammonium by anammox. This modest deviation could be caused by a preferential degradation of nitrogen-rich organic moieties in the anoxic waters (van Mooy et al. 2002; Dalsgaard et al. 2003). However, the low denitrifi-

cation rates off Iquique cannot be explained by this mechanism alone, because such an explanation would require an unrealistically low C : N ratio for mineralization, and sediment-trap studies off Iquique have not revealed any marked deviation from Redfield C : N ratios of mineralization for organics sinking through the oxygen-deficient water column (Pantoja et al. 2004).

Our results are most likely explained by an uncoupling of anammox and denitrification that results from an uncoupling of the different steps of the denitrification pathway. Thus, abundant evidence from the oxygen-deficient waters of the tropical Pacific indicates that the reduction of nitrate to nitrite often proceeds at a much higher rate than the subsequent reduction of nitrite to N_2, which results in nitrite accumulation. In a batch incubation of water from the eastern tropical North Pacific, nitrite reduction started only after 3 days of incubation, when nitrate reduction had produced 20 μmol L^{-1} nitrite, and the nitrate concentration had decreased to $\sim$8 μmol L^{-1} (Goering and Cline 1970). Similarly, Richards and Broenkow (1971), who compared in situ concentrations in the water column of Darwin Bay, Galapagos, at two visits separated by 2 months, observed the reduction of 8 μmol L^{-1} nitrate to nitrite, whereas only 4 μmol L^{-1} nitrate + nitrite had been lost to N_2. Ammonium concentrations increased very little, and the occurrence of anaerobic ammonium oxidation was suggested. Also, with water from locations off Peru, nitrate reduction was active in batch incubations, whereas nitrite reduction to N_2O was not detected by the acetylene-inhibition technique (Lipschultz et al. 1990).

Although the rates were too low to detect changes in nitrate or nitrite concentrations during the first part of our incubations, we observed a strong dominance of nitrate reduction over nitrite reduction toward the end, with sharp increases in nitrite concentrations in most incubations and incipient denitrification in only a small subset of these incubations. Consistent with these results and the earlier findings, our observations during the first part of the incubations could represent a situation in which carbon mineralization and removal of fixed nitrogen occur through the combination of nitrate reduction and anammox, as indicated by the following reactions:

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 212NO_3^- \rightarrow 106CO_2 + 212NO_2^- + 16NH_3 + H_3PO_4 + 106H_2O \quad \text{(Reaction 1)}$$

$$16NH_4^+ + 16NO_2^- \rightarrow 16N_2 + 32H_2O \quad \text{(Reaction 2)}$$

Sum:

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 212NO_3^- + 16H^+ \rightarrow 106CO_2 + 196NO_2^- + 16N_2 + H_3PO_4 + 138H_2O \quad \text{(Reaction 3)}$$

With a production ratio of nitrite to N_2 of 12.25 : 1 in the combined Reaction 3, nitrite production rates, corresponding to the maximum anammox rates of 0.2–0.7 nmol L^{-1} h^{-1} (Fig. 5), should be 0.003–0.009 μmol L^{-1} h^{-1}, which would generally be too low to be detected as changes in nitrite concentrations during our incubations.

Our results are similar to those reported most recently from oxygen-deficient waters over the Namibian Shelf (Kuypers et al. 2005). There, in similar incubations, denitrification was also not detected while anammox rates ranged from 0.4 to 7.5 nmol L^{-1} h^{-1}. These results were hypothesized to reflect a slower recovery of denitrification than of anammox after exposure to oxygen, as may occur through frequent intrusions of oxygenated waters. In situ oxygen concentrations at depths with anammox activity ranged from 0.3 to 9 μmol L^{-1}, and high-resolution hydrographic profiles indicated highly dynamic oxygen conditions. Fluctuating oxygen concentrations are less likely to explain the results from the Chilean margin. Thus, the samples with anammox activity were all retrieved at depths where oxygen was not detectable. Furthermore, a comparison of density gradients indicates that vertical mixing was weaker in the Chilean waters than off Namibia. The density gradient at depths with anammox off Iquique exceeded those off Namibia (Fig. 1) ($d\sigma_t/dz$ of 0.002–0.006 kg m^{-4} at 50–100 m in this study compared with $\leq$0.001 kg m^{-4} off Namibia, as calculated from density data for depths with $<$10 μmol L^{-1} O_2 [Kuypers et al. 2005]). In general, the coastal transition zone off northern Chile is characterized by low-eddy kinetic energy that results in a stable stratification of the water column, with very little vertical mixing (Hormazabal et al. 2004). Regardless of which mechanisms lie behind the apparent absence of denitrification in the two areas, the results together emphasize the need for a better understanding of the regulation of the denitrification pathway as a whole.

