The effect of organic carbon on fixed nitrogen loss in the eastern tropical South Pacific and Arabian Sea oxygen deficient zones

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Abstract
The three major oxygen deficient zones (ODZs) of the world oceans (eastern tropical North and South Pacific [ETNP and ETSP, respectively], and Arabian Sea [AS]) host the vast majority of pelagic fixed nitrogen (N) loss and up to half of total marine N loss. The input of organic matter is an important control on the absolute and relative importance of the two main pathways of N removal (denitrification and anammox). We investigated the response of N loss in the ETSP and AS ODZs to additions of organic matter in the form of glucose and naturally derived dissolved and particulate organic matter (DOM and POM, respectively). In the ETSP ODZ, the addition of glucose stimulated denitrification (1.6-fold increase after 5 d) but not anammox (14-fold decrease after 5 d). In the AS ODZ, only POM, not DOM, significantly increased rates of denitrification at the base of the oxycline (5.4–6.4-fold increase after 2 d), but not at the secondary nitrite maximum. These results suggest that denitrification was generally limited by organic matter supply at the time of this study in both the ETSP and AS ODZs, although the lability of the organic matter supplied was important. Interestingly, 15N2 produced in ETSP and AS incubations was not binomially distributed relative to the reactants after the influence of anammox was taken into account, suggesting an unknown production mechanism or pathway of N removal.

The vast majority of pelagic fixed nitrogen (N) removal occurs in three major oxygen deficient zones (ODZs) of the world: the eastern tropical North and South Pacific (ETNP and ETSP, respectively), and the Arabian Sea (AS). Although they comprise < 1% of the total volume of the ocean (Codispoti et al. 2001), the ODZs are responsible for at least a quarter of total marine N loss (Codispoti et al. 2001). Wind-driven upwelling stimulates high productivity in the overlying waters of the ODZs, which sinks and fuels substantial respiration at depth. Combined with poor ventilation of these regions, the result is a depletion of water-column oxygen to the extent that it becomes thermodynamically favorable for microbes to utilize NO3−, IO3− (Farrenkopf and Luther 2002), oxidized metals (Luther et al. 1997; Moffett et al. 2007), and SO42− (Canfield et al. 2010) as electron acceptors during organic matter oxidation. Within the open-ocean ODZs, the majority of organic matter respired is ultimately coupled to the reduction of NO3−. Although the SO42− concentration is orders of magnitude higher than the NO3− concentration, there is no accumulation of H2S, implying reduced S is efficiently reoxidized, likely via direct or indirect coupling to the reduction of NO3− (Canfield et al. 2010). Numerous transformations of fixed N can occur; however, once converted to N2O or N2, N becomes biologically inaccessible except to N2-fixing microbes.

The two major pathways of fixed N loss in the ODZs are denitrification and the anaerobic oxidation of ammonium (anammox). Denitrification is a heterotrophic process that proceeds via a stepwise process in which organic carbon oxidation is coupled to the sequential reduction of NOx to gaseous end products: NO3− → NO2− → NO → N2O (g) → N2 (g). Anammox is an autotrophic process, gaining energy from the oxidation of NH4+ to N2 using NO2−. Because of the importance of the three major ODZs to the balance of fixed N in the ocean, there has been substantial interest in determining the contribution of these two processes to total pelagic N removal, especially given the potential of these regions to change with changing climate.

