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Anammox and denitrification in the oxygen minimum zone of the eastern South Pacific

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Abstract

We quantified the removal of fixed nitrogen as N_2 production by anammox and N_2 and N_2O production by denitrification over a distance of 1900 km along the coasts of Chile and Peru, using short-term incubations with ^{15}N-labeled substrates. The eastern South Pacific contains an oxygen minimum zone (OMZ) characterized by an anoxic, nitrate- and nitrite-rich layer of ~ 200-m thickness below 30–90 m of oxic water. Anammox and denitrification were almost exclusively recorded when the in situ O_2 concentration was below detection, indicating that the induction of these processes is highly oxygen sensitive. Anammox was detected in 70% of the samples from anoxic depths. Denitrification was detected in fewer samples, but maximum rates were an order of magnitude higher than those of anammox. In our incubations denitrification was responsible for 72% of the total N_2 production and 77% of the total removal of fixed nitrogen including N_2O production. However, at the individual depths it could be one or the other process that was responsible for all of the nitrogen removal. Anammox activity was highest just below the oxic–anoxic interface and declined exponentially with depth, whereas no depth dependence was discerned for denitrification. Denitrification resulted in net production of N_2O in some of the samples and consumption of added $^{15}N_2O$ in others. Together with the accumulation of NO_2^- this indicates that denitrification must be seen as a sequence of individually regulated reactions, each of which may start and stop depending on the electron donor input, while anammox is much less variable. The highly patchy distribution of denitrification contributes to explain the apparent imbalances between ammonium sources and sinks suggested by previous ^{15}N-based studies in OMZs.

The oxygen minimum zones (OMZs) in the oceans are major players in the marine nitrogen cycle, which account for 20–40% of the oceanic reactive nitrogen loss (Gruber and Sarmiento 1997; Codispoti et al. 2001). This primarily takes place in parts of the eastern tropical North and South Pacific (ETSP) and the Arabian Sea, in water masses that are so low in O_2 that nitrate reduction occurs. Such conditions are only found in about 0.1% of the ocean volume (Codispoti 2007). Nitrate reduction leads to characteristic high concentrations of NO_2^-, which in turn is further reduced to gaseous N_2O and N_2 in anaerobic metabolisms such as denitrification or anaerobic ammonium oxidation (anammox) (Codispoti and Christensen 1985; Thamdrup et al. 2006). In the eastern South Pacific (ESP) the oxygen-depleted, nitrite-rich zone is found along the coasts of Chile and Peru as an up to 300-m-thick layer starting 50–100 m below the surface (Wooster et al. 1965; Silva et al. 2009; Thamdrup et al. 2012).

Removal of fixed nitrogen has been documented extensively for OMZs using a variety of approaches. Many studies have calculated the nitrogen deficit assuming that deviations of the ratio of dissolved inorganic nitrogen to PO_4^{3-} from the Redfield ratio (Redfield et al. 1963) is due to loss of fixed nitrogen (Gruber and Sarmiento 1997; Deutsch et al. 2001; Codispoti et al. 2005). Nitrogen removal in the ETSP has also been estimated from electron transport system activity (Codispoti and Packard 1980) and by assuming that denitrification was 30% of the measured NO_3^- reduction (Lipschultz et al. 1990). Recently these approaches have been supplemented by direct rate measurements of nitrogen removal from the water column off Chile (Thamdrup et al. 2006; Galan et al. 2009) and Peru (Hamersley et al. 2007; Lam et al. 2009) using ^{15}N-tracer techniques that allow discrimination between denitrification and anammox as sinks for fixed nitrogen. Denitrification is the reduction of NO_3^- via NO_2^-, NO, and N_2O to N_2 and was generally believed to be responsible for the nitrogen deficit until anammox was discovered, first in wastewater treatment plants (Mulder et al. 1995), and later in marine sediments (Thamdrup and Dalsgaard 2002; Dalsgaard et al. 2005) and anoxic water columns (Dalsgaard et al. 2003; Kuypers et al. 2003). The anammox process also removes fixed nitrogen but via a different biochemical pathway in which bacteria catalyze N_2 formation from NO_2^- and NH_4^+.

The recent applications of the ^{15}N tracer technique (references above) have identified anammox as the only N_2 producing process in the ETSP in all but two incubations: one using Exetainers (Thamdrup et al. 2006) and one using a large-volume bag (Ward et al. 2009). In contrast to this, Ward et al. (2009) and Bulow et al. (2010) found canonical denitrification to be the dominant N_2 producing process in the Arabian Sea OMZ, applying the same techniques. While these measurements were typically made within 24 h, denitrification has frequently been observed in longer incubations, after a lag phase of 24–36 h, which indicates that denitrifiers are present but not active (Goering and Cline 1970; Kuypers et al. 2005; Thamdrup et al. 2006).

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Mineralization of organic matter is an important source of NH_4^+ for anammox; the process that is suggested to be responsible for the general lack of NH_4^+ accumulation in the OMZs (Richards 1965; Dalsgaard et al. 2003). In fact, NH_4^+ concentrations are generally lower inside the OMZ core than in the overlying oxycline (Thamdrup et al. 2006; this study; Fernandez et al. 2009). Theoretically, if C and N are mineralized at a 106 : 16 ratio with mineralization coupled to denitrification, and anammox consumes all the NH_4^+ from mineralization, anammox and denitrification will be responsible for 29% and 71% of the N_2 production, respectively. The first direct measurements of the two processes under OMZ conditions in Golfo Dulce, a coastal bay connected to the eastern North Pacific OMZ, were overall consistent with such a stoichiometric coupling of the two processes (Dalsgaard et al. 2003). Thus, the apparent lack of denitrification in the ETSP is surprising and raises the question as to how NH_4^+ for anammox is produced. Several explanations have been offered. Dissimilatory nitrate reduction to nitrite contributes to ammonium production through mineralization (Thamdrup et al. 2006; Lam et al. 2009; Jensen et al. 2011), but the stoichiometry of nitrite accumulation and fixed nitrogen depletion in OMZs excludes this process as the main ammonium source (Thamdrup et al. 2006). Dissimilatory nitrate reduction to ammonium (DNRA) has been demonstrated to be active and suggested to be important both as a mineralization process and as a source of NH_4^+ for anammox in OMZs off Peru (Lam et al. 2009), Namibia (Kartal et al. 2007), and Oman (Jensen et al. 2011). Lately also sulfate reduction has been shown to contribute to mineralization in the OMZ off Chile, but it produced less than a quarter of the NH_4^+ needed by anammox (Canfield et al. 2010). Finally, it has been suggested that denitrification is actually active, as found in Golfo Dulce, but that anammox and denitrification are separated in space and time, with denitrification occurring mainly at higher rates arising from episodic inputs of organic matter and that sampling a few stations and depths may easily miss these hot spots (Thamdrup et al. 2006; Ward et al. 2008, 2009).

Earlier studies of denitrification and anammox in the ETSP OMZ have each focused on relatively few stations in a limited part of the OMZ: two stations off Chile at 20°S (Thamdrup et al. 2006), five stations off Peru at 9–12°S (Hamersley et al. 2007), one station off Chile at 20°S (Galan et al. 2009), and two stations off Peru at 12°S and 15°S (Ward et al. 2009). Here we report results of the investigation of the processes responsible for removal of fixed nitrogen in the ETSP and into subtropical waters along the coasts of Chile and Peru between 26°S and 12°S. The measurements were made as frequently as possible in oxygen-deficient waters on the 1900-km-long cruise track parallel to the coast outside the continental shelf. The aim of the study was to explore the regional variation in rates of denitrification and anammox in order to improve our understanding of how these processes are regulated in situ and assess their importance as oceanic sinks for fixed nitrogen. The coverage of our survey should also increase the chances of encountering hot spots of activity, where "missing" denitrification might be detected.

Methods

Study area—Sampling took place from 12 to 26 February 2007 at 11 stations along the coasts of Chile and Peru from 26.3°S to 12.5°S at a distance of 24 to 117 km from the shore (Table 1; Fig. 1). A detailed survey of the oxygen conditions during the cruise is published elsewhere (Thamdrup et al. 2012): oxygen was depleted to the detection limit (0.09 μmol L^{-1} at most stations and 0.01 μmol L^{-1} at some, depending on oxygen sensors) in a zone of $\geq$ 150-m thickness beginning at depths of 50–150 m along the cruise track from Sta. G02 at 26.3°S to 10°S, with an interruption at Sta. G03, 23.6°S, where oxygen levels fluctuated from $<$ 0.09 to 3.2 μmol L^{-1}. At G01, 29.3°S, oxygen did not reach below 25 μmol L^{-1} and ^{15}N incubations were not performed there. The oxygen-depleted layer was found between densities (σ_θ) of 26.15 and 26.7–26.8 kg m^{-3} mainly associated with the equatorial subsurface water mass as typical for the region (Silva et al. 2009). We refer to this layer as the OMZ core. In the northern part of the transect between 12°S and 14°S, thinner, completely oxygen-depleted water layers were located at shallower depths at σ_θ values of 25.93–25.94 kg m^{-3}, separated from the main OMZ core by a layer with up to 1.6 μmol O_2 L^{-1}.

Sampling from the OMZ—Water was sampled from the OMZ using a Seabird conductivity–temperature–depth (CTD) rosette system with 30-liter Niskin bottles (OceanTest Equipment). At each station, three depths were chosen for sampling for incubations based on the oxygen distribution recorded during the downcast by two SBE43 oxygen sensors. The depths were aimed at representing the uppermost depth of oxygen depletion, the approximate depth of the nitrite maximum within the OMZ core, and an intermediate depth between these two. A detailed post-cruise evaluation of oxygen distributions based on data from highly sensitive switchable trace amount oxygen (STOX) sensors (Revsbech et al. 2009, 2011; Thamdrup et al. 2012) revealed that some of the samples were taken at depths with some oxygen present. At Sta. G15 incubations were performed from six depths to better resolve the depth dependence of the processes. At each station a more detailed concentration profile (8–12 sampling depths) of NO_3^-, NO_2^-, and NH_4^+ was determined, and profiles of temperature, salinity, and chlorophyll fluorescence were recorded from the CTD.

