Revising the nitrogen cycle in the Peruvian oxygen minimum zone

Phyllis Lam1,2, Gaute Lavika3, Marlene M. Jensen1,2, Jack van de Vossenberg4, Markus Schmid1,3, Dagmar Woebken1,4, Dmitri Gutierrez2, Rudolf Amann3, Mike S. M. Jetten5, and Marcel M. M. Kuypers

1Max Planck Institute for Marine Microbiology, D-28359 Bremen, Germany; 2Department of Microbiology, WRR, Radboud University Nijmegen, 6500 HC Nijmegen, The Netherlands; and 3Dirección de Investigaciones Oceanográficas, Instituto del Mar del Perú, Esquina Gamarra y General Valle S/N, Chucuito, Callao 2, Peru

Edited by David M. Karl, University of Hawaii, Honolulu, HI, and approved January 21, 2009 (received for review December 8, 2008)

The oxygen minimum zone (OMZ) of the Eastern Tropical South Pacific (ETSP) is of the 3 major regions in the world where oceanic nitrogen is lost in the pelagic realm. The recent identification of anammox, instead of denitrification, as the likely prevalent pathway for nitrogen loss in this OMZ raises strong questions about our understanding of nitrogen cycling and organic matter remineralization in these waters. Without detectable denitrification, it is unclear how NH4+ is remineralized from organic matter and sustains anammox or how secondary NO3− maxima arise within the OMZ. Here we show that in the ETSP-OMZ, anammox obtains 67% or more of NO2− from nitrate reduction, and 33% or less from aerobic ammonia oxidation, based on stable-isotope pairing experiments corroborated by functional gene expression analyses. Dissimilatory nitrate reduction to ammonium was detected in an open-ocean setting. It occurred throughout the OMZ and could satisfy a substantial part of the NH4+ requirement for anammox. The remaining NH4+ came from remineralization via nitrate reduction and probably from microaerobic respiration. Altogether, deep-sea NO3− accounted for only ~50% of the nitrogen loss in the ETSP, rather than 100% as commonly assumed. Because oceanic OMZs seem to be expanding because of global climate change, it is increasingly imperative to incorporate the correct nitrogen-loss pathways in global biogeochemical models to predict more accurately how the nitrogen cycle in our future ocean may respond.

N
itrogen often is a limiting nutrient to biological production in the oceans, and nitrogen cycling is linked intimately to biological CO2 sequestration via various feedback loops (1, 2). In the conventional paradigm of oceanic nitrogen cycling, dinitrogen gas (N2) becomes bioavailable via N2 fixation. This fixed nitrogen remains in the oceans in various organic and inorganic forms until it is lost to the atmosphere when facultative anaerobic microorganisms respire nitrate (NO3−) in the absence of oxygen and produce N2. Known as “heterotrophic denitrification,” this process for decades has been the only known pathway for oceanic nitrogen loss. This paradigm now is challenged by the recent findings of anammox, the anaerobic ammonium (NH4+) oxidation by nitrate (NO3−) to yield N2 (3), as the likely predominant pathway for nitrogen loss in oceanic oxygen minimum zones (OMZs) off Namibia, Peru, and Chile (4–7). Although OMZ waters constitute only about 0.1% of the ocean volume worldwide, 20% to 40% of the total loss of oceanic nitrogen is estimated to occur in these zones (2, 8, 9).

Anammox is a chemolithoautotrophic process that fixes inorganic carbon with the energy harnessed from N2 production, as opposed to the degradation of organic matter in heterotrophic denitrification. Denitrification is a stepwise reduction process involving a number of intermediates (NO3−→NO2−→NO→N2O→N2), but only when the process proceeds all the way to N2 does it meet the strict definition of denitrification (10). Apart from being a nitrogen sink, heterotrophic denitrification is regarded as the major remineralization pathway in the OMZs, such that heterotrophic bacteria release NH4+ from organic matter when anaerobically respiring NO3−. Nonetheless, the expected NH4+ accumulations have not been observed in the OMZs (11). Although the occurrence of anammox could explain this lack of NH4+ accumulation, the exact NH4+ sources for anammox become unclear without detectable denitrification (4, 7). Moreover, processes leading to secondary NO3− maxima (as opposed to primary NO3− maxima that occur at shallower depths and probably result from phytoplankton growths) and their interactions with anammox in the OMZs are also poorly understood.

