Revisiting nitrification in the Eastern Tropical South Pacific: A focus on controls

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Abstract Nitrification, the oxidation of ammonium (NH$_4^+$) to nitrite (NO$_2^-$) and to nitrate (NO$_3^-$), is a component of the nitrogen (N) cycle internal to the fixed N pool. In oxygen minimum zones (OMZs), which are hotspots for oceanic fixed N loss, nitrification plays a key role because it directly supplies substrates for denitrification and anaerobic ammonia oxidation (anammox), and may compete for substrates with these same processes. However, the control of oxygen and substrate concentrations on nitrification are not well understood. We performed onboard incubations with $^{15}$N-labeled substrates to measure rates of NH$_4^+$ and NO$_2^-$ oxidation in the eastern tropical South Pacific (ETSP). The spatial and depth distributions of NH$_4^+$ and NO$_2^-$ oxidation rates were primarily controlled by NH$_4^+$ and NO$_2^-$ availability, oxygen concentration, and light. In the euphotic zone, nitrification was partially photoinhibited. In the anoxic layer, NH$_4^+$ oxidization was negligible or below detection, but high rates of NO$_2^-$ oxidation were observed. NH$_4^+$ oxidation displayed extremely high affinity for both NH$_4^+$ and oxygen. The positive linear correlations between NH$_4^+$ concentration and NH$_4^+$ oxidation potential. The depth distribution of ammonia oxidizers responds to in situ NH$_4^+$ concentrations or supply by adjusting their population size, which determines the NH$_4^+$ oxidation potential. The depth distribution of ammonia oxidizers responds to in situ NH$_4^+$ concentrations or supply by adjusting their population size, which determines the NH$_4^+$ oxidation potential.

1. Introduction

Many facets of the nitrogen (N) cycling in the oceanic oxygen minimum zones (OMZs, oxygen concentrations < ~5 μM), have been studied during the past three decades. One of the primary motivations to understand N cycling in OMZs is that these regions account for a significant portion of fixed N removal from the ocean (25–58%) [DeVries et al., 2012] despite their small volume (~0.1% of total ocean volume) [Codispoti et al., 2001]. While tremendous progress has been made in understanding N cycling processes, incomplete knowledge of controls on each specific process has fueled ongoing studies on OMZs. Nitrification is one of the key N cycling processes that is still not well understood in OMZs.

Nitrification consists of two sequential steps: the oxidation of ammonium (NH$_4^+$) to nitrite (NO$_2^-$) and then to nitrate (NO$_3^-$). Nitrification could directly supply substrates for denitrification and anaerobic ammonia oxidation (anammox), or compete for substrates with the same processes. In the eastern tropical South Pacific (ETSP), NH$_4^+$ oxidation was suggested to account for 6–33% of the total NO$_2^-$ production in the upper OMZ [Lam et al., 2009]. A following study in the same region showed that NH$_4^+$ oxidation supplied only ~7% of the total NO$_2^-$ production [Kalvelage et al., 2013]. These data highlight the uncertainty about the contribution of NH$_4^+$ oxidation to NO$_2^-$ supply for denitrification and anammox.

NH$_4^+$ oxidation