^{15}N incubations—Water for incubation experiments was taken from the Niskin bottles as soon as the rosette was on deck and always before any other sampling from the same bottles. The water was allowed to flow through a piece of Tygon tubing to the bottom of a 250-mL glass bottle and allowed to overflow for three volume changes. The bottles were then closed without bubbles or headspace with butyl rubber stoppers and immediately brought to a laboratory at constant temperature within 2°C of the in situ temperature. Time from sampling until start of the incubation was $<$ 3 h, except for the $^{15}N_2O$ amendment experiment at G17 where it was 5 h.

Table 1. Sampling depths, positions, water depths, and chemical characteristics of the sites where ^{15}N incubations were made. At Sta. G01 no incubations were made and the lowest recorded O_2 concentration is indicated. The detection limit for O_2 was 0.01 μmol L^{-1} for the STOX sensor (G03, G10, G17) and 0.09 μmol L^{-1} for the Seabird sensor (Thamdrup et al. 2012). na, not available.

Station	Depth (m)	Latitude (°S)	Longitude (°W)	Water depth (m)	Distance from coast (km)	NH_4^+ (nmol L^{-1})	NO_2^- (μmol L^{-1})	NO_3^- (μmol L^{-1})	O_2 (μmol L^{-1})
G01	208	29°17.11′	71°52.98′	3646	39	na	na		>28
G02	90	26°17.75′	71°16.80′	4935	60	50	3.6	24.9	<0.09
	100					16	5.7	12.4	<0.09
	150					17	9.2	6.7	<0.09
G03	100	23°37.35′	70°52.71′	3022	30	25	0.0	22.9	5.20
	125					5	2.1	17.3	0.09
	150					17	0.0	20.6	0.40
G04	50	20°03.47′	70°45.28′	1480	64	22	0.2	22.7	0.29
	70					7	4.6	10.9	<0.09
	100					27	7.8	3.1	<0.09
G05	60	18°30.04′	71°00.14′	1203	51	16	0.0	21.1	6.18
	70					23	0.3	23.2	<0.09
	100					35	6.0	9.1	<0.09
G07	60	17°05.14′	72°25.13′	1222	28	46	2.2	na	<0.09
	80					42	6.1	na	<0.09
	100					42	8.6	na	<0.09
G09	80	15°30.02′	75°44.77′	3135	58	140	0.4	21.2	7.76
	100					21	1.7	18.6	<0.09
	200					42	4.8	17.9	<0.09
G10	75	14°23.05′	76°23.99′	313	24	22	0.0	na	7.77
	100					8	3.8	na	<0.01
	150					44	5.5	na	<0.01
G15	30	13°52.29′	76°48.27′	750	35	68	0.8	24.2	<0.09
	60					27	0.3	22.9	<0.09
	101					19	0.0	28.9	1.12
	120					96	5.0	15.6	0.70
	150					16	0.1	19.7	<0.09
	201					22	5.0	20.2	<0.09
G16	30	14°16.59′	76°47.60′	2426	56	62	0.3	26.4	1.79
	60					2	0.0	29.4	2.59
	101					4	0.0	27.6	0.42
G17	31	14°09.82′	77°25.72′	5153	117	5684*	6.6	24.6	<0.01
	60					21	0.1	30.2	0.23
	111					15	3.9	11.7	<0.01
G22	60	12°26.59′	77°36.97′	583	57	15	1.0	18.6	<0.09
	75					5	3.4	16.6	<0.09
	100					11	0.2	16.2	<0.09

* This NH_4^+ concentration was probably a result of contamination.

Fig. 1. Map of the sampling area in the eastern South Pacific with the sampling stations indicated.

The ^{15}N incubations were made as described earlier by spiking with ^{15}N-labeled substrates, purging with helium for 15 min, and transferring the water to ten 12-mL glass vials (Labco Exetainers) in parallel for incubation without a headspace (Dalsgaard et al. 2003; Thamdrup et al. 2006). Two amendments were applied at all stations and depths: (1) $^{15}NO_2^-$ and (2) $^{15}NH_4^+$, both at 5 μmol L^{-1} final concentration. Furthermore, at Sta. G17 and G22 a third treatment was applied in which double-labeled $(^{15}N)_2O$ (99% Cambridge Isotope Laboratories) was added to a final concentration of 1 μmol L^{-1}. The Exetainers were prepared as above but without adding any ^{15}N label, and 100 μL of a 120 μmol L^{-1} $^{15}N_2O$ solution was injected into each Exetainer through the septum. The ^{15}N mole fraction in each incubation with $^{15}NO_2^-$ or $^{15}NH_4^+$ was determined from the concentrations before and after addition of the ^{15}N-labeled compound. The ^{15}N mole fraction in the $^{15}NH_4^+$ experiments was 0.96–0.99, except at G17, 31-m depth, where it was 0.46. In the $^{15}NO_2^-$ experiments it was 0.26–0.99. The labeling with $^{15}N_2O$ was not determined directly, but it was assumed to be 0.99 assuming that all natural N_2O was removed during the 15-min helium purging. This may be an overestimate of the actual labeling since some of the natural N_2O may be left after the purging. However, the highest in situ N_2O concentration found at the incubation depths was 75 nmol L^{-1} (G17, 60-m depth; L. Farías et al. unpubl.) and, in the extreme case that all this was still present after purging, the overestimation of the N_2O labeling is only 6%. Incubation time for all incubations varied from 36 to 42 h during which the 10 Exetainers were sacrificed in pairs at approximately equally spaced time intervals. Incubation was stopped by withdrawing 5 mL of water with a syringe and needle while simultaneously allowing helium to flow into the Exetainer through a second needle and finally adding 100 μL of a 50% (w : v) $ZnCl_2$ to inhibit biological activity (Dalsgaard et al. 2003). The 5 mL of water was immediately analyzed for the NO_2^- concentration.

Analytical—Nitrite was analyzed on board with a colorimetric technique (Grasshoff et al. 1983), NO_3^- + NO_2^- was quantified on frozen samples as NO after reduction in hot acidic vanadium chloride (Bramann and Hendrics 1989) (NOx analyzer model 42c; Thermo Environmental Instruments) and ammonium was quantified fluorometrically by the orthophthaldialdehyde method (Holmes et al. 1999) on a Turner Designs Trilogy fluorometer (Turner Designs Inc.). Nitrogen isotopes in N_2 were analyzed by coupled gas chromatography–isotope ratio mass spectrometry (GC-IRMS) with a custom-made GC setup coupled to a Thermo Delta V Plus mass spectrometer (Thermo), and the excess $^{14}N^{15}N$ and $^{15}N^{15}N$ was quantified as described by Thamdrup and Dalsgaard (2000). The GC system consisted of a manual injection port connected to, in series, a combined ascarite-$Mg(ClO_4)_2$ trap for CO_2 and H_2O, a Porapak R chromatographic column, a 600°C reduced copper column removing O_2, and a second $Mg(ClO_4)_2$ trap. The GC system was coupled to the IRMS via a Thermo ConFlo-III interface. ^{15}N-labeled N_2O was also quantified with the system after separation from N_2 on the GC column and quantitative reduction to N_2 on the hot copper column with a total sensitivity about half that of N_2.

Calculations of anammox and denitrification—Concentrations of N_2 produced by anaerobic ammonium oxidation ($N_{2anammox}$) and denitrification ($N_{2denitrification}$) were calculated from the ^{15}N mole fraction of the source compound (F_x) and the excess ^{15}N-labeled N_2 ($^{14}N^{15}N_{xs}$ and $^{15}N^{15}N_{xs}$) according to Thamdrup and Dalsgaard (2002). Briefly, in the $^{15}NH_4^+$ incubations the N_2 production by ammonium oxidation was calculated as:

$$N_{2anammox} = {}^{14}N^{15}N_{xs} \times (FNH_4)^{-1} \quad (1)$$

where FNH_4 is the mole fraction of ^{15}N in the NH_4^+ pool in the $^{15}NH_4^+$ incubations. In the $^{15}NO_2^-$ incubations N_2 production by denitrification was calculated as:

$$N_{2denitrification} = {}^{15}N^{15}N_{xs} \times (FNO_2)^{-2} \quad (2)$$

where FNO_2 is the mole fraction of ^{15}N in the NO_2^- pool for $^{15}NO_2^-$ incubations. Rates were calculated as the slope of the linear regression of the ^{15}N-N_2 concentrations as a function of time for the entire length of the incubation. Rates were tested to be larger than zero, applying a *t*-test ($p < 0.05$). Detection limits were estimated as the rate needed to give a *p*-value of < 0.05, with the average measured standard error for rates in the range from 0 to 2 times the minimum significant measured rate, and were 0.03 nmol N_2 L^{-1} h^{-1} for anammox and 0.10 nmol N_2 L^{-1} h^{-1} for denitrification.

Results

Oxygen and nutrient concentrations—Incubations for anammox and denitrification were performed at a total of

36 sampling locations (Table 1). At six of the stations the upper sample contained O_2 up to 7.7 μmol L^{-1} and at two stations, G15 and G17, O_2 was below the detection limit at the upper sampling depth but appeared again deeper, with the deeper samples representing the OMZ core at $\sigma_\theta >$ 26.1 kg m^{-3} while the shallower samples were from the secondary oxygen-depleted layer. Nitrite concentrations at the depths sampled for incubations were generally high (up to 9.2 μmol L^{-1}) when O_2 was absent and < 0.5 μmol L^{-1} in the presence of O_2. The maximum NO_2^- concentration in the OMZ core increased from north to south from 2 μmol L^{-1} at 10°S to 11 μmol L^{-1} at 20°S (G04) (data not shown), but this trend was not clearly reflected at the depths used for incubation. Ammonium was below 100 nmol L^{-1} except for the shallowest depths at G09 and G17 (Table 1).