The combination of Reactions 1 and 2 may explain our results and represent the metabolic state of the water column off Iquique at the time of sampling. However, it cannot represent a persistent situation for a wider part of the ETSP, because the combined Reaction 3 generates much less N_2 and, thereby, a much lower DIN deficit relative to the amount of nitrite accumulated than observed in the core of the oxygen-deficient zone, where the nitrogen deficiency is $\sim$3 times greater than the nitrite concentration (Fig. 2). The discrepancy is further illustrated by modeling of the depth distributions of nitrite and DIN deficits in Peruvian waters as influenced by vertical mixing (Anderson et al. 1982). This model showed that the ratio of nitrite reduction to nitrate reduction is generally $>$0.3 under conditions similar to those observed off Iquique, rather than 0.08, as in Reactions 1 and 2. The model approach of Anderson and coworkers (1982) may even overestimate the discrepancy between nitrate and nitrite reduction because horizontal advection is often the dominant transport mechanism for the nitrogen species, rather than the vertical mixing assumed in the model, and the horizontal export of nitrite from the ETSP is insignificant relative to the exported DIN deficit (Codispoti and Christensen 1985). Denitrification to N_2, thus, remains the simplest explanation for most of the DIN missing in oxygen-deficient zones. (See Devol et al. [2006] for a discussion of this issue for the Arabian Sea.)

The general lack of denitrification in our incubations may be related to a heterogeneous distribution of the process. Water-column chemistry integrates mineralization processes over longer timescales and in larger water bodies than our 1-day to 2-day incubations of <1 liter of water per station and depth. Thus, high rates of denitrification could be associated with pulses of sedimentation of fresh detritus or with organic-rich aggregates that were generally not captured by our sampling. The detection of denitrification in a single sample from 55 m at Sta. 1, 24 March, both in incubations with $^{15}NO_2^- + {}^{14}NH_4^+$ and in incubations with $^{15}NO_3^-$, and coinciding with the highest anammox rates found in this study (Fig. 5), would be consistent with such a patchy distribution of denitrification. The very high rates of anoxic N_2O cycling observed off Iquique in March 2003 (Castro-González and Farías 2004) could possibly represent a more extreme example of such a situation.

In conclusion, our results provide good evidence for the activity of the anammox process in the eastern tropical South Pacific, at rates that suggest that anammox is an important sink for fixed nitrogen there. This finding adds an important new location to the database with previous demonstrations of significant anammox in other oxygen-deficient waters (Dalsgaard et al. 2003; Kuypers et al. 2005), in anoxic basins (Kuypers et al. 2003), and in marine sediments (Thamdrup and Dalsgaard 2002; Dalsgaard et al. 2005), which emphasizes the importance of the process in the nitrogen cycle.

The absence of denitrification in our incubations is interpreted as a transient phenomenon and is analogous to findings in the Benguela upwelling area (Kuypers et al. 2005), although the reasons for this absence may not be the same in the two areas. These findings together emphasize that a better understanding of the removal of fixed nitrogen from the oceans requires detailed investigations of the regulation of both anammox and denitrification under environmentally relevant conditions.

References

Anderson, J. J., A. Okubo, A. S. Robbins, and F. A. Richards. 1982. A model for nitrite and nitrate distributions in oceanic oxygen minimum zones. Deep-Sea Res. Part I Oceanogr. Res. Pap. **29:** 1113–1140.

Bange, H. W., S. W. A. Naqvi, and L. A. Codispoti. 2005. The nitrogen cycle in the Arabian Sea. Progr. Oceanogr. **65:** 145–158.

Bower, C. E., and T. Holm-Hansen. 1980. A salicylate-hypochlorite method for determining ammonia in seawater. Can. J. Fish. Aquat. Sci. **37:** 794–798.

Braman, R. S., and S. A. Hendrix. 1989. Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium(III) reduction with chemiluminescence detection. Anal. Chem. **61:** 2715–2718.

Brandes, J. A., and A. H. Devol. 2002. A global marine-fixed nitrogen isotopic budget: Implications for Holocene nitrogen cycling. Glob. Biogeochem. Cycles **16**, 1120, doi: 10.1029/2001GB001856.

Castro-González, M., G. Braker, L. Farías, and O. Ulloa. 2005. Communities of *nir*S-type denitrifiers in the water column of the oxygen minimum zone in the eastern South Pacific. Environ. Microbiol. **7:** 1298–1306.