15N-labeling experiments have suggested that anammox is responsible for the majority of N2 production in the ETSP (Thamdrup et al. 2006; Hamersley et al. 2007; Kalvelega et al. 2013) and AS (Jensen et al. 2011) ODZs. In contrast, other studies in the ETSP (Dalsgaard et al. 2012) and the AS ODZs (Nicholls et al. 2007; Ward et al. 2009) identified heterotrophic denitrification to be the dominant N loss pathway. There is evidence that both denitrification and anammox are regulated by the availability of organic matter in the ODZs (Ward et al. 2008; Kalvelega et al. 2013), and this discrepancy in the relative contributions of denitrification and anammox to N removal in the ETSP and AS ODZs has been ascribed to the differing responses of anammox and denitrifying bacteria to organic matter.
availability (Thamdrup et al. 2006; Ward et al. 2009; Dalsgaard et al. 2012). Seasonally changing wind patterns in all three ODZs give rise to temporally varying productivity, which, in turn, affect the timing and quantity of organic matter reaching the ODZs (Lee et al. 1998). If the flux of organic matter to the ODZs is linked to surface productivity, the degree to which carbon is available to microbes in either the ETSP or AS ODZ would also be both spatially and temporally heterogeneous. Denitrifiers have been found to be abundant and diverse, capable of rapid growth in response to episodic inputs of organic matter (Ward et al. 2008). Anammox bacteria grow more slowly (Van de Graaf et al. 1995) and may maintain a lower though more constant rate of N removal in the ODZs. Although anammox is itself an autotrophic process, the substrates anammox depends upon (i.e., NH$_4^+$ and NO$_2^-$) are produced by the oxidation of organic matter. NH$_4^+$ is generated at each step of the successive reduction of N-oxides during heterotrophic denitrification. Additionally, dissimilatory nitrate reduction to ammonium (Lam et al. 2009; Jensen et al. 2011) and sulfate reduction (Canfield et al. 2010), both heterotrophic processes, may supply anammox with NH$_4^+$. NO$_2^-$ is formed via the reduction of NO$_3^-$ during the oxidation of organic matter. NO$_3^-$ might also be produced by NH$_4^+$ oxidation (Kalvelage et al. 2013); however, this pathway depends on the availability of NH$_4^+$ and thus, organic matter oxidation.

The objectives of this work were to investigate the effect of organic matter quality and availability on the rates of N loss in the ETSP and AS ODZs. In the ETSP ODZ, we measured the rates and relative contributions of denitrification and anammox in incubations with and without the addition of a simple organic compound (glucose). In the AS ODZ, we used freshly collected dissolved organic matter (DOM) and sinking particulate organic matter (POM) from sediments traps deployed directly above the ODZ in order to assess the response of N$_2$ production to naturally derived organic matter. We chose these forms of organic matter because although bacteria most readily take up DOM, a significant source of DOM in the interior of the ocean is the degradation of POM. In addition to acting as a source of DOM, bacteria directly colonize POM and take advantage of living adjacent to both substrate and other bacteria performing complementary redox transformations (Karl et al. 1984).

**Methods**

*Study site and sample collection—* Incubation experiments were carried out at one station in the ETSP ODZ aboard the R/V Knorr (October–November 2005) and at two stations in the AS ODZ aboard the R/V Roger Revelle (September–October 2007; Table 1). Samples were collected using a rosette of 10 liter or 30 liter Niskin bottles equipped with dissolved oxygen (O$_2$) sensors calibrated by Winkler titrations, in addition to conductivity, temperature, and pressure sensors.

Sampling depths were chosen based on O$_2$ and nitrite (NO$_2^-$) concentrations; at the secondary NO$_3^-$ maximum (SNM), and at the shallowest depth where O$_2$ was undetectable (base of the oxycline; in AS only). The base of the oxycline was chosen based on previous studies, which have reported the highest rates of N$_2$ production at the top of the ODZ, possibly due to the relatively high flux of POM compared with deeper in the ODZ. The SNM was also sampled because it is associated with the most oxygen depleted waters (Thamdrup et al. 2012) and has classically been regarded as a zone of active N loss (Codispoti and Christensen 1985).

Incubations were carried out in duplicate in acid-washed, large-volume, gas-tight trilaminate bags (Pollution Management Corporation) fitted with three-way stopcocks. Prior to sampling, bags were flushed at least three times with CO$_2$ to eliminate O$_2$ from any possible headspace and evacuated. Approximately 8 liters of water were gravity-fed into each bag directly from a Niskin bottle, taking care to prevent any contact with the atmosphere. The headspace of each Niskin bottle was continuously flushed with CO$_2$ through the vent to prevent atmospheric O$_2$ contamination during sampling. Bags received additions of $^{15}$N-labeled NO$_3^-$ (in ETSP only), NO$_2^-$ (in AS only), or NH$_4^+$ (in both, Table 2).

In the ETSP, DOM in the form of glucose was also added to $^{15}$NO$_3^-$ and $^{15}$NH$_4^+$ labeled incubations to a final concentration of 2 μmol C L$^{-1}$. In the AS experiments, $^{15}$NO$_3^-$ labeled incubations were amended with POM and DOM collected in situ from the AS ODZ (*see* below for

**Table 1.** Station locations, sampling depths, and associated hydrographic characteristics. [O$_2$] measured by Seabird O$_2$ sensor, detection limit $\sim$ 1 μmol L$^{-1}$. Detection limits of NO$_3^-$, NO$_2^-$, and NH$_4^+$ were 0.08, 0.01, and 0.07 μmol L$^{-1}$, respectively. ETSP—eastern tropical South Pacific; AS—Arabian Sea; SNM—secondary NO$_3^-$ maximum; nd—not detected.