Anammox and denitrification—Anammox, identified as $^{14}N^{15}N$ production in the $^{15}NH_4^+$ incubations, was detected at all stations except G03 (Fig. 2A), which turned out to have O_2 concentrations above the detection limit at all three sampling depths. The anammox process was generally only found if the in situ O_2 concentration was below detection (Figs. 2A, 3A), with G15 and G16 being exceptions where O_2 was 0.42–1.72 μmol O_2 L^{-1} (Fig. 2; Table 1). No lag phase in the production of ^{15}N-labeled N_2 was observed. Significant N_2 production by this process was observed in 19 out of the 36 samples tested and in 16 of 23 from anoxic depths, and ranged from 0.04–0.86 nmol L^{-1} h^{-1} with a mean of 0.24 ± 0.06 (standard error) nmol N_2 L^{-1} h^{-1} for the 19 positive incubations and 0.13 ± 0.04 nmol N_2 L^{-1} h^{-1} for all 36 incubations, assuming a rate of zero for the nonsignificant ones. Denitrification to N_2, identified as $^{15}N^{15}N$ production from $^{15}NO_2^-$, was found in only eight of the 36 incubations, seven of 23 from anoxic depths, and with a higher variability in rates from 0.11 to 7.9 nmol N_2 L^{-1} h^{-1} (Fig. 2B). The mean was 1.45 ± 0.95 nmol N_2 L^{-1} h^{-1} for the eight positive incubations and 0.32 ± 0.23 nmol N_2 L^{-1} h^{-1} for all incubations, assuming a value of zero for those that were nonsignificant. Dinitrogen production by denitrification was only detected in one instance when O_2 was present in situ (G05; Figs. 2B, 3B) with a rate of 0.15 nmol N_2 L^{-1} h^{-1} or 10% of the mean of the measured rates. Production of N_2O from denitrification in the $^{15}NO_2^-$ incubations was found on three stations in a total of seven incubations, almost as often as N_2 production (Fig. 2C), and denitrification to N_2 was not detected in any of these. Thus, denitrification to either N_2O or N_2 occurred in 42% of the samples. Unlike N_2 production by anammox and denitrification, O_2 was present in situ up to 2.6 μmol L^{-1} in more than half of the incubations where N_2O production was found (Figs. 2C, 3C). The mean rate of N_2O production in the seven positive incubations was 0.56 ± 0.26 nmol N_2 L^{-1} h^{-1} but only 0.11 ± 0.06 nmol N_2 L^{-1} h^{-1} overall, assuming a rate of zero for the 29 incubations where production was not detected. No lag phase was observed in the incubations resulting in significant N_2 or N_2O production by denitrification. Based on the mean rates of all 36 incubations denitrification accounted for 72% of the total N_2 production by both anammox and denitrification. Inclusion of N_2O production in the denitrification estimate yielded a mean gas production rate by this process of 0.43 ± 0.23 nmol N_2 L^{-1} h^{-1}, and denitrification thus accounted for 77% of the removal of fixed nitrogen in these 36 incubations. It should be noted that these mean values include depths where either one or the other process was responsible for all of the nitrogen removal.

Vertical variation of the processes—Anammox was generally detected in the shallowest anoxic sample from all stations, and rates were highest in the upper part of the OMZ core and decreased with depth below the oxic–anoxic interface (the depth where O_2 changed from detectable to not detectable) (Fig. 4). Denitrification did not show a systematic variation with depth. Production of N_2 by anammox and denitrification and of N_2O production by the latter occurred mainly at σ_θ values between 26.1 and 26.4 kg m^{-3}, with a few exceptions at lower densities (Fig. 5).

The variation in process rates with depth was investigated in more detail at Sta. G15 where six depths were sampled (Fig. 6). Like G17, this station had a secondary oxygen depletion, from 55–69 m at σ_θ values of 25.9 kg m^{-3}, above the OMZ core which had σ_θ values $>$ 26.1 kg m^{-3} and was found below 150 m. Oxygen concentrations were below detection at the two upper and two lower sampling depths, whereas the two central ones had O_2 concentrations of around 1 μmol L^{-1}. Both N_2 production by anammox and N_2O production by denitrification were found at G15, while N_2 production by denitrification was not detected. The anammox rates were highest in the upper oxygen-depleted zone, but anammox was also detected in the OMZ core and in the intermediate sample with 0.7 μmol O_2 L^{-1} in situ. Anammox was not detected in the shallow oxygen minimum at G17, but here denitrification was active. Production of N_2O was only seen at two depths at G15 (Fig. 6), and the rates were substantially lower than the anammox rates. At the deepest sampling depth neither N_2 nor N_2O production was found.

Regional variation of the processes—There were no systematic meridional trends in anammox and denitrification rates (Fig. 2) and rates did not correlate with concentrations of NO_3^-, NO_2^-, NH_4^+, or chlorophyll *a* at the sampling depths whether all rates or only rates from anoxic depths were included (data not shown). The stations with the three highest denitrification rates (G02, G10, G04) all had relatively high chlorophyll *a* concentrations in the water column above the OMZ core (Fig. 7). On the other hand, G15 and G22 also had high chlorophyll concentrations higher in the water column, but denitrification rates were much lower or not detectable. There was no correlation between anammox or N_2O production rates and the chlorophyll *a* distribution.

Nitrous oxide metabolism—The addition of double-labeled $(^{15}N)_2O$ resulted in the formation of double-labeled $^{15}N^{15}N$ with no lag phase (Fig. 8). The increase in $^{15}N^{15}N$ was linear through the first 20–25 h of the incubation, after which it increased significantly. The $^{15}N^{15}N$ production rates were estimated from the initial, linear part of the curve. The reduction of added N_2O to N_2 was observed at the shallowest incubation depth at both G17 and G22 but

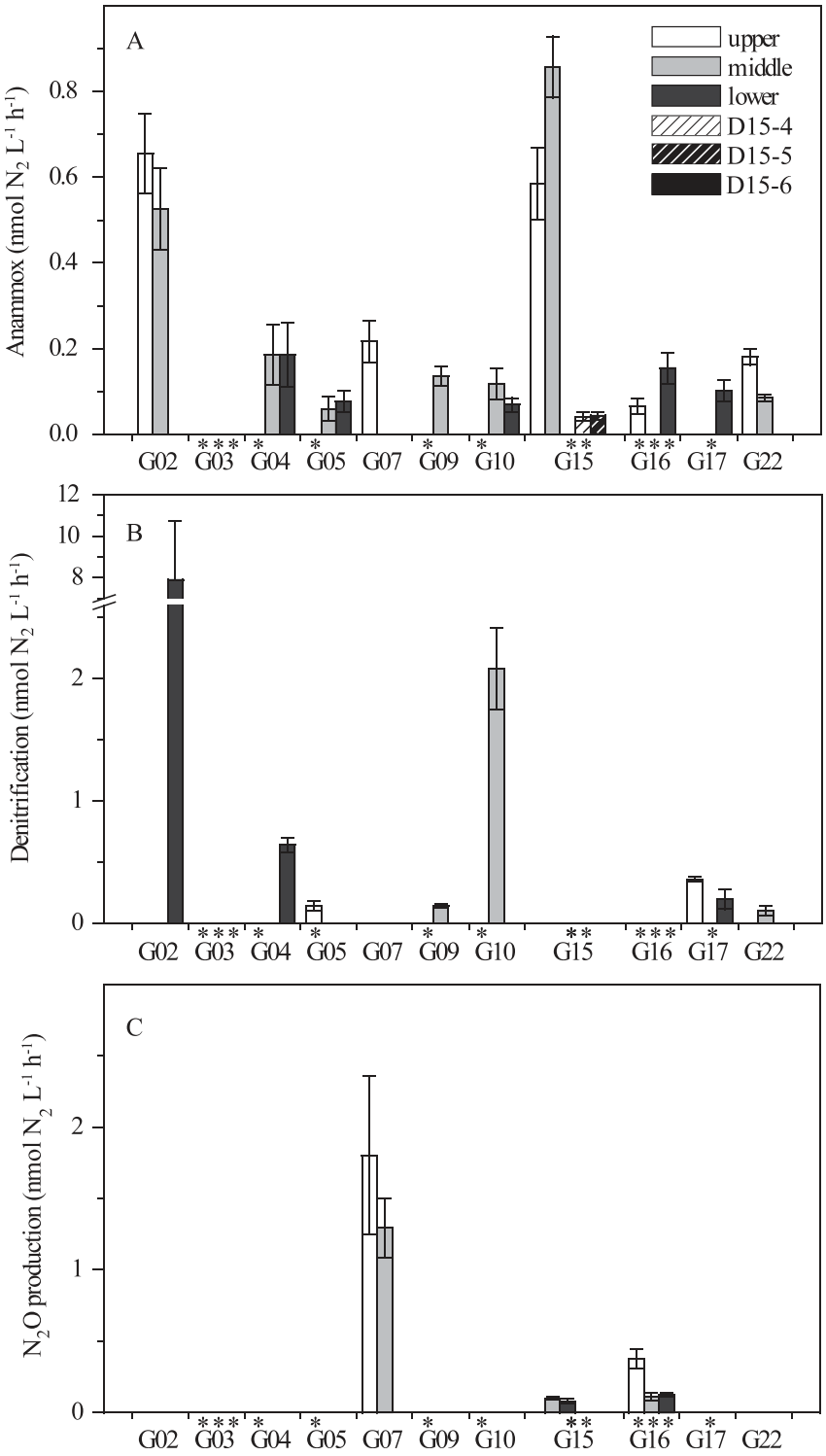

Fig. 2. (A) Anammox determined from $^{15}NH_4^+$ amendments, (B) N_2 production by denitrification determined from $^{15}NO_2^-$ amendment, and (C) N_2O production by denitrification determined from $^{15}NO_2^-$ amendment. For each station the rates are shown for the three depths analyzed (upper, middle, and lower), except for Sta. G15, where also the rates for depths 4, 5, and 6 are shown (D15-4, D15-5, and D15-6). The actual depths sampled are shown in Table 1. Asterisks indicate that O_2 was detectable in situ (actual O_2 concentrations are given in Table 1). Error bars show the standard error of the slope of the regression of produced N_2 or N_2O by each process as a function of incubation time.