———, and L. Farías. 2004. N_2O cycling at the core of the oxygen minimum zone off northern Chile. Mar. Ecol. Progr. Ser. **280:** 1–11.

Chapman, P., and L. V. Shannon. 1985. The Benguela ecosystem. 2: Chemistry and related processes. Oceanogr. Mar. Biol. **23:** 183–251.

Cline, J. D., and F. A. Richards. 1972. Oxygen deficient conditions and nitrate reduction in eastern tropical North-Pacific Ocean. Limnol. Oceanogr. **17:** 885–900.

Codispoti, L. A., J. A. Brandes, J. P. Christensen, A. H. Devol, S. W. A. Naqvi, H. W. Paerl, and T. Yoshinary. 2001. The oceanic fixed nitrogen and nitrous oxide budgets: Moving targets as we enter the anthropocene? Sci. Mar. **65:** 85–105.

———, and J. P. Christensen. 1985. Nitrification, denitrification and nitrous-oxide cycling in the eastern tropical South-Pacific ocean. Mar. Chem. **16:** 277–300.

———, G. E. Friederich, T. T. Packard, H. E. Glover, P. J. Kelly, R. W. Spinrad, R. T. Barber, J. W. Elkins, and others. 1986. High nitrite levels off northern Peru—a signal of instability in the marine denitrification rate. Science **233:** 1200–1202.

———, and T. T. Packard. 1980. Denitrification rates in the eastern tropical South-Pacific. J. Mar. Res. **38:** 453–477.

———, and F. A. Richards. 1976. Analysis of horizontal regime of denitrification in eastern tropical North Pacific. Limnol. Oceanogr. **21:** 379–388.

Copin-Montegut, C., and P. Raimbault. 1994. The Peruvian upwelling near 15°S in August 1986—results of continuous measurements of physical and chemical properties between 0 and 200 m depth. Deep-Sea Res. Part I Oceanogr. Res. Pap. **41:** 439–467.

Dalsgaard, T., D. E. Canfield, J. Petersen, B. Thamdrup, and J. Acuña-Gonzalez. 2003. N_2 production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. Nature **422:** 606–608.

———, and B. Thamdrup. 2002. Factors controlling anaerobic ammonium oxidation with nitrite in marine sediments. Appl. Environ. Microbiol. **68:** 3802–3808.

———, ———, and D. E. Canfield. 2005. Anaerobic ammonium oxidation (anammox) in the marine environment. Res. Microbiol. **156:** 457–464.

Deuser, W. G., E. H. Ross, and Z. J. Mlodzinska. 1978. Evidence for and rate of denitrification in Arabian Sea. Deep-Sea Res. **25:** 431–445.

Devol, A. H., S. W. A. Naqvi, and L. A. Codispoti. 2006. Nitrogen cycling in the suboxic waters of the Arabian Sea,, p. 283–310. *In* L. N. Neretin [ed.], Past and present water column anoxia. NATO science series, IV earth and environmental sciences. v. 64, Springer.

Dugdale, R. C., J. J. Goering, R. T. Barber, R. L. Smith, and T. T. Packard. 1977. Denitrification and hydrogen-sulfide in Peru upwelling region during 1976. Deep-Sea Res. **24:** 601–608.

Engström, P., T. Dalsgaard, S. Hulth, and R. C. Aller. 2005. Anaerobic ammonium oxidation by nitrite (anammox): Implications for N_2 production in coastal marine sediments. Geochim. Cosmochim. Acta **69:** 2057–2065.

Falkowski, P. G., R. T. Barber, and V. Smetacek. 1998. Biogeochemical controls and feedbacks on ocean primary production. Science **281:** 200–206.

FIADEIRO, M., AND J. D. H. STRICKLAND. 1968. Nitrate reduction and occurrence of a deep nitrite maximum in ocean off west coast of South America. J. Mar. Res. **26:** 187–201.

GOERING, J. J., AND J. D. CLINE. 1970. A note on denitrification in seawater. Limnol. Oceanogr. **15:** 306–309.

GRASSHOFF, K. 1983. Determination of nitrite, p. 139–42. *In* K. Grasshoff, M. Ehrhardt, and K. Kremling [eds.], Methods of seawater analysis. Verlag Chemie.

GRUBER, N., AND J. L. SARMIENTO. 1997. Global patterns of marine nitrogen fixation and denitrification. Glob. Biogeochem. Cycles **11:** 235–266.