<table>
<thead>
<tr>
<th>Station</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Bottom depth (m)</th>
<th>Sampling depth (m)</th>
<th>Feature</th>
<th>[O$_2$] (μmol L$^{-1}$)</th>
<th>[NO$_3^-$] (μmol L$^{-1}$)</th>
<th>[NO$_2^-$] (μmol L$^{-1}$)</th>
<th>[NH$_4^+$] (μmol L$^{-1}$)</th>
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<tbody>
<tr>
<td>ETSP</td>
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<tr>
<td>20</td>
<td>13.3’S</td>
<td>77’W</td>
<td>788</td>
<td>260</td>
<td>SNM</td>
<td>nd</td>
<td>24.3</td>
<td>4.4</td>
<td>0.1</td>
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<td>AS</td>
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</tr>
<tr>
<td>1</td>
<td>19.4’N</td>
<td>66.7’E</td>
<td>3095</td>
<td>100</td>
<td>base of oxycline</td>
<td>nd</td>
<td>22.3</td>
<td>1.9</td>
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<td>150</td>
<td>SNM</td>
<td>nd</td>
<td>15.1</td>
<td>5.7</td>
<td>nd</td>
</tr>
<tr>
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<td>64’E</td>
<td>3900</td>
<td>150</td>
<td>base of oxycline</td>
<td>nd</td>
<td>10.7</td>
<td>3.7</td>
<td>nd</td>
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<td>200</td>
<td>SNM</td>
<td>nd</td>
<td>7.6</td>
<td>7.7</td>
<td>nd</td>
</tr>
</tbody>
</table>
Ambient and tracer concentrations of $\text{NO}_3^-$, $\text{NO}_2^-$, and $\text{NH}_4^+$ in all incubations were measured by autoanalyzer using standard colorimetric techniques (Strickland and Parsons 1972). Additionally, in the ETSP incubations, $\text{NO}_3^-$ and $\text{NO}_2^-$ were measured by autoanalyzer at $\approx 12$ h intervals for the length of the incubation. Detection limits of $\text{NO}_3^-$, $\text{NO}_2^-$, and $\text{NH}_4^+$ were 0.08, 0.01, and 0.07 $\mu$mol L$^{-1}$, respectively.

$^{15}$N-labeled incubations—Samples for the determination of $^{29}$N$_2$ and $^{30}$N$_2$ were taken following the method of Emerson et al. (1999). Approximately 150 mL of water was drawn from the Niskin bottles (representing initial N$_2$ gas and isotope concentrations) or from a bag into 300 mL HgCl$_2$-poisoned, pre-evacuated glass flasks equipped with 9 mm, gas-tight, single o-ring valves (Louwers–Hapert). The flasks were returned to the University of Washington where they were weighed and dissolved gases were equilibrated with the headspace of the flask at a constant temperature for 24 h. The headspace gases were transferred to a stainless steel finger immersed in liquid He, during which water and CO$_2$ were trapped cryogenically. Samples were analyzed on a Finnigan Delta XL dual-inlet isotope ratio mass spectrometer for mass ratios 29:28, 30:28, 28:40, relative to an in-house gas standard with known gas and isotope ratios. Anammox and denitrification rates were calculated from the production of $^{15}$N-labeled N$_2$ following de Brabandere et al. (2013).

Statistical analyses—The effects of treatments on the rates of N$_2$ production in the AS incubations were examined using an analysis of variance (ANOVA). The factors affecting N$_2$ production that were considered were location (Sta. 1 or 2), depth (base of oxycline or SNM), $^{14}$NH$_4^+$ (with or without), and organic matter (control, POM, and DOM). A post hoc comparison using Tukey’s HSD test was used to evaluate differences in organic matter treatments. All statistical analyses were performed using SPSS version 13.