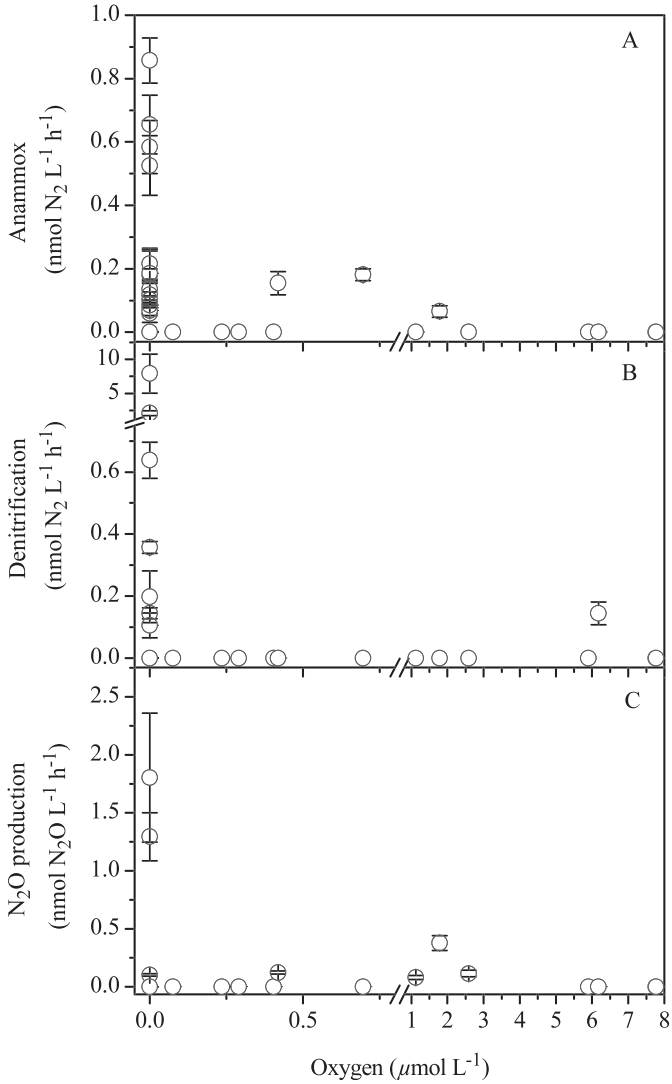

Fig. 3. (A) Anammox determined from $^{15}NH_4^+$ amendment, (B) N_2 production by denitrification determined from $^{15}NO_2^-$ amendment, and (C) N_2O production by denitrification determined from $^{15}NO_2^-$ amendment as a function of in situ O_2 concentration. Error bars as in Fig. 2.

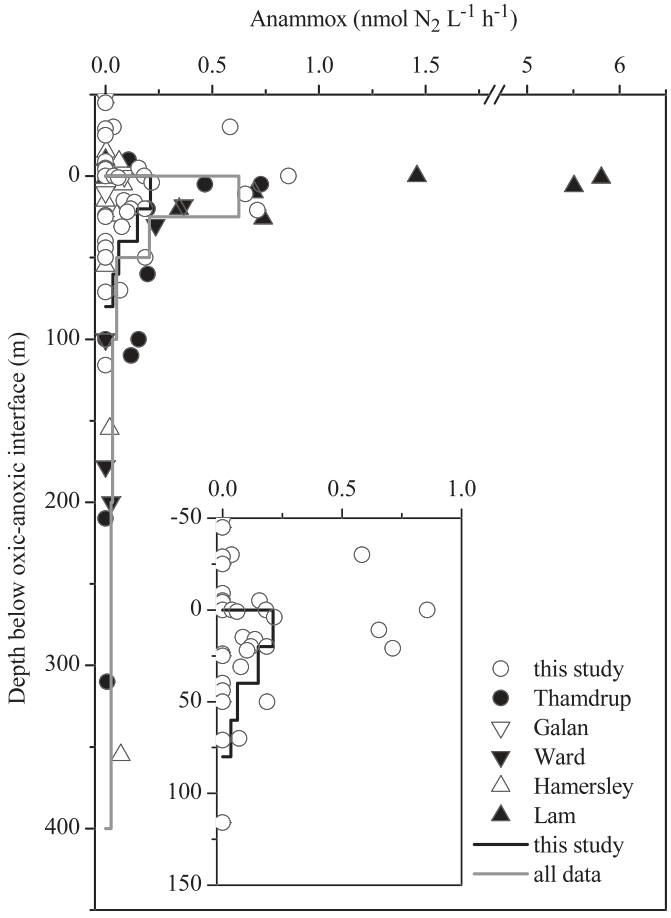

Fig. 4. Rates of anammox as a function of depth below the oxic–anoxic interface (relative depth) in each activity profile. Data from the present study and Thamdrup et al. (2006), Galan et al. (2009), Ward et al. (2009), Hamersley et al. (2007), and Lam et al. (2009) as indicated. All data are from the eastern South Pacific. Data from this study were divided into four depth intervals (0–20-m, 20–40-m, 40–60-m, and 60–80-m relative depth) and the average for each interval is plotted as the black line. Data from all studies were divided into depth intervals (0–25-m, 25–50-m, 50–100-m, 100–200-m, and 200–400-m relative depth) and the average for each depth interval is plotted as the gray line. Data from the present study alone are shown in the inset.

not deeper (Fig. 9). The highest N_2O reduction rate of 0.34 nmol L^{-1} h^{-1} was found in the upper oxygen-depleted layer at G17. This layer was not present at G22. Denitrification to N_2O was not found at these two stations, but complete denitrification of NO_2^- to N_2 was active at both and co-occurred with N_2O reduction at G17.

Discussion

The general hydrographic conditions during the cruise were typical for the Humboldt Current System outside El Niño and La Niña periods, although the cruise took place immediately after a moderate El Niño event that peaked in November–December 2006 (Climate Prediction Center Internet Team, http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ensoyears.shtml). Monthly mean sea surface temperatures for February 2007 in the region along the cruise track were at or slightly (< 1°C) above the normal for February (mean for 1985–1997; National Aeronautics and Space Administration Earth Observations, http://neo.sci.gsfc.nasa.gov/Search.html?pg=6&datasetId=AMSRE_SSTAn_M). The monthly averaged Aqua Moderate Resolution Imaging Spectroradiometer (MODIS) satellite-based distribution of chlorophyll in the region was also very similar to the monthly climatology for February 2003–2011, with a tendency towards higher-than-normal concentrations near the coast north of 14°S in 2007 (OceanColor Web, http://oceancolor.gsfc.nasa.gov/cgi/l3). Also the distributions of oxygen and nitrite were similar to earlier reports (Thamdrup et al. 2012).

Distribution of anammox and denitrification in the ESP—This first survey of N_2 producing processes covering almost the entire meridional extent of the ESP OMZ showed a

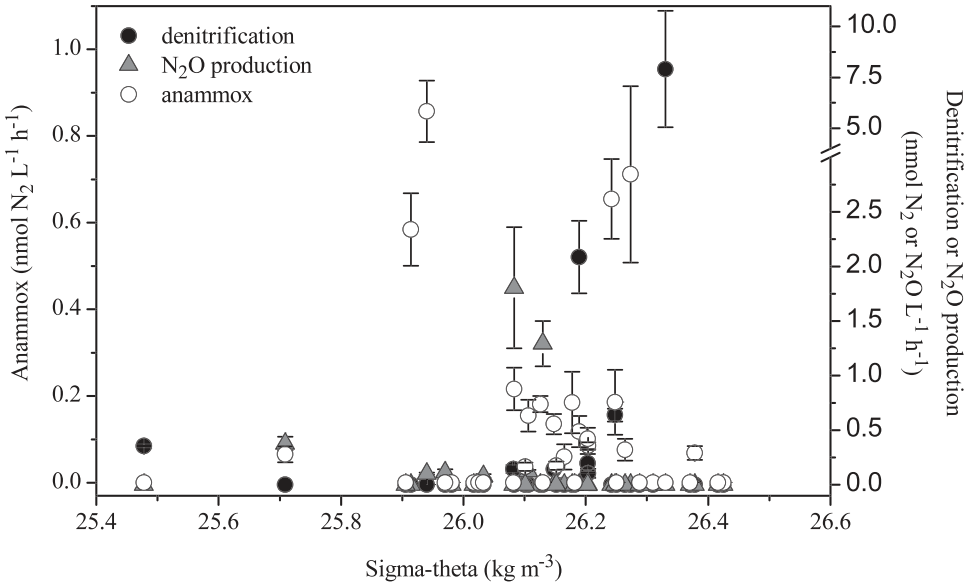

Fig. 5. N_2 production by anammox and N_2 and N_2O production by denitrification as a function of density expressed as sigma-theta (σ_θ).

relatively homogeneous distribution of anammox between stations and its consistent presence at shallow anoxic depths. The process was found at all stations except G03, where only oxic depths were sampled, and at 70% of the anoxic depths. The anoxic depths where anammox was not detected were generally the deeper ones in the profiles (Fig. 2A) as anammox rates rapidly attenuated with depth below the oxic–anoxic interface (Fig. 4).