Two microbial processes may lead to NO3− production: anaerobic nitrate reduction and aerobic ammonia oxidation. Nitrate reduction to NO3− has been measured previously as a proxy for denitrification in the Eastern Tropical South Pacific (ETSP) (12), but its significance as a standalone process has not been evaluated thus far. Direct coupling between anammox and aerobic ammonia oxidation was reported for the Black Sea suboxic zone even though oxygen concentrations were below detection limits (13). Given the similar suboxic conditions and nitrogen availability, nitrification–anammox coupling also would be highly probable in oceanic OMZs. Meanwhile, in the absence of detectable denitrification in the ETSP, NH4+ for anammox still would have to be remineralized from organic matter via other microbial processes. If nitrate reduction indeed occurs as a heterotrophic process, it also would release NH4+. Another possible source of NH4+ is dissimilatory nitrate reduction to ammonium (DNRA). Until its recent detection in the Namibian inner-shelf bottom waters (14), most studies on DNRA were restricted to fully anoxic, sulfide-rich environments; its potential occurrence in the open ocean remains unexplored.

Here we aimed to assess the microbial processes responsible for the generation of NO3− and NH4+ for anammox in the ETSP OMZ off Peru and the microorganisms involved. Along a 12°S-transect from the inner shelf to offshore open ocean, anammox was detected throughout the OMZ with especially high rates in the upper part of the OMZ on mid-shelf (4). Strong deficits of fixed nitrogen,
Results and Discussion

Functional Gene Expression Analyses for Anammox. Based on the whole-genome data of an enriched marine anammox bacterium, Candidatus Scalindua sp. T23 (17), primers were designed to target specifically the putative cytochrome cd-containing nitrite reductase gene (nirS) that is unique to Candidatus Scalindua but is distinct from denitrifier nirS. The encoding enzyme, similar to that of the anammox bacterium Candidatus Kuenenia stuttgartiensis, is believed to be responsible for the initial nitrite reduction to nitric oxide in anammox (18). In fact, Scalindua nirS genes were detected in the Peruvian OMZ in an abundance significantly correlated with that determined by 16S-rRNA gene-targeted quantitative PCR (4) (Pearson correlation $r = 0.84$, $P < 0.0001$). Furthermore, Scalindua-nirS was strongly expressed, as determined by quantitative RT-PCR, especially in the upper part of the OMZ where anammox rates were high (Fig. 2B), and was positively correlated with anammox bacterial abundance (Spearman $R = 0.66$, $P < 0.05$) (4).

Based on the high-expression-to-gene ratio (mRNA:DNA) of typical denitrifier nirS when detected (mean values $= 63\%$ sequence identity) (supporting information (SI) Fig. S2), these expressed Scalindua-nirS were fairly diverse, but all clustered with the nirS present in the Candidatus Scalindua genome assembly (73%–93% nucleotide sequence identity) and in 2 sequences obtained from the Arabian Sea (19); however, they were clearly different from typical denitrifier nirS ($\geq 63\%$ sequence identity) (supporting information (SI) Fig. S2). Despite the high-expression-to-gene ratio (mRNA:DNA) of typical denitrifier nirS when detected (mean values $= 63\%$ sequence identity) (supporting information (SI) Fig. S2), these expressed Scalindua-nirS were fairly diverse, but all clustered with the nirS present in the Candidatus Scalindua genome assembly (73%–93% nucleotide sequence identity) and in 2 sequences obtained from the Arabian Sea (19); however, they were clearly different from typical denitrifier nirS ($\geq 63\%$ sequence identity) (supporting information (SI) Fig. S2).