Results

Hydrographic conditions—At the ETSP study site, the ODZ (defined as the minimum value reached by the Seabird oxygen sensor mounted to the sampling rosette) extended from $\approx 75$ to 400 m. The mixed layer was shallow ($< 20$ m, defined as a change in $\sigma_0 > 0.03$ kg m$^{-3}$ [de Boyer Montégut et al. 2004]), sea surface temperature (SST) was relatively low (15.3°C), and $[\text{NO}_3^-]$ and $[\text{PO}_4^{3-}]$ were relatively high in surface waters (5.9 and 0.8 $\mu$mol L$^{-1}$, respectively), all of which are indicative of active upwelling. $[\text{NO}_2^-]$ was detectable at the surface (0.2 $\mu$mol L$^{-1}$) and formed a broad secondary maximum within the ODZ (maximum 4.4 $\mu$mol L$^{-1}$). A distinct primary $\text{NO}_2^-$ maximum (PNM) was not detected, probably due to the sampling distribution being too coarse to resolve the narrow peak. $[\text{NH}_4^+]$ was as high as 0.3 $\mu$mol L$^{-1}$ in the mixed layer, decreasing to below detection ($< 0.07$ $\mu$mol L$^{-1}$) in and below the ODZ.

The AS field campaign took place at the end of the high-productivity southwest monsoon, although conditions were

<table>
<thead>
<tr>
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<th>$^{15}$N-tracer*</th>
<th>Treatment</th>
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<tbody>
<tr>
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<td>20 SNM</td>
<td>$^{15}$NO$_3^-$ (2)</td>
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<td></td>
<td></td>
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<td>Glucose</td>
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<td></td>
<td></td>
<td>$^{15}$NH$_4^+$ (1)</td>
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<td></td>
<td></td>
<td>$^{15}$NH$_4^+$ (1)</td>
<td>Glucose</td>
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<tr>
<td>AS</td>
<td>1 SNM, base of oxycline</td>
<td>$^{15}$NO$_3^-$ (5)</td>
<td>—</td>
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<td>$^{15}$NO$_3^-$ (5)</td>
<td>POM *</td>
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<td>$^{15}$NO$_3^-$ (5)</td>
<td>DOM *</td>
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<td>$^{15}$NH$_4^+$ (5)</td>
<td>$^{14}$NO$_2^-$</td>
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<td>2 SNM, base of oxycline</td>
<td>$^{15}$NO$_3^-$ (5)</td>
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<td>$^{15}$NO$_3^-$ (5)</td>
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<td>$^{15}$NO$_3^-$ (5)</td>
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<td>$^{15}$NO$_3^-$ (5)</td>
<td>POM + $^{14}$NH$_4^+$ (5)</td>
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<td>$^{15}$NO$_3^-$ (5)</td>
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<td>$^{15}$NO$_3^-$ (5)</td>
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<td>$^{15}$NH$_4^+$ (5)</td>
<td>$^{14}$NO$_2^-$</td>
</tr>
</tbody>
</table>

* Target concentration of $^{15}$N tracer and $^{14}$NH$_4^+$ in parentheses ($\mu$mol L$^{-1}$). † Both organic matter treatments came from sediment traps deployed $\approx 20$ and 50 m above the ODZ proper at Sta. 1 and 2, respectively. See text for details of preparation.
already transitioning to the more oligotrophic autumn intermonsoon as indicated by relatively high SST (28.8°C) and a shallow mixed layer (40 m) in which \([\text{NO}_3^-]\) and \([\text{PO}_4^{3-}]\) were undetectable. Hydrographic conditions were previously reported by Ward et al. (2009), which we will summarize here. A distinct PNM was present at the base of the mixed layer (maximum 0.9 and 2.8 μmol L\(^{-1}\), at Sta. 1 and 2, respectively), underlain by a much larger SNM (maximum 5.7 and 7.7 μmol L\(^{-1}\), at Sta. 1 and 2, respectively), which extended from ~100–150 to 400 m. At the more northern station sampled (Sta. 1), O\(_2\) was undetectable from ~100 to 800 m. At the more southern station sampled (Sta. 2), the ODZ was slightly thinner, extending from 150 to 800 m. \([\text{NH}_4^+]\) was as high as 0.6 μmol L\(^{-1}\) in the mixed layer, decreasing to below detection (< 0.07 μmol L\(^{-1}\)) in and below the ODZ.

**N transformations and loss in ETSP ODZ incubations**—
At Sta. 20 in the ETSP, in the incubations with glucose, the initial concentration of 24 μmol L\(^{-1}\) NO\(_3^-\) was rapidly decreased to < 1 μmol L\(^{-1}\) in < 3 d, and undetectable by 5.5 d (Fig. 1A). There was some NO\(_3^-\) accumulation until 1.5 d, after which NO\(_3^-\) was quickly consumed to undetectable levels by 4.5 d (Fig. 1B). This pattern of nutrient consumption was in contrast to the incubation in which no organic carbon was added. In these incubations, NO\(_3^-\) remained unchanged or even slightly increased until ~3 d, after which NO\(_3^-\) steadily decreased to undetectable levels by 11.5 d. Nitrite decreased ~ 1 μmol L\(^{-1}\) for 1 d before slowly increasing to maximum values by 9.5–10 d followed by rapid consumption to undetectable levels by 11.5 d.