The rates, depth distribution, and ubiquity of anammox in the OMZ core were consistent with previous studies in the ETSP (Fig. 4). Only two samples from previous studies showed much higher anammox rates (Hamersley et al. 2007; Lam et al. 2009). These samples were from a station over the Peruvian shelf about 30 km off Lima at a water depth of 141 m, and the high activity was associated with NH_4^+ concentrations close to 5 μmol L^{-1} and a relatively high turbidity. Thus, the conditions were not typical for the pelagic OMZ. High anammox rates were also associated with high turbidity over the Namibian Shelf (Kuypers et al. 2005), probably influenced by sediment resuspension. While the few data available from previous studies might indicate more anammox activity off Peru than off northern Chile, our nearly synoptic survey does not support general meridional trends.

This study provides the first extensive documentation of denitrification in the ESP using short-term $^{15}NO_2^-$ incubations. Only two published incubations have shown significant denitrification, both at a rate of 0.7 nmol N_2 L^{-1} h^{-1} (Thamdrup et al. 2006; Ward et al. 2009), which is within the range found in the present study. Denitrification did not show any relation to depth, whether or not the previously published rates were included. It occurred less frequently, and reached much higher maximum values than anammox, whether N_2 or N_2O was the product (Fig. 2), and was generally much more variable. As for anammox, the data revealed no meridional trends.

The DNRA has recently been invoked as an important sink for NO_3^- or NO_2^-, estimated to supply 7% to 34% of the NH_4^+ needed for the anammox process at an offshore station in the Peruvian OMZ at 12°S (Lam et al. 2009) and an even higher fraction over the Omani Shelf (Jensen et al. 2011). The presence of DNRA could potentially have methodological implications for the $^{15}NO_2^-$ incubations since $^{15}NH_4^+$ produced by DNRA may be coupled with $^{15}NO_2^-$ through anammox yielding $^{15}N^{15}N$, which will be interpreted as resulting from denitrification (Kartal et al. 2007). This shortcut will be most important in NH_4^+-poor environments, such as those studied here (Table 1), where the $^{15}NH_4^+$ from DNRA can rapidly label the NH_4^+ pool. However, since anammox is responsible for the $^{15}N^{15}N$ production that may be interpreted as denitrification, the magnitude of this error cannot exceed the anammox rate (determined independently in the $^{15}NH_4^+$ incubations). In our study high rates of denitrification were only seen when anammox rates were low (Fig. 10) and, therefore, only a small part of this denitrification could be anammox in disguise. The situation is different for the low rates of denitrification (four incubations, Fig. 10), which occurred together with similar rates of anammox, and here DNRA could potentially have played a role. Since production of $^{15}NH_4^+$ by DNRA would cause a gradual increase of the ^{15}N labeling of the NH_4^+ pool, the $^{30}N_2$ production rate by the coupled DNRA–anammox should increase over time. However, there was only a slight indication of increasing $^{30}N_2$ production during the incubation in one of the four incubations where this might be a problem, whereas the increase in $^{30}N_2$ concentration was linear over time in the other three (data not shown). We further constrained the potential significance of this false denitrification through simple numerical simulations, in which we calculated the development of the concentration of $^{15}NH_4^+$ and the resulting production of $^{15}N^{15}N$ during an incubation with addition of $^{15}NO_2^-$. In the simulations, NH_4^+ was produced by mineralization and DNRA and consumed by anammox at constant rates, $R_{[process]}$, and

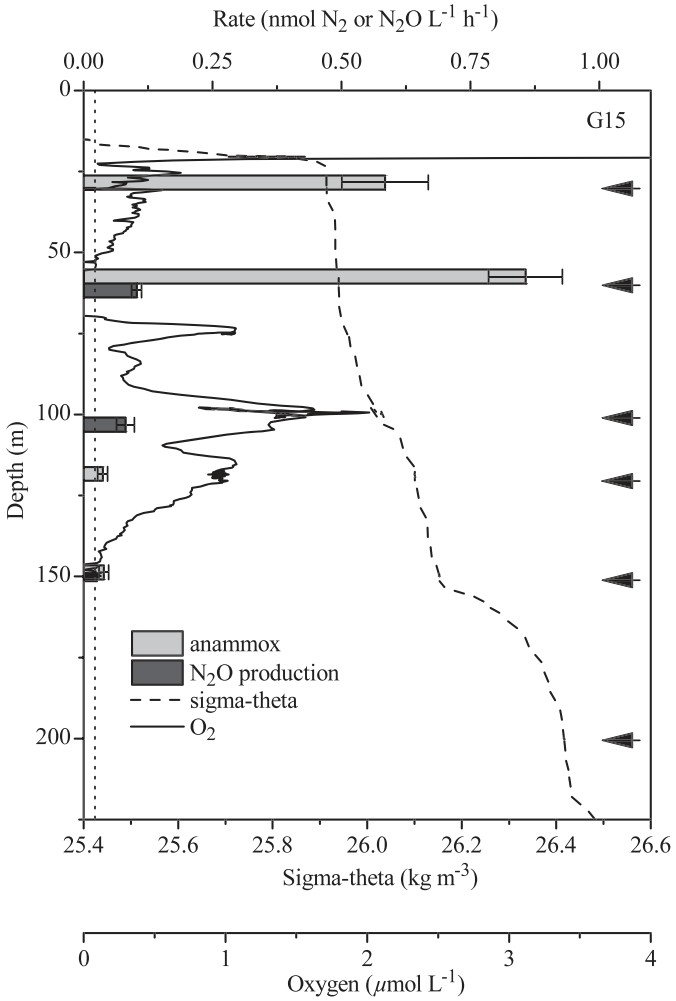

Fig. 6. Depth profile at Sta. G15 of anammox determined from $^{15}NH_4^+$ amendments, N_2O production by denitrification determined from $^{15}NO_2^-$ amendment, density as sigma-theta, and oxygen concentration. Denitrification to N_2 was below detection. The detection limit of the oxygen sensor is shown as a vertical dashed line (0.09 μmol L^{-1}). Note that the oxygen concentration was below the detection limit at the upper sampling depth (30 m). Arrows indicate sampling depths. Error bars show the standard error of the mean.

$^{15}NH_4^+$ was produced by DNRA and consumed by anammox at rates determined by the total rate and the mole fraction of ^{15}N, FNO_2, and FNH_4, in the reactants:

$$\frac{d[NH_4^+]}{dt} = R_{\text{Mineralization}} + R_{\text{DNRA}} - R_{\text{Anammox}} \quad (3)$$

$$\frac{d[^{15}NH_4^+]}{dt} = F_{NO2} \times R_{\text{DNRA}} - FNH_4 \times R_{\text{Anammox}} \quad (4)$$

The production of $^{15}N^{15}N$ by anammox was found as:

$$\frac{d[^{15}N^{15}N]}{dt} = F_{NO2} \times FNH_4 \times R_{\text{Anammox}} \quad (5)$$

The simulations were carried out in a discrete time interval approximation and the apparent rate of denitrification for a 40-h incubation was calculated according to Eq. 2. Even in the extreme case where DNRA produced NH_4^+ at the same rate as it was consumed by anammox, and the rate of mineralization was zero, i.e., DNRA was the only source of NH_4^+, the false denitrification was only 15% of the anammox rate for a typical anammox rate of 0.2 nmol N_2 L^{-1} h^{-1}, NH_4^+ concentration of 20 nmol L^{-1}, and 95% ^{15}N-labeling of the NO_2^- pool. We thus conclude that the high quantitative importance of denitrification found in this study is not an artifact due to coupled DNRA–anammox.

Vertical distribution of the processes—The upper oxic–anoxic interface of the contiguous OMZ core was located at σ_θ values of 26.1 kg m^{-3} along the entire cruise track (Farías et al. 2007; Thamdrup et al. 2012), and significant rates of gas production by anammox and denitrification were generally found only when σ_θ was higher than 26.1 kg m^{-3} (Fig. 5). We mainly found nitrogen removal at σ_θ values between 26.1 and 26.4 kg m^{-3}, with a few exceptions at slightly lower values. This agrees well with Deutsch et al. (2001), who estimated nitrogen removal from nutrient fields and found maximum nitrogen removal at these densities with lower rates at both lower (25.7 kg m^{-3}) and higher (26.6 kg m^{-3}) densities. Lam et al. (2009) detected anammox between 25.9 and 26.4 kg m^{-3} and in one case at 26.8 kg m^{-3}. It thus appears that removal of fixed nitrogen is generally limited to the OMZ core or to secondary O_2-depleted layers as observed at G15 and G17 (*see* below).