Sources of Nitrite. Nitrate, the preferred electron acceptor after $O_2$, was reduced to $NO_3^-$ at high rates ($\geq 3.07 \pm 26\text{ nM d}^{-1}$) throughout

**Fig. 1.** Hydrochemical properties along an east–west transect at 12°S: distribution of (A) nitrate, (B) nitrite, (C) ammonium, (D) $N^*$, (E) phosphate, and (F) oxygen plotted against neutral density ($\text{kg m}^{-3}$). Black-filled circles denote discrete sampling depths at Stations 1 to 7. Station numbers circled in red indicate sampling stations from which the parallel $^{15}$N-rate measurements and gene expression data presented in the current study were obtained.
the OMZ, thereby providing anammox with NO$_2$ (Fig. 2). The measured rates of nitrate reduction were congruent with previously reported values (12) and usually were greater than those of anammox, sometimes by more than an order of magnitude, except in the lower oxic zone offshore (Station 7). Further evidence for nitrate reduction was given by the high abundance and strong expression of the membrane-bound nitrate reductase gene, narG, within the OMZ. The expressed sequences at the anammox rate maximum (Station 4) were verified to be narG by cDNA cloning and comparative sequence analyses. They were affiliated with environmental clones obtained from soils or estuarine sediments, or some with known denitrifiers and nitrate reducers (Fig. S4A). Both the abundance and expression of narG exceeded those of Scalindua-nirS (Figs. 2 and S3), but the mRNA:DNA ratio of narG was far below that of Scalindua-nirS (mean = 1% and 49%, respectively). This difference might reflect the facultative nature of nitrate-reducing (narG) potential despite its relative ubiquity among microbes, if the stability of the 2 types of mRNA were similar. Nevertheless, the transcriptional regulatory network and behavior for these 2 genes in various microbes are not sufficiently understood at this point to verify this interpretation further. Although periplasmic nitrate reductase (NAP), unlike the membrane-bound counterpart (NAR), is not necessarily used in respiratory nitrate reduction (20), the expression of the encoding gene (napA) also was considerably elevated at anammox depths. The identities of these expressed napA genes were confirmed via cDNA sequence analyses. Their closest relatives included estuarine sediment clones and the photosynthetic, nitrate-reducing α-proteobacterium Rhodobacter capsulatus (Fig. S4B). Nitrate reduction is the first essential step in denitrification, but more organisms are capable of nitrate reduction than of complete denitrification (10). Hence, the finding of nitrate reduction but no denitrification in the Peruvian OMZ is not surprising.
theless, do indicate that both groups are actively involved and at
tent characteristic rate-to-gene-expression relationships, our data
anammox. Because different groups of organisms may have differ-
however, was that crenarchaeal

parts with respect to gene abundance, but at lower expression levels

tively (Fig. S5). Similar to the Black Sea suboxic zone (13),

affiliated with

it was undetectable in the lower OMZ offshore (Station 7) (Fig.

added in parallel incubations, indicating the occurrence of mi-
allylthiourea, an inhibitor of aerobic ammonium oxidation, was

rates (17–144 nM N d

Lam et al. PNAS

15N-rate measurements corroborated by gene expression analyses

production, in the upper OMZ, but it was

were more abundant than its bacterial counter-

were released for every mole of

Aerobic ammonium oxidation produced at least 65% (> 100% in

all but 2 cases) of the NO2 required for anammox, or 6% to 33% of

the total NO2 production, in the upper OMZ, but it was

undetectable in the lower OMZ offshore (Station 7), based on

Sources of Ammonium. Apart from NO2 production, nitrate reduc-
tion as a heterotrophic process involves the degradation of organic

nitrate reduction could meet a substantial proportion

requirement by anammox on shelf stations (16%–100%

Table 1. Estimated depth-integrated NO and NH4+ sources and sinks in the Peruvian OMZ, calculated as net fluxes with the unit of