Samples for \(^{15}\text{N}_2\) production were taken at 5.7 d (Fig. 2). In the control incubations (only \(^{15}\text{N}\) tracers added), the average denitrification rate over the length of the incubation was 2.6 nmol N\(_2\) h\(^{-1}\), and the average anammox rate was 0.9 nmol N\(_2\) h\(^{-1}\) over the course of the incubation. In the incubations amended with glucose, the average denitrification rate was 4.2 nmol N\(_2\) h\(^{-1}\) during the length of the incubation and the average anammox rate was 0.06 nmol N\(_2\) h\(^{-1}\) over the course of the incubation.

**Sediment-trap organic matter composition in the AS**—AS DOM (trap leachates) had an atomic C:N ratio of 9.3. This ratio is higher than that of the particulate trap flux, which had an average C:N ratio of 7.8.

**N loss in AS ODZ incubations**—The addition of \(^{14}\text{NH}_4^+\) had no significant effect on \(^{29}\text{N}_2\) or \(^{30}\text{N}_2\) production in the AS regardless of station, depth, or organic matter addition (\(F_{1,21} = 0.486, p > 0.05, \text{data not shown}\)); therefore, all rates with and without organic matter additions are presented as an average of the treatments with and without \(^{14}\text{NH}_4^+\).

In the AS incubations, samples for \(^{15}\text{N}_2\) production were taken at ~2 d. \(^{15}\text{N}_2\) production rates by anammox (in \(^{15}\text{NH}_4^+\) amended incubations) and denitrification (in \(^{15}\text{NO}_3^-\) amended incubations with no organic matter additions) were previously published in Ward et al. (2009). We present them here as the ‘control’ to assess the effect of organic matter additions on N\(_2\) production rates. At Sta. 1, in the control incubations (no organic matter additions), the average denitrification rates over the length of the incubations were 0.21 ± 0.14 and 0.15 ± 0.06 nmol N\(_2\) h\(^{-1}\), at the base of the oxycline and SNM, respectively (Fig. 3A). At Sta. 2, in the control incubations, the average denitrification rates over the course of the incubations were 0.06 ± 0.02 and 0.05 ± 0.02 nmol N\(_2\) h\(^{-1}\), at the base of the oxycline and SNM, respectively (Fig. 3B).

The two organic carbon treatments, DOM and POM collected freshly from sediment traps, had different effects on denitrification rates (determined from \(^{30}\text{N}_2\) production) in the AS ODZ (Fig. 3A,B). Tukey’s post hoc test revealed that denitrification rates with and without DOM were not significantly different (\(p > 0.05\)). The addition of POM from sediment traps significantly increased denitrification rates at both stations relative to the control (\(p < 0.05\)) at the base of the oxycline only (\(F_{1,16} = 10.255, p < 0.01\));
however, the response of denitrification to the addition of POM was not significantly different between the two stations ($F_{1,10} = 0.565, p > 0.05$). Average denitrification rates in the POM treatment at the base of the oxycline was 6.4 times larger than the control at Sta. 1 and 5.4 times larger at Sta. 2.

Parallel incubations with $^{15}$NH$_4^+$ only without any additions of organic matter were carried out at each station and depth in the AS (Fig. 3A,B), which allowed the determination of the control anammox rate. At Sta. 1, the average anammox rates during incubations were 0.005 ± 0.0004 and 0.007 ± 0.0009 nmol N$_2$ h$^{-1}$, at the base of the oxycline and SNM, respectively. At Sta. 2, the average anammox rates during incubations were 0.009 ± 0.008 and 0.06 ± 0.05 nmol N$_2$ h$^{-1}$, at the base of the oxycline and SNM, respectively.