Anammox rates have previously been found to be highest in the upper part of the OMZ (Thamdrup et al. 2006; Hamersley et al. 2007) and this was also evident here when anammox rates were plotted vs. depth below the oxic–anoxic interface (relative depth), both for the data from this study and for all the previously published data from the ETSP (Fig. 4). The attenuation of anammox rates with depth was broadly consistent with the attenuation of mineralization rates in shallow aphotic waters (Andersson et al. 2004; Arístegui et al. 2005). The relatively large database and consistent distribution of anammox rates in the equatorial South Pacific allow us estimate an average depth-integrated rate. As discussed below, we interpret the rates measured above the oxic–anoxic interface as biased by the incubation procedure and exclude them from the calculations. For all measurements below the oxic–anoxic interface we assumed a value of zero for the depths where the process was not detected. The anammox rates from the present study were averaged for the relative depth intervals 0–20 m, 21–40 m, 41–60 m, and 61–80 m (Fig. 4, inset) and the total N_2 production by anammox per unit area was found to be 9.1 ± 3.1 (standard error) μmol N_2 m^{-2} h^{-1}, with no anammox detected in any incubation below 80-m relative depth. However, previously published data for the ETSP found anammox to greater depth below the oxic–anoxic interface (Fig. 4) and we include those in the further calculations. We assumed a value of zero when rates were below detection as above, and calculated mean rates based on all available data for the relative depth intervals 0–25 m, 26–50 m, 51–100 m, 101–200 m, and 201–400 m. The

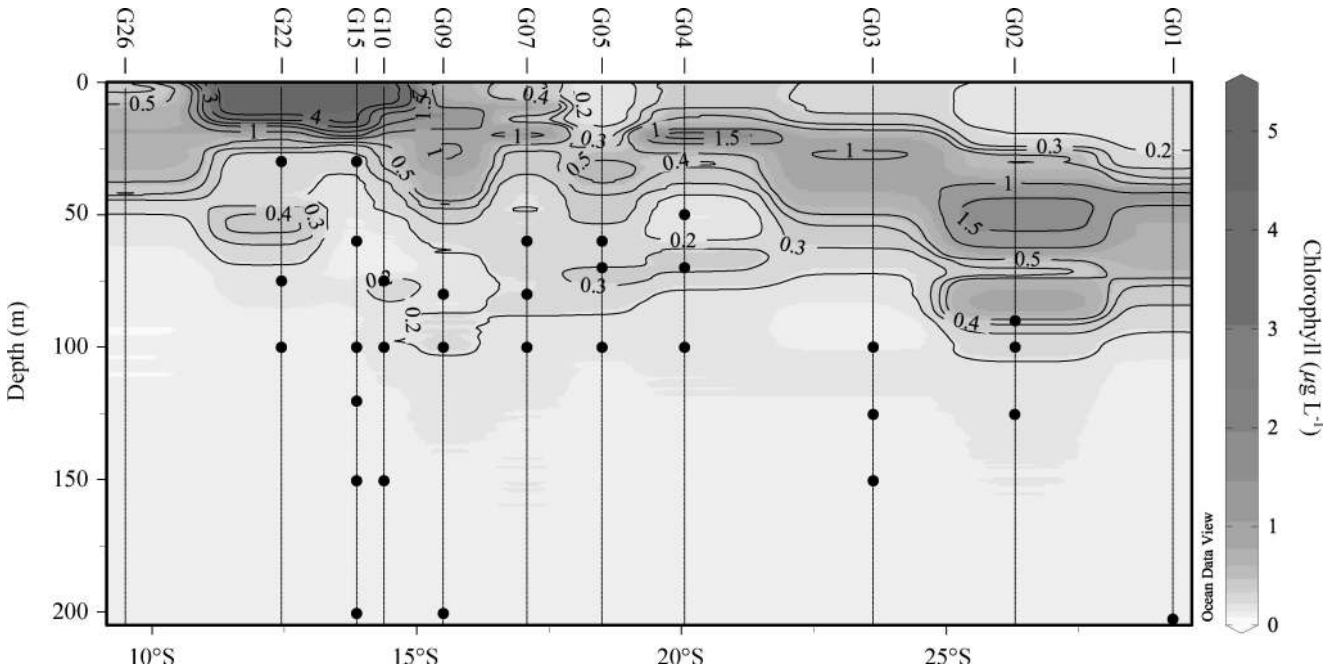

Fig. 7. Chlorophyll *a* concentrations as a function of depth and distance along the cruise track. Data interpolation was done in Ocean Data View.

resulting mean anammox rate per unit area was 32.0 ± 8.9 μmol N_2 m^{-2} h^{-1}.

As discussed above, the evidence for denitrification in the ESP, until this cruise, was very limited. The sources of ammonium for anammox have instead been suggested to be the mineralization of organic matter coupled to nitrate reduction to nitrite, DNRA, sulfate reduction, and, in the case of DNRA, the reduction of nitrite itself. While all of these processes have been shown to be active in the ETSP OMZ, their activity was not sufficient to cover the ammonium demand of anammox at the measured rates and stoichiometries (Thamdrup et al. 2006; Lam et al. 2009; Canfield et al. 2010). In the incubations from the present study, however, denitrification accounted for 77% of the removal of fixed nitrogen (N_2 + N_2O production), which is close to the 71% that would be expected if denitrification and anammox were directly coupled (Dalsgaard et al. 2003). The high contribution was driven mainly by a few measurements of very high rates (Fig. 2), and the patchy distribution likely explains that the process was missed in earlier studies, which covered fewer locations.

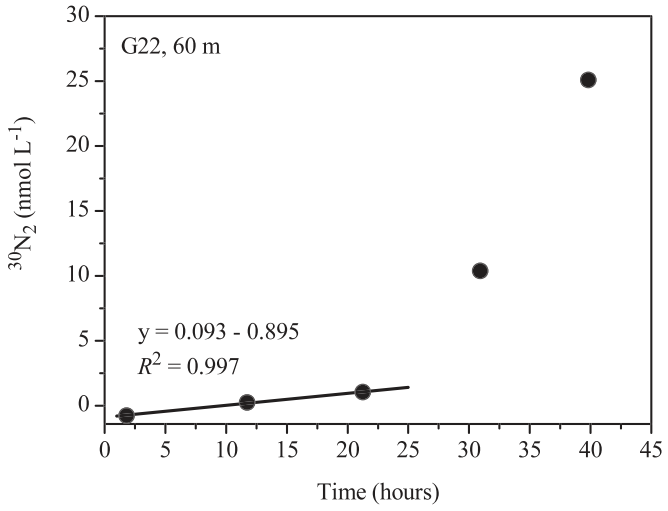

Fig. 8. Example of the reduction of double-labeled $(^{15}N)_2O$ to $^{30}N_2$ at Sta. G22, 60-m depth. A regression line was fitted through the data points from the first 22 h to estimate the initial production rate.

Given the very patchy distribution of denitrification in the system, we cannot make a robust estimate of the average integrated rate based on incubation results. Nonetheless, we find it useful to attempt a comparison of rates of denitrification to anammox rates from the present study and to rates from the literature. In a highly skewed data set such as our denitrification rates, the median would often be chosen as the best representation of an average. In this case the median would be zero, and clearly not give a proper representation of our recorded rates. Instead we calculate the arithmetic mean of the rates, which is subsequently used for estimating the overall N-loss rates as contributed by anammox or denitrification. Because of the lack of a clear relation of denitrification rates with depth, we calculated an overall denitrification rate as a mean of the measured rates, assuming a value of zero for depths where the process was not detected. In the present data set the deepest detected rate was at 71-m relative depth, and assuming that denitrification occurred from the oxic–anoxic interface to 80-m relative depth, as for anammox above, the overall denitrification rate equaled 52 ± 30 μmol N_2 m^{-2} h^{-1}. From earlier application of the isotope pairing technique in the ETSP only two significant denitrification rates are available (Thamdrup et al. 2006; Ward et al. 2009). However, incubations for denitrification were made in parallel to the anammox measurements

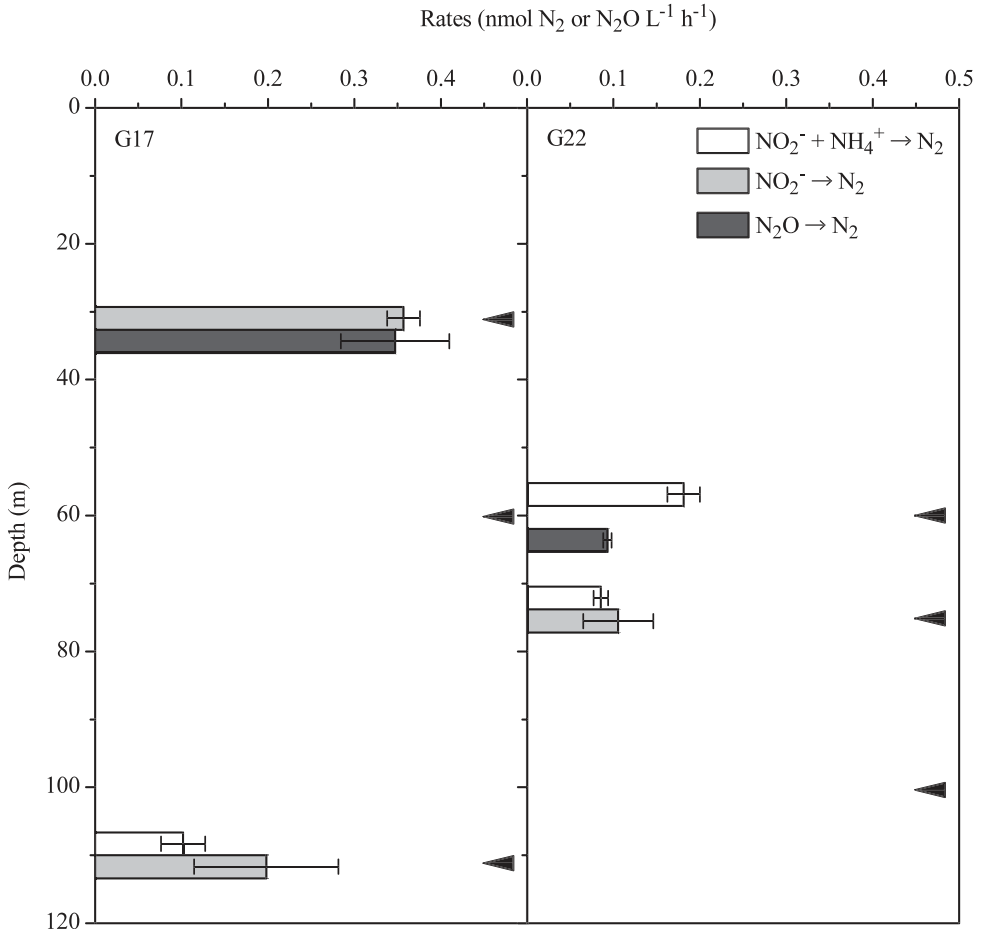

Fig. 9. Depth profiles at Sta. G17 and G22 of anammox from $^{15}NH_4^+$ amendments, N_2 production by denitrification from NO_2^- amendments, and N_2O reduction to N_2 from $^{15}N_2O$ amendment. N_2O production by denitrification in $^{15}NO_2^-$ amendments was not detected. Arrows indicate sampling depths. Error bars as in Fig. 2.