GÜVEN, D., A. DAPENA, B. KARTAL, M. C. SCHMID, B. MAAS, K. VAN DE PAS-SCHOONEN, S. SOZEN, R. MENDEZ, AND OTHERS. 2005. Propionate oxidation by and methanol inhibition of anaerobic ammonium-oxidizing bacteria. Appl. Environ. Microbiol. **71:** 1066–1071.

HOLMES, R. M., A. AMINOT, R. KEROUEL, B. A. HOOKER, AND B. J. PETERSON. 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Can. J. Fish. Aquat. Sci. **56:** 1801–1808.

HORMAZABAL, S., G. SHAFFER, AND O. LETH. 2004. Coastal transition zone off Chile. J. Geophys. Res. Oceans **109**, C01021, doi:10.1029/2003JC001956.

HULTH, S., R. C. ALLER, D. E. CANFIELD, T. DALSGAARD, P. ENGSTRÖM, F. GILBERT, K. SUNDBÄCK, AND B. THAMDRUP. 2005. Nitrogen removal in marine environments: Recent findings and future research challenges. Mar. Chem. **94:** 125–145.

JETTEN, M. S. M., I. CIRPUS, B. KARTAL, L. VAN NIFTRIK, K. T. VAN DE PAS-SCHOONEN, O. SLIEKERS, S. HAAIJER, W. VAN DER STAR, AND OTHERS. 2005. 1994–2004: 10 years of research on the anaerobic oxidation of ammonium. Biochem. Soc. Trans. **33:** 119–123.

KUYPERS, M. M. M., G. LAVIK, D. WOEBKEN, M. SCHMID, B. M. FUCHS, R. AMANN, B. B. JØRGENSEN, AND M. S. M. JETTEN. 2005. Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation. Proc. Natl. Acad. Sci. U. S. A. **102:** 6478–6483.

———, ———, AND B. THAMDRUP. 2006. Anaerobic ammonium oxidation in the marine environment,, p. 311–335. *In* L. N. Neretin [ed.], Past and present water column anoxia. NATO science series, IV earth and environmental sciences. v. 64, Springer.

———, A. O. SLIEKERS, G. LAVIK, M. SCHMID, B. B. JORGENSEN, J. G. KUENEN, J. S. S. DAMSTE, M. STROUS, AND OTHERS. 2003. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. Nature **422:** 608–611.

LIPSCHULTZ, F., S. C. WOFSY, B. B. WARD, L. A. CODISPOTI, G. FRIEDRICH, AND J. W. ELKINS. 1990. Bacterial transformations of inorganic nitrogen in the oxygen-deficient waters of the eastern tropical South-Pacific ocean. Deep-Sea Res. Part I Oceanogr. Res. Pap. **37:** 1513–1541.

LUTHER, G. W, III, B. SUNDBY, B. L. LEWIS, AND P. J. BRENDEL. 1997. Interactions of manganese with the nitrogen cycle: Alternative pathways to dinitrogen. Geochim. Cosmochim. Acta **61:** 4043–4052.

MOLINA, V., L. FARIAS, Y. EISSLER, L. A. CUEVAS, C. E. MORALES, AND R. ESCRIBANO. 2005. Ammonium cycling under a strong oxygen gradient associated with the oxygen minimum zone off northern Chile (~23° S). Mar. Ecol. Progr. Ser. **288:** 35–43.

MORALES, C. E., J. L. BLANCO, M. BRAUN, H. REYES, AND N. SILVA. 1996. Chlorophyll-a distribution and associated oceanographic conditions in the upwelling region off northern Chile during the winter and spring 1993. Deep-Sea Res. Part I Oceanogr. Res. Pap. **43:** 267–289.

———, S. E. HORMAZABAL, AND J. L. BLANCO. 1999. Interannual variability in the mesoscale distribution of the depth of the upper boundary of the oxygen minimum layer off northern Chile (18–24°S): Implications for the pelagic system and biogeochemical cycling. J. Mar. Res. **57:** 909–932.

MURRAY, J. W., L. A. CODISPOTI, AND G. E. FRIEDERICH. 1995. Oxidation-reduction environments—the suboxic zone in the Black Sea, p. 157–176. *In* C. P. Huang, C. R. O'Melia, and J. J. Morgan [eds.], Aquatic chemistry: interfacial and interspecies processes. Advances in chemistry series. American Chemical Society.

NAQVI, S. W. A. 1994. Denitrification processes in the Arabian Sea. Proc. Indian Acad. Sci. Earth Planet. Sci. **103:** 279–300.