Discussion

Effect of dissolved organic carbon (glucose) on N loss in the ETSP—Concentrations of dissolved organic carbon (DOC) in the Pacific Ocean range from as high as 70 μmol L$^{-1}$ in the low-latitude surface ocean to < 40 μmol L$^{-1}$ in the deep ocean (Hansell and Carlson 1998). The amount of glucose added to incubations in this study (2 μmol C L$^{-1}$) was only a small fraction of the DOC naturally present. However, this ambient DOC is thought to be very old (4000–6000 yr B.P.), estimated from radiocarbon measurements (Druffel et al. 1992), indicating that the majority of this DOC is refractory and is returned to the deep un-respired after a complete ocean mixing cycle. Glucose (C$_6$H$_{12}$O$_6$) is a simple carbohydrate (readily utilizable by microorganisms as an energy source) that yields ATP via glycolysis; thus, even a relatively small amount of glucose may fuel relatively high rates of bacterial respiration.

At Sta. 20 in the ETSP, both the control and glucose-amended incubations exhibited NO$_3^-$ consumption and NO$_2^-$ accumulation and consumption in a classic denitrifying sequence, as observed in bacterial cultures, which is controlled by the enzyme kinetics of each step of denitrification (Betlach and Tiedje 1981). The addition of glucose to these incubations stimulated NO$_3^-$ and NO$_2^-$ reduction by supplying substrate to a carbon-limited system (Fig. 1A,B). The average denitrification rate measured by the production of $^{15}$N$_2$ was 1.6 times higher in the incubations with the addition of glucose as compared with those without glucose (Fig. 2). Conversely, the average anammox rate was an order of magnitude lower in the glucose treatments compared with the control. Although glucose does not contain organic N, which would be remineralized to NH$_4^+$ potentially for anammox, it is unlikely that anammox bacteria in these incubations were NH$_4^+$-limited because of the addition of 1 μmol L$^{-1}$ $^{15}$NH$_4^+$ tracer.

These results support the findings of Dalsgaard et al. (2012) in the ETSP ODZ, who hypothesize a lack of close coupling between denitrification and anammox based on the observation that denitrification rates were high when anammox rates were low and vice versa. Dalsgaard et al. (2012) argue that the lack of close coupling may be due to the relative response times of the different microorganisms involved: denitrifiers are fast-growing and can respond quickly to episodic inputs of organic matter, whereas anammox bacteria are relatively slow-growing, which limits the rate of their response to increased substrate; however, they subsequently maintain lower rates for longer periods of time.

Effect of NH$_4^+$ on denitrification in the AS—Previous researchers have also found no significant increase in the anammox rate (determined from $^{29}$N$_2$ production) in $^{15}$NO$_3^-$ labeled incubations with $^{14}$NH$_4^+$ compared with those without $^{14}$NH$_4^+$ (Kuypers et al. 2005; Thamdrup et al. 2006). This is somewhat unexpected because [NH$_4^+$] in both the Benguela upwelling and ETSP ODZs is frequently below detection, suggesting anammox could be limited by [NH$_4^+$]. Thamdrup et al. (2006) speculated that no stimulation of anammox by the addition of NH$_4^+$ may reflect extremely efficient ammonium uptake by anammox bacteria, which would lead to reaching saturating concentrations at a relatively low [NH$_4^+$].

Effect of dissolved organic matter on denitrification in the AS—The absence of a significant response from the addition of DOM indicated that denitrifiers in the AS ODZ were either restricted in their ability to respire the DOM added because of its possibly refractory nature (Druffel et al. 1992), or did not receive sufficient additional DOM to cause a response (Fig. 3A,B). Because of the significant increase in denitrification rates by the addition of POM (see discussion in following section), it is unlikely that
denitrification rates at Sta. 1 was triple that of Sta. 2 (1.1
addition of POM. However, the absolute increase in
2, respectively), suggesting the original bacterial population
stations was similar (6.4- vs. 5.4-fold increase at Sta. 1 and
Additional, although double the amount of POM was
additions in this study exceeded the estimated POC
addition of only the particulate
matter relative to suspended POM or DOM. A significant
increase in denitrification rates was observed at the base of
the oxycline only, and not at the SNM, at both stations;
this suggests that it was the POM itself, not particle-
associated denitrifiers, that gave rise to increased denitrifi-
cation rates. If the stimulation were due to new bacteria
introduced with the POM, a comparable absolute increase
in rates at both depths at each station would be expected.
Additionally, although double the amount of POM was
added to incubations at Sta. 1 relative to Sta. 2, the
proportional increase in the average denitrification rates
relative to the control at the base of the oxycline of both
stations was similar (6.4- vs. 5.4-fold increase at Sta. 1 and
2, respectively), suggesting the original bacterial population
in each incubation had comparable responses to the
addition of POM. However, the absolute increase in
denitrification rates at Sta. 1 was triple that of Sta. 2 (1.1
vs. 0.3 nmol L$^{-1}$ h$^{-1}$, respectively), although double the
POM was added at Sta. 1 relative to Sta. 2, further
suggesting that the increased denitrification rates were due
to a stimulation of the original bacterial population in the
bags and not due to bacteria introduced with the POM.