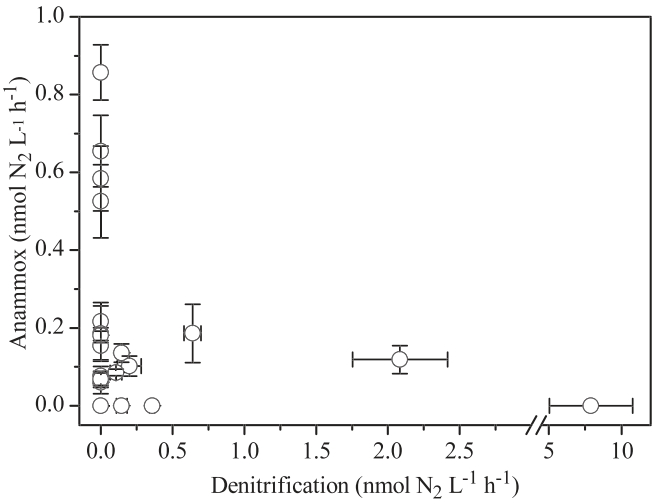

Fig. 10. Anammox determined from $^{15}NH_4^+$ amendments as a function of denitrification determined from $^{15}NO_2^-$ amendments in parallel incubations from the same depth and station. Error bars as in Fig. 2.

mentioned above, resulting in 20 further measurements at anoxic depths within the relevant depth range, with rates below the detection limit. Assigning these a value of zero, pooling them with the two significant rates from the literature and with data from the present study, and assuming that denitrification occurred to a depth of 180 m (deepest recorded rate was at 170-m relative depth), the estimated overall denitrification rate was 59 ± 32 μmol N_2 m^{-2} h^{-1}. Adding the contribution from anammox to this, we obtain a total N_2 production of 91 ± 33 μmol N_2 m^{-2} h^{-1} and a contribution from anammox of 35%. Thus, this exercise shows that even if the previous, mainly negative results for denitrification are included, an anammox contribution close to the stoichiometric prediction of 29% is within the limits of the data set.

Interactions of anammox and denitrification—We did not observe a close coupling of anammox and denitrification in the individual incubations as one process only attained high rates when the rate of the other process was low (Fig. 10). Mineralization of organic matter coupled to denitrification may still be the source of NH_4^+ for anammox, although the processes may be temporally and/or spatially separated.

The electron acceptor for denitrification, NO_3^- or NO_2^-, is readily available and denitrification is probably limited by the availability of organic matter (Ward et al. 2008). Denitrification is thus most likely coupled to episodic inputs of organic matter from the photic zone (Thamdrup et al. 2006; Ward et al. 2008, 2009), and NH_4^+ released through mineralization is only later transformed by anammox. The omnipresent and relatively fast-growing denitrifiers (Strohm et al. 2007; Ward et al. 2008) may respond rapidly, whereas the slower growth rate of anammox bacteria (Jetten et al. 1999; Kuenen 2008) in combination with the very special biochemistry of the process (Kartal et al. 2011) might cause a slow response of the anammox process. As a consequence of its fast response, denitrification may reach higher process rates than anammox and may also deplete the electron donor pool, resulting in periods and water volumes without denitrification and, therefore, a patchy distribution of the process. Higher maximum rates of denitrification than anammox are indeed what we find in the present data set, where the maximum value of denitrification was nine times higher than that of anammox. This proposed coupling of the processes through shorter periods with higher denitrification rates and longer periods with lower anammox rates will cause NH_4^+ concentrations to fluctuate, with accumulation during the denitrification phase and depletion during the anammox phase. With the mean N_2 production rate by anammox in this study of 0.13 nmol N_2 L^{-1} h^{-1}, the NH_4^+ consumption over, e.g., 1 month, will be around 95 nmol L^{-1}. The actual NH_4^+ concentrations found in this study varied from 2–140 nmol L^{-1} (Table 1), so NH_4^+ accumulated to levels allowing anammox to operate for up to at least 1 month in the absence of NH_4^+ production.

There was no correlation between denitrification rates and chlorophyll fluorescence at the sampling depth as a proxy of the freshest organic matter. The high denitrification rates were all found at stations with high chlorophyll levels in the overlying water, and the highest rate at G02 occurred below a relatively deep chlorophyll maximum, which could represent a subducted and potentially decaying algal bloom (Fig. 7), but even at the high-chlorophyll stations, the rates were patchily distributed. Thus, sampling at high spatial and temporal resolution is required for a more detailed analysis of the proposed coupling between algal blooms and bursts of denitrification.

Anammox is generally believed to be responsible for maintaining the very low levels of NH_4^+ found in the OMZ core in this and most of the studies from the ETSP and the Arabian Sea referred to above. It might thus be expected that anammox rates would be directly controlled through the NH_4^+ concentration. The affinity for NH_4^+ in anammox bacteria is not well known and the current best estimate of a half-saturation value is < 5 μmol L^{-1} based on enrichments from bioreactors (Strous and Jetten 2004). However, while anammox rates show some correlation to the in situ NH_4^+ concentration when compared across OMZs (Lam and Kuypers 2011), the short-term kinetics of the process remain unknown. In $^{15}NH_4^+$ incubations NH_4^+ concentrations are typically increased by two orders of magnitude over the in situ values, but anammox rates from such incubations are most often not significantly higher than those measured in parallel incubations with $^{15}NO_2^-$ (Thamdrup et al. 2006; Hamersley et al. 2007 [two out of four stations]; Galan et al. 2009), and in some experiments anammox rates measured with $^{15}NO_2^-$ even exceed rates from $^{15}NH_4^+$ incubations (Hamersley et al. 2007 [two out of four stations]; Bulow et al. 2010). In the present study there was no clear correlation between anammox rates measured in $^{15}NH_4^+$ and $^{15}NO_2^-$ incubations (not shown), primarily because of the higher detection limit in the latter, which also resulted in a lower number of recorded significant anammox rates in $^{15}NO_2^-$ incubations. In the nine incubations where both treatments gave significant rates, there was a tendency for the $^{15}NO_2^-$ incubations to give higher rates than $^{15}NH_4^+$ incubations at the lowest NH_4^+ concentrations. The measured anammox rates do, therefore, not appear limited by the NH_4^+ concentrations. This suggests that the K_m for NH_4^+ uptake by anammox bacteria is extremely low so that they are near saturation at in situ conditions, or, alternatively, that these bacteria have an intrinsic limitation, which hinders a rapid response to changes in NH_4^+ concentrations within the timeframe of the incubation. However, the anammox rates may in the long run still be constrained by the NH_4^+ availability, through a combination of fluctuating NH_4^+ concentrations and the low growth potential of the organisms. Doubling times of 15–30 d are common under laboratory conditions (Van Der Star et al. 2008), and in the OMZ, doubling times may be expected to be substantially longer. Given the time scale of the NH_4^+ fluctuations proposed above and expected doubling time, the populations of anammox bacteria will not be able to grow to a density that fully exploits the higher NH_4^+ concentrations resulting from bursts in mineralization. Instead, the density of these organisms may be reduced during the low NH_4^+ periods, depending on how well they survive NH_4^+ starvation, and slowly increase when NH_4^+ availability is higher. This also agrees well with the relatively low variation found in anammox rates in this and earlier studies.

Effects of oxygen—Anammox and denitrification occurred almost exclusively when O_2 concentrations in situ were below detection (Figs. 2, 3). In the incubations the concentrations of ^{15}N-labeled N_2 increased linearly from the start, and we argue that this reflects the in situ activity, and that lack of detection of the processes at oxic depths indicates that they were not active at these depths in situ. Furthermore, we suggest that the lack of activity at oxic depths was caused by O_2 inhibiting the induction and synthesis of the enzymes needed for the processes. If the enzymes had been present the processes would probably have become active once O_2 concentrations were lowered in the experimental setup, as often seen in experiments with short-term exposure to oxygen for both anammox (Strous et al. 1997; Egli et al. 2001) and denitrification (Bonin et al. 1989; Gao et al. 2010). If the enzymes were not present in situ they might have been produced during the incubation, but the rates showed no evidence for this. Most likely, the induction was so slow that the activity was not seen during the incubation. In *Paracoccus denitrificans*, 10–24 h was

needed to reach full activity of denitrification under optimal conditions at 30°C in the laboratory (Baumann et al. 1996, 1997), and the lower temperature and oligotrophic conditions in our incubations would likely have slowed down the induction even further. As obligate anaerobes, anammox bacteria, on the other hand, may not have been present at oxic depths. Their density decreases rapidly with increasing O_2 concentrations up through the oxycline in most studies in both the ETSP (Hamersley et al. 2007; Galan et al. 2009) and other systems (Kuypers et al. 2003, 2005). This may explain the absence of anammox in the upper sampling depths where O_2 was present (Fig. 2A; G04, G05, G09, G10).

While we intended the incubations to be anoxic, the potential effect of trace amounts of oxygen must be considered. Tests showed that flushing the water in the 250-mL bottles for 15 min removed $> 99\%$ of the O_2 (not shown), so the water was extremely low in O_2 before being transferred to the Exetainers for incubation. However, the water may take up O_2 from the atmosphere when dispensed into the incubation vials and the butyl rubber septum in the Exetainers may release O_2 to the water during the incubation and the O_2 concentrations may in the worst case reach a few hundred nanomoles per liter (De Brabandere et al. 2012). Earlier, tests have shown that O_2 concentrations were below 200 nmol L^{-1} during incubations applying the same protocol as here (Dalsgaard et al. 2003). Even if traces of oxygen were present in our incubations, the fact that we find denitrification and anammox indicates that our handling and incubation procedure created sufficiently low O_2 concentrations for the processes to occur.