PANTOJA, S., J. S. SEPULVEDA, AND H. E. GONZALEZ. 2004. Decomposition of sinking proteinaceous material during fall in the oxygen minimum zone off northern Chile. Deep-Sea Res. Part I Oceanogr. Res. Pap. **51:** 55–70.

RICHARDS, F. A., J. J. ANDERSON, AND J. D. CLINE. 1971. Chemical and physical observations in Golfo Dulce, an anoxic basin on the Pacific coast of Costa Rica. Limnol. Oceanogr. **16:** 43–50.

———, AND W. W. BROENKOW. 1971. Chemical changes, including nitrate reduction, in Darwin Bay, Galapagos archipelago, over a 2-month period, 1969. Limnol. Oceanogr. **16:** 758–765.

RISGAARD-PETERSEN, N., AND S. RYSGAARD. 1995. Nitrate reduction in sediments and waterlogged soil measured by ^{15}N techniques, p. 287–310. *In* K. Alef and P. Nannipieri [eds.], Anaerobic microbial activities in soil. Academic.

ROBINSON, C, AND P. J. LE B. WILLIAMS. 2005. Respiration and its measurement in surface marine waters, p. 147–180. *In* P. del Giorgio [ed.], Respiration in aquatic ecosystems. Oxford Univ. Press.

SCHMID, M. C., B. MAAS, A. DAPENA, K. VAN DE PAS-SCHOONEN, J. VAN DE VOSSENBERG, B. KARTAL, L. VAN NIFTRIK, I. SCHMIDT, AND OTHERS. 2005. Biomarkers for in situ detection of anaerobic ammonium-oxidizing (anammox) bacteria. Appl. Environ. Microbiol. **71:** 1677–1684.

STROUS, M., J. A. FUERST, E. H. M. KRAMER, S. LOGEMANN, G. MUYZER, K. T. VAN DE PAS-SCOONEN, R. WEBB, J. G. KUENEN, AND OTHERS. 1999. Missing lithotroph identified as new planctomycete. Nature **400:** 446–449.

THAMDRUP, B., D. E. CANFIELD, T. G. FERDELMAN, R. N. GLUD, AND J. K. GUNDERSEN. 1996. A biogeochemical survey of the anoxic basin Golfo Dulce, Costa Rica. Rev. Biol. Tropical **44:** 19–33.

———, AND T. DALSGAARD. 2000. The fate of ammonium in anoxic manganese oxide-rich marine sediment. Geochim. Cosmochim. Acta **64:** 4157–4164.

———, AND ———. 2002. Production of N_2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. Appl. Environ. Microbiol. **68:** 1312–1318.

TRIMMER, M., J. C. NICHOLLS, AND B. DEFLANDRE. 2003. Anaerobic ammonium oxidation measured in sediments along the Thames Estuary, United Kingdom. Appl. Environ. Microbiol. **69:** 6447–6454.

———, ———, N. MORLEY, C. A. DAVIES, AND J. ALDRIDGE. 2005. Biphasic behavior of anammox regulated by nitrite and nitrate in an estuarine sediment. Appl. Environ. Microbiol. **71:** 1923–1930.

TYRRELL, T., AND M. I. LUCAS. 2002. Geochemical evidence of denitrification in the Benguela upwelling system. Cont. Shelf Res. **22:** 2497–2511.

VAN DE GRAAF, A. A., P. DE BRUIJN, L. A. ROBERTSON, M. S. M. JETTEN, AND J. G. KUENEN. 1997. Metabolic pathway of anaerobic ammonium oxidation on the basis of ^{15}N studies in a fluidized bed reactor. Microbiol. **143:** 2415–2421.

———, A. Mulder, P. De Bruijn, M. S. M. Jetten, L. A. Robertson, and J. G. Kuenen. 1995. Anaerobic oxidation of ammonium is a biologically mediated process. Appl. Environ. Microbiol. **61:** 1246–1251.

van Mooy, B. A. S., R. G. Keil, and A. H. Devol. 2002. Impact of suboxia on sinking particulate organic carbon: Enhanced carbon flux and preferential degradation of amino acids via denitrification. Geochim. Cosmochim. Acta **66:** 457–465.

ZoBell, C. E., and Q. Anderson. 1936. Observations on the multiplication of bacteria in different volumes of stored sea water and the influence of oxygen tension and solid surfaces. Biol. Bull. **71:** 324–342.

Zumft, W. G. 1997. Cell biology and molecular basis of denitrification. Microbiol. Mol. Biol. Rev. **61:** 533–616.

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