The flux of organic matter is correlated to the rate of
fixed N removal in the ETSP and AS ODZs (Jensen et al.
2011; Kalvelage et al. 2013), suggesting organic matter is an
important control on this process. Further supporting this
conclusion is the observation that rates of N$_2$ production
generally decrease with increasing depth in both these
regions (Thamdrup et al. 2006; Jensen et al. 2011;
Dalsgaard et al. 2012), following the trend of decreasing
organic matter flux with increasing depth (Martin et al.
1987). Additionally, carbon limitation of denitrification in
the ETSP has been directly measured in incubations (Ward
et al. 2008). In the present study, we hypothesize that the
significant stimulation of denitrification at the base of the
oxycline only, and not the SNM, by the addition of POM is
due to increased respiration leading to elevated organic
carbon demand at the shallower depth.

The magnitude of the POM additions (2.6 and
1.3 nmol C L$^{-1}$ at both depths at Sta. 1 and 2, respectively)
can be evaluated in the context of ambient POC fluxes and
mineralization rates in the AS. The Martin equation
(Martin et al. 1987):

$$P_{OC} \text{ flux at depth } z = P_{OC} \text{ flux at 100 m} \times \left(\frac{z}{100}\right)^{-b} \quad (1)$$

was used to estimate the POC flux to the depths sampled in
this study. Temporal patterns of productivity and POC
export in the AS are dominated by seasonal monsoonal
cycles with the highest productivity and export during the
Northeast and Southwest monsoons (Lee et al. 1998). A
$^{234}$Th-based average export from a depth, location, and
season comparable to this study (100 m at an open-ocean
AS ODZ station during the SW monsoon) is
10.85 mmol C m$^{-2}$ d$^{-1}$ (Lee et al. 1998). An estimate of
the attenuation coefficient (b) in Eq. 1 at the same open-
ocean AS ODZ station is 0.74 (Berelson 2001). In order to
specify a depth interval over which POC is consumed, the
8 liter bag incubation is assumed to be a cube with a height
of 0.2 m. Using these values, the average in situ rates of
POC consumption in these incubations would be 0.08, 0.04,
and 0.02 nmol C L$^{-1}$ d$^{-1}$ at 100, 150, and 200 m. These
calculated POC consumption rates are comparable to
measured bacterial carbon demand associated with different
forms of POC (Smith et al. 1992). Although the POC
additions in this study exceeded the estimated POC
consumption rate at all depths, it may be that the base of
the oxycline harbors a more active and/or denser bacterial
population relative to the deeper SNM due to a chronically
larger POC flux and, thus, was able to produce a larger
response to the addition of fresh POC.

*Anammox and the source of excess $^{29}$N$_2$ production in ETSP and AS incubations*—During the 2005 R/V *Knorr*
cruise to the ETSP, additional $^{15}$NO$_3^-$ amended bag
incubations were carried out at other stations using the
same method described here (data not shown). After 2 d,
$^{29}$N$_2$ and $^{30}$N$_2$ produced by denitrification were binomially
distributed after taking the production due to anammox
into account, such that the total $^{29}$N$_2$ produced was equal
to the sum of $^{29}$N$_2$ from anammox (determined from a
parallel incubation with $^{15}$NH$_4^+$) and $^{29}$N$_2$ from
denitrification (predicted from $^{30}$N$_2$ assuming a binomial
distribution of $N_2$ relative to the initial fraction labeled of $^{15}$NO$_3^-$).

However, this was not true after 5.7 d in the ETSP
incubations (Fig. 2) and in all of the AS incubations after
2 d (Fig. 4A,B). In these incubations, more $^{29}$N$_2$ was
produced than could be accounted for based on the
fraction of the initial NO$_3^-$ that was labeled, after taking
into account the production due to anammox. We name
this excess the residual $^{29}$N$_2$ ($\Delta_{\text{resid}}$). It is defined as the
difference between the total measured $^{29}$N$_2$ production rate
and the $^{29}$N$_2$ production rate predicted from the sum of anammox and denitrification rates, assuming that the isotopic composition of the products is binomially distributed relative to the reactants.