It is well known that anammox is sensitive to short-term exposure to O_2, although there is disagreement about the levels needed to inhibit the process. In O_2 amendment experiments Kalvelage and coworkers (2011) found that O_2 concentrations required to inhibit anammox 50% varied from 11 to 16 μmol L^{-1} in the Namibian OMZ and from 2 to 11 μmol L^{-1} in the Peruvian OMZ. In the Black Sea, anammox was reduced by 7–8% at about 1 μmol O_2 L^{-1} and was fully inhibited by 13 μmol O_2 L^{-1} (Jensen et al. 2008), whereas full inhibition occurred already at 1 μmol O_2 L^{-1} in bioreactor experiments (Strous et al. 1997). Given these sensitivities to short-term exposure to O_2 and the expected levels of O_2 contamination in the Exetainers, it is unlikely that the O_2 in the incubations may have caused significant underestimation of in situ anammox rates.

The estimates of the inhibitory effects of O_2 on denitrification are also quite variable. Denitrification has been reported to be active at O_2 concentrations up to 2–4 μmol O_2 L^{-1} in pure cultures of bacteria isolated from the Eastern Tropical North Pacific OMZ (Devol 1978). Kuypers et al. (2005) attributed the absence of denitrification in the Benguela upwelling to frequent oxygenation but did not indicate which concentrations would be inhibitory. In Mariager Fjord, denitrification was fully inhibited at 8 μmol O_2 L^{-1} (Jensen et al. 2009). Pure cultures of denitrifying bacteria exhibit highly variable sensitivities towards O_2, e.g., a 50% inhibition of the reduction of NO_2^- to N_2O in *Pseudomonas nautica* occurred at 18 μmol O_2 L^{-1} (Bonin et al. 1989) and 50% inhibition of *Pseudomonas denitrificans* was observed at $\sim$ 10 μmol O_2 L^{-1} (Robertson et al. 1995). Although the effects of a few hundred nanomoles O_2 per liter, as expected in the incubations, have not been examined directly, we assume that this would probably only lead to small underestimations of in situ process rates.

With a single exception, all cases of N_2O and N_2 production recorded with detectable O_2 in situ were found at G15 and G16 (Fig. 2), which had more complicated distributions of O_2 than the other stations and a weaker stratification as indicated by a lower gradient in σ_θ with depth (Fig. 6; data for G16 not shown), suggesting a recent mixing of oxic and anoxic water. Under these conditions, it is likely that the organisms originating from the anoxic water still possessed the anammox or denitrification enzymes, and the lowering of O_2 in the incubations would have activated the processes, if they were not already active in situ. On these two stations only partial denitrification to N_2O was seen, which agrees very well with the fact that nitrous oxide reductase is the most oxygen-sensitive enzyme in the denitrification pathway (Betlach and Tiedje 1981; Bonin et al. 1989; Thamdrup and Dalsgaard 2008). Given the strict relation between denitrification or anammox and O_2 in Fig. 3, we assume that any significant process rates recorded at oxic depths were artifacts induced by the lowering of O_2 concentrations in the incubations. At G15 and G17, anammox or denitrification to N_2 or N_2O were found above the proper OMZ core in secondary O_2-depleted layers separated from the core by an oxic layer. At G15 the O_2 concentrations at the two upper sampling depths were below detection even though the σ_θ values were around 25.9 kg m^{-3} and only the two lower sampling depths were in the proper OMZ core with σ_θ values above 26.1 kg m^{-3} (Fig. 6). The rate measured at the intermediate oxic sampling depth was probably induced by the incubation technique as discussed above. At G17 the situation was similar with a less dense water layer (σ_θ = 25.48 kg m^{-3}, data not shown) with O_2 below detection at the upper sampling depth separated from the OMZ core by an oxic layer (Fig. 2; Table 1). These results are somewhat contradictory to those of Kalvelage et al. (2011), who found anammox in anoxic incubations of Peruvian OMZ water with O_2 in the low micromolar range. This may represent true aerobic anammox or this may, as in our experiments, be due to recent mixing events and expression of activity of organisms originating from anoxic waters.

Our results point to a high sensitivity of both anammox and denitrification to oxygen in the OMZ, with both processes almost only taking place when in situ O_2 concentrations were below the detection limits (10 or 90 nmol L^{-1}, Table 1). We interpret this as the O_2 concentration below which these anaerobic processes are induced. Active anammox and denitrifying bacteria may continue their metabolism for some time when exposed to low oxygen levels, but the activity will decline over time if gene expression and enzyme synthesis are suppressed. The O_2 concentrations needed to inhibit the processes in already active organisms, in the experiments cited above, were apparently much higher than the ones needed to inhibit the induction of the processes. A combined experimental analysis of gene expression and

activity of the anammox and denitrifier communities at nanomolar O_2 levels is clearly needed.

Nitrous oxide metabolism—From the $^{15}NO_2^-$ incubations it was clear that denitrification produced N_2O as the end-product in some cases but also that N_2O reduction must have been active in the incubations where N_2 was produced from NO_2^-, assuming that N_2 production occurred through the normal denitrification pathway. Incubations with $(^{15}N)_2O$ confirmed this and showed that the double ^{15}N-labeled N_2O was reduced to $^{15}N^{15}N$ (Fig. 8) in agreement with findings in the Arabian Sea (Bulow et al. 2010). The increase in $^{15}N^{15}N$ was linear over the first 22 h but then accelerated dramatically. We assume that the linear section is a measure of the in situ N_2O reduction activity, although the rate may be stimulated by the increase in N_2O concentration from 13–75 nmol L^{-1} (L. Farías et al. unpubl.) in situ to 1 μmol L^{-1} in the incubations. The much higher rate observed later in the incubation was interpreted as an artifact induced by conditions in the bottles, similar to previously observed bottle effects (Kuypers et al. 2005; Thamdrup et al. 2006).

Nitrous oxide was both produced and consumed in this study (Figs. 2, 9), which agrees well with the general understanding of OMZs as sites of active N_2O cycling (Castro-Gonzalez and Farias 2004; Farías et al. 2009; Naqvi et al. 2010). We only found N_2O accumulation in situations where N_2 production by denitrification was not detected and vice versa (Fig. 2), indicating that N_2O reduction was either highly active or not active at all. In agreement with this, the reduction of ^{15}N-labeled N_2O only took place in some of the incubations (Fig. 9). These observations, together with the general accumulation of NO_2^- from NO_3^- reduction in the OMZ (this study and, for example, Codispoti et al. 1986), emphasize that denitrification, the reduction of NO_3^- to N_2 through NO_2^-, NO, and N_2O, should be viewed as a series of individual processes that are not necessarily simultaneously active. The term "stop and go" denitrification has previously been assigned to accumulation of N_2O (Naqvi et al. 2000; Codispoti et al. 2001, 2005), but it may be used more widely to describe situations where parts of the denitrification pathway become active after an electron donor input, stopping when this has been consumed with whatever intermediates that have accumulated (primarily NO_2^-, N_2O), and continuing the reaction sequence once electron donor is again supplied. It is the reduction of NO_2^- to N_2O that removes fixed nitrogen, and this is therefore the key reaction from a nitrogen budget perspective. However, the generally lower N_2O concentrations in the OMZ core than around the upper oxic–anoxic interface indicate a net consumption inside the OMZ (Farías et al. 2009; Naqvi et al. 2010) and, therefore, that most of the N_2O is ultimately consumed within the OMZ, although there may be a net production in some situations.

Nitrogen budget—Previously several attempts have been made to estimate the nitrogen removal in the ESP OMZ using a variety of approaches. We wish to compare those removal rates to the direct estimates of denitrification and anammox, keeping in mind that rates scaled from milliliters to several thousand cubic kilometers must have large uncertainties. Rates in the present study and the rates from previous studies (*see* Fig. 4) were measured at 24–117 km and 15–200 km from the coast, respectively. The rates did not vary systematically with distance from the coast and we argue that these rates can be applied to the 175-km-wide inner part of the ESP OMZ, which was previously estimated to account for 76% of the fixed nitrogen removal in the OMZ core (Codispoti and Packard 1980). However, upwelling and primary production may be especially strong in the innermost 20 km (Yuras et al. 2005) and anammox and denitrification may be higher in this area, which is not covered by the present data set. Based on the distribution of the secondary nitrite maximum, the area of the inner zone was estimated to 326,000 km^2 (Codispoti and Packard 1980). Comparison of nitrite and oxygen distributions during our cruise confirmed that elevated nitrite is a robust indicator of anoxic conditions, and the extent of the anoxic region along the coast was similar to the previous studies (Thamdrup et al. 2012). Our estimated depth-integrated rate of N_2 production of 91 μmol N_2 m^{-2} h^{-1} was 50% of the rate estimated by Codispoti and Packard (1980) based on electron transport system (ETS) activity, and consequently our total upscaled removal of fixed nitrogen of 7.3 Tg N yr^{-1} was similarly half the previous estimate of 14.7 Tg yr^{-1} (Codispoti and Packard 1980). Although substantial biases may be associated with the ETS technique, the ETS-based estimate of fixed nitrogen removal in the entire ETSP of 25 Tg yr^{-1} matches an alternative estimate of 26 Tg N yr^{-1} based on nutrient fields (Deutsch et al. 2001). Possible explanations for these differences include potential methodological biases as discussed above, but also temporal and spatial variability as strongly emphasized by our results. Further investigations of the coupling between inputs of fresh organic detritus and nitrogen transformations in the OMZ core are needed to quantify these dynamics.

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