mmol N m⁻² d⁻¹

Table 1. Estimated depth-integrated NO and NH₄⁺ sources and sinks in the Peruvian OMZ, calculated as net fluxes with the unit of

mmol N m⁻² d⁻¹

<table>
<thead>
<tr>
<th>Sources and Sinks</th>
<th>Inner Shelf: Station 2</th>
<th>Mid-shelf: Station 4</th>
<th>Offshore: Station 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper OMZ 25–50 m</td>
<td>Lower OMZ 50–94 m</td>
<td>Overall OMZ</td>
</tr>
<tr>
<td></td>
<td>Upper OMZ 25–60 m</td>
<td>Lower OMZ 60–140 m</td>
<td>Overall OMZ</td>
</tr>
<tr>
<td></td>
<td>Upper OMZ 25–100 m</td>
<td>Lower OMZ 100–600 m</td>
<td>Overall OMZ</td>
</tr>
<tr>
<td>NO3⁻ sources</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₄⁺ oxidation</td>
<td>1.6</td>
<td>0.6</td>
<td>2.2</td>
</tr>
<tr>
<td>NO2⁻ oxidation</td>
<td>3.8</td>
<td>4.3</td>
<td>8.1</td>
</tr>
<tr>
<td>NO2⁻ reduction</td>
<td>5.1</td>
<td>9.9</td>
<td>15.0</td>
</tr>
<tr>
<td>Total</td>
<td>6.7</td>
<td>10.5</td>
<td>17.2</td>
</tr>
<tr>
<td>NO3⁻ sinks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*NO2 oxidation</td>
<td>3.8</td>
<td>4.3</td>
<td>8.1</td>
</tr>
<tr>
<td>Anammox</td>
<td>0.6</td>
<td>0.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td>4.4</td>
<td>5.2</td>
<td>9.6</td>
</tr>
<tr>
<td>NH₄⁺ oxidation</td>
<td>1.6</td>
<td>0.6</td>
<td>2.2</td>
</tr>
<tr>
<td>*Assimilation</td>
<td>0.5</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Anammox</td>
<td>0.6</td>
<td>0.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td>2.7</td>
<td>2.0</td>
<td>4.7</td>
</tr>
<tr>
<td>NH₄⁺ sinks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₄⁺ oxidation</td>
<td>1.6</td>
<td>0.6</td>
<td>2.2</td>
</tr>
<tr>
<td>*Assimilation</td>
<td>0.5</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Anammox</td>
<td>0.6</td>
<td>0.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td>2.7</td>
<td>2.0</td>
<td>4.7</td>
</tr>
</tbody>
</table>

*From Lipschultz et al. (12).

**Amounts of NH₄⁺ produced based on the measured ¹⁵NO₂⁻ - reduction rates and stoichiometry of Eq. 1.

***Amounts of NH₄⁺ produced based on the measured ¹⁵N-DNRA rates and stoichiometry of Eq. 2.

⁴Amounts of additional NH₄⁺ source(s) required to achieve an assumed NH₄⁺ balance.

⁵Likely attributed to DNRA in this case.

Despite the very low to nondetectable oxygen concentrations (conventional detection limit: 1.5–2 μM), high ¹⁵NH₄⁺ oxidation rates (17–144 nM N d⁻¹), measured as ¹⁵NO₂⁻ production in ¹⁵NH₄⁺/¹⁰⁶NO₂⁻ incubations, were observed within the upper OMZ along with high anammox rates (16–279 nM N d⁻¹) (4), sometimes even exceeding those in shallower oxic depths (e.g., Stations 4 and 7) (Fig. 2D). No significant ¹⁵NO₂⁻ production was observed when allythiourea, an inhibitor of aerobic ammonium oxidation, was added in parallel incubations, indicating the occurrence of microaerobic ammonium oxidation. Although ¹⁵NH₄⁺ oxidation was still detectable in the lower OMZ on shelf stations (Stations 2 and 4), it was undetectable in the lower OMZ offshore (Station 7) (Fig. 2D). These results were consistent with some previous reports on nitrification in the ETSP (12, 21). Further support for microaerobic (≤ 10 μM O₂) NH₄⁺ oxidation was provided by an independent study of amoA expression in unmanipulated water samples. The functional gene amoA encodes for the subunit A of ammonia monooxygenase, a key enzyme in aerobic ammonium oxidation that requires oxygen for activation. Strong expression of amoA was exhibited by both crenarchaeal and bacterial ammonia oxidizers, especially in the upper OMZ (Fig. 2D). The expressed crenarchaeal amoA formed 2 subclusters with other marine pelagic sequences, whereas the expressed β- and γ-proteobacterial amoA were affiliated with Nitrosospira spp. and Nitrosococcus oceansi, respectively (Fig. S5). Similar to the Black Sea suboxic zone (13), crenarchaeal amoA was more abundant than its bacterial counterparts with respect to gene abundance, but at lower expression levels (Fig. S3). There were tight associations between anammox and crenarchaeal amoA gene abundance based on correlation (Spearman R = 0.57, P < 0.005) and principal component analyses (SI Text, Table S1, and Fig. S6). The difference in the Peruvian OMZ, however, was that crenarchaeal amoA was expressed alongside anammox. Because different groups of organisms may have different characteristic rate-to-gene-expression relationships, our data here could not determine the relative importance of bacterial versus crenarchaeal ammonia oxidizers in nitrification. These data, nonetheless, do indicate that both groups are actively involved and at which depths where individual groups are more likely to be active.
associations were supported further by principal component analyses (SI Text).

Nevertheless, a large NH4\textsuperscript{+} source still was unaccounted for in the upper OMZs at all stations where the highest anammox rates were measured, as well as in the lower OMZ offshore (Station 7). Another potential NH4\textsuperscript{+} source could be DNRA, in which NH4\textsuperscript{+} originates from both NO3\textsuperscript{-} and organic matter:

\[
(\text{CH}_2\text{O})_{106}(\text{NH}_3)_16\text{H}_2\text{PO}_4 + 53 \text{NO}_3^- + 122 \text{H}^+ \rightarrow 106 \text{CO}_2 \\
+ 69 \text{NH}_4^+ + 53 \text{H}_2\text{O} + \text{H}_3\text{PO}_4
\]  

[2]

Indeed, significant 15NH4\textsuperscript{+} production could be detected in 15NO3\textsuperscript{-} incubations throughout the OMZ, with the highest rates reported for the upper OMZ on the shelf (Fig. 2E) coinciding with high anammox rates. The biomarker functional gene for DNRA, cytochrome c nitrite reductase gene nrfA, also was strongly expressed throughout the OMZ (Fig. 2F). These expressed sequences were verified to be nrfA (Fig. S7) by cDNA sequence analyses. Their phylogenetic affiliations with known sequences perhaps are not very informative at this point, because most nrfA sequences currently available in public databases come from genome sequences of culture collections in which the majority of cultures are pathogens. Most research on DNRA to date has focused on strictly anoxic environments, but DNRA never has been identified as a significant NO3\textsuperscript{-} sink in an open-ocean setting and linked to nitrogen loss. In the Peruvian OMZ, our measured DNRA rates were sufficient to fuel 7% to 134% and 7% to 34% of the NH4\textsuperscript{+} needed by anammox at the shelf and offshore stations, respectively.

Although nitrate reduction and DNRA combined appeared to produce more than enough NH4\textsuperscript{+} for anammox in the lower OMZ on the shelf, if all potential NH4\textsuperscript{+} sources and sinks were considered, some sources of NH4\textsuperscript{+} still needed to be identified at all stations (Table 1). The occurrence of ammonia oxidation and nitrite oxidation (12, 21), particularly in the upper OMZ, strongly suggested microaerobic conditions. In fact, oxygen concentrations up to \(\approx 10 \mu\text{M} (\approx 0.25 \text{ ml l}^{-1})\) were detected in the lower OMZ on mid-shelf (Stations 3–5) (Figs. 1 and S1). Nitrate reduction may be less sensitive to oxygen than the subsequent steps in the denitrification sequence (\(\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO}_\text{aq}^{-} \rightarrow \text{N}_2\)) (22), and anammox bacteria have been found to be microaerotolerant (active up to \(\approx 10 \mu\text{M} \text{O}_2\)) in the marine environment (23). Therefore, the detection of nitrate reduction and anammox was in line with the suggested microaerobic conditions in the upper OMZ just below the oxycline, as well as in the lower OMZ on the shelf. Lipschultz et al. (12) also pointed out the possible presence of oxygen in the ETSP OMZ and detected nitrate reduction therein. However, the exact extent of oxygen penetration in the OMZ would require further verification with more sensitive oxygen measurements (detection limit \(\approx 1.5–2 \mu\text{M}\)). Because oxygen is the most preferred electron acceptor, microaerobic remineralization of organic matter could proceed and release more NH4\textsuperscript{+} than nitrate reduction and DNRA at these depths. Its occurrence also would be consistent with the elevated levels of NH4\textsuperscript{+} in the upper boundaries of the OMZs, as well as in the lower OMZ on mid-shelf where O2 seemed to be slightly elevated (Fig. 1). Even at the anammox rate maxima, the required remineralization would need less than 0.7 \mu\text{M} of O2, or less than 1.2 \mu\text{M} taking into account the O2 requirement by ammonia oxidation, a level that remains below the limits of conventional methods of O2 detection. Such microaerobic remineralization could release enough NH4\textsuperscript{+} to fulfill the remaining needs for NH4\textsuperscript{+} on the shelf and in the upper OMZ offshore.

In the lower OMZ offshore (Station 7), the low to nondetectable nitrification rates and amoA expression indicated that microaerobic remineralization is not significant. Although the presence of relatively high 14NO3\textsuperscript{-} concentrations in our incubations enabled us to capture most, if not all, of the 15NO3\textsuperscript{-} produced for nitrate reduction and ammonium oxidation rate measurements, the same did not always apply for the 15NH4\textsuperscript{+} production measurements for DNRA. The ambient NH4\textsuperscript{+} concentrations were especially close to or below detection level in the lower OMZ offshore, so that some of the 15NH4\textsuperscript{+} produced in the 15NO3\textsuperscript{-} incubations might have been taken up by other NH4\textsuperscript{+}-consuming processes and gone undetected. Thus, the net DNRA rates measured are likely be lower than the actual gross rates. Consequently, DNRA, a process that does not consume oxygen, might be an even more important source of NH4\textsuperscript{+} in the offshore lower OMZ. This possibility also would be consistent with the increase in nrfA expression and DNRA rates with depth within this zone, where nitrate reduction rates (as well as narG and naraA expression) were reduced, but anammox rates remained comparable to those in the overlying upper OMZ. On the other hand, the possibilities that anammox bacteria might themselves perform DNRA in the presence of small organic compounds (14) or that NH4\textsuperscript{+} might be released in fermentative reactions cannot be fully excluded at this point.

**Fig. 3.** A revised nitrogen cycle in the Peruvian OMZ. Anammox (yellow) has been found to be the predominant pathway for nitrogen loss and was coupled directly to nitrate reduction (red) and aerobic ammonia oxidation (the first step of nitrification, green) for sources of NO3\textsuperscript{-}. The NH4\textsuperscript{+} required by anammox originated from DNRA (blue) and remineralization of organic matter via nitrate reduction and probably from microaerobic respiration. Microaerobic conditions, at least in the upper part of the OMZ, were suggested by the occurrence of nitrification, which diminishes in importance from shelf to open ocean and in the lower OMZ. In contrast, NH4\textsuperscript{+} production caused by nitrate reduction and DNRA became increasingly important in the lower OMZ and offshore. Assim (gray) denotes assimilation. Remin (brown) denotes remineralization. Nitrogen fixation (gray dashes) might be coupled spatially to nitrogen loss near the OMZ but has not been assessed in this study.

**Conclusions and Perspectives**

A considerably different and complex picture of nitrogen cycling has emerged in the Peruvian OMZ (Fig. 3). Our results based on both 14N-incubation experiments and molecular analyses indicate that anammox is the predominant pathway for nitrogen loss (4) and is coupled directly to multiple aerobic and anaerobic nitrogen transformations. Nitrate reduction provides anammox with NO2\textsuperscript{-} and NH4\textsuperscript{+} for denitrification. The ambivalent NH4\textsuperscript{+} concentrations were especially close to or below detection level in the lower OMZ offshore, so that some of the 15NH4\textsuperscript{+} produced in the 15NO3\textsuperscript{-} incubations might have been taken up by other NH4\textsuperscript{+}-consuming processes and gone undetected. Thus, the net DNRA rates measured are likely be lower than the actual gross rates. Consequently, DNRA, a process that does not consume oxygen, might be an even more important source of NH4\textsuperscript{+} in the offshore lower OMZ. This possibility also would be consistent with the increase in nrfA expression and DNRA rates with depth within this zone, where nitrate reduction rates (as well as narG and naraA expression) were reduced, but anammox rates remained comparable to those in the overlying upper OMZ. On the other hand, the possibilities that anammox bacteria might themselves perform DNRA in the presence of small organic compounds (14) or that NH4\textsuperscript{+} might be released in fermentative reactions cannot be fully excluded at this point.
alized nitrogen. Remineralized NH$_4^+$ thus may play a much more important role in oceanic nitrogen loss than previously thought. It would require the remineralization of about 3.5 to 7 times the amount of Redfieldian organic matter (C:N:P = 106:16:1) (24) than the estimates based on denitrification stoichiometry. However, because of the constraints imposed by other closely associated elemental cycles (e.g., carbon and phosphorus) (2), such an increase in the remineralization of Redfieldian organic matter may not be realistic. Alternatively, remineralized NH$_4^+$ might come from preferential degradation of organic nitrogen over carbon in suboxic settings (25), or the remineralization of nitrogen-enriched organic matter might result from the spatially coupled N$_2$ fixation over the OMZ (26, 27). In either case, calculations of nitrogen loss based on nitrate deficit alone would be underestimates, possibly explaining the discrepancies between the estimates of nitrogen loss based on nitrate deficits and excess N$_2$ (see ref. 8). However, the degree of such underestimations would need evaluated further via larger-scale experiments and modeling studies. The OMZs are expanding in global oceans (28), and more ocean volumes are becoming subjected to nitrogen loss. At the same time, atmospheric anthropogenic nitrogen input is increasing rapidly (29). In theory, this additional input would increase marine primary production and thus marine CO$_2$ sequestration (29), but whether positive or negative feedbacks may ensue via subsequent remineralization of organic nitrogen and nitrogen losses becomes an urgent research question. At this time of rapid global change, it is increasingly imperative to incorporate the correct nitrogen-loss mechanisms in global biogeochemical models, in order to more accurately assess the current oceanic nitrogen balance accurately and to more precisely predict the closely linked nitrogen and carbon cycles in the future Ocean will respond.

**Materials and Methods**

**Water Sampling and $^{15}$N-Isotope Pairing Experiments.** Water sampling was conducted in April 2005. Details of site descriptions, sampling, physico-chemical analyses, and $^{15}$N stable-isotope pairing experiments measuring anammox and the denitrification rate have been described previously (4). In the $^{15}$N incubations, the rates of nitrate reduction and aerobic ammonia oxidation were determined as net $^{15}$NO$_3^-$ production in the $^{15}$NO$_3^-$ and $^{15}$NH$_4^+$–$^{14}$NO$_2^-$ incuba-