Nicholls et al. (2007) in the AS found the isotopic composition of $^{15}$N$_2$O produced by denitrification was binomially distributed relative to the starting pool of NO$_3^-$ . However, similar to the results in this study, $^{15}$N$_2$ production could not be predicted by the binomial distribution. Trimmer and Purdy (2012) measured N$_2$ production that was not via canonical denitrification or anammox, and hypothesized amine groups on allylthiourea were directly oxidized to N$_2$ by NO$_3^-$ . In incubations with $^{15}$NO$_3^-$ in the ETSP, de Brabandere et al. (2013) observed $^{15}$N$_2$ production that was non-binomially distributed after production by anammox had been taken into account. These researchers speculated that production of $^{29}$N$_2$ via denitrification in the $^{15}$NO$_3^-$ incubations might be underestimated if denitrifiers reduced ambient NO$_3^-$ directly to N$_2$ intracellularly, without allowing the NO$_3^-$ to mix completely with the ambient NO$_3^-$ pool ('nitrite shunting').

Production of $^{29}$N$_2$ in excess of the predicted binomial distribution indicates that the $^{15}$N-labeled fraction of the reactant pool is not well-known. To further explore possible mechanisms responsible for the production of $\Delta_{\text{resid}}$, we calculated the average $^{15}$N-labeled fraction of the reactant pool that would have been required to produce the observed $^{29}$N$_2$ and $^{30}$N$_2$, with no $\Delta_{\text{resid}}$ assuming the anammox rates measured in the control incubations remained constant across all treatments (this is reasonable considering the slow growth rate of anammox bacteria relative denitrifiers) and $^{15}$N$_2$ was binomially distributed relative to the reactants. In the ETSP incubations, the fraction of the NO$_3^-$ pool that was $^{15}$N-labeled was $\sim 0.1$. In order to produce the observed distributions of $^{29}$N$_2$ and $^{30}$N$_2$ at 5.7 d, the $^{15}$N-labeled fraction of the reactant pool must have been much lower: 0.002. In the AS incubations, the fraction of the NO$_3^-$ pool $^{15}$N-labeled was between 0.4 and 0.7. For $\Delta_{\text{resid}} = 0$, the fraction of the reactant pool $^{15}$N-labeled must have been 0.001–0.5. These results indicate that direct oxidation of organic N to N$_2$ is probably not the only mechanism producing the observed distributions of $^{15}$N$_2$, given that an organic $^{14}$N pool up to 1000 times greater than the $^{15}$NO$_3^-$ concentration would be required. Thus in this study, ‘nitrite shunting’ (de Brabandere et al. 2013) is a reasonable mechanism to explain a significant portion of the $\Delta_{\text{resid}}$. At 2 d in the ETSP incubations, $^{29}$N$_2$ and $^{30}$N$_2$ were binomially distributed, which is consistent with ‘nitrite shunting’ not affecting the predicted distribution of $^{29}$N$_2$ and $^{30}$N$_2$ because $^{15}$NO$_3^-$ was used as the tracer. At 5.7 d, $\Delta_{\text{resid}}$ becomes significant, which may be due to changes in the fraction $^{15}$N-labeled of the substrates, suggested by significant changes in the concentrations of NO$_3^-$ and NO$_2^-$ . Additionally, similar to denitrification rates, $\Delta_{\text{resid}}$ was significantly affected by only the addition of POM ($p < 0.005$); no significant effect was observed between stations, depths, or with the addition of NH$_4^+$ . This response suggests a heterotrophic source for $\Delta_{\text{resid}}$.

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References


KALVELAGE, T., AND OTHERS. 2013. Nitrogen cycling driven by
DRUFFEL, E. R. M., P. M. WILLIAMS, J. E. BAUER, AND J. R.
DE BOYER MONTEGUT, C., G. MADEC, A. S. FISCHER, A. LAZAR,
LAM, P., AND OTHERS. 2009. Revising the nitrogen cycle in the
LEE, C., AND OTHERS. 1998. Particulate organic carbon fluxes:
LUTHER, G. W., B. SUNDBY, B. L. LEWIS, P. J. BRENDEL, AND N.
SILVERBERG. 1997. Interactions of manganese with the
LUTHER, G. W., B. SUNDBY, B. L. LEWIS, P. J. BRENDEL, AND N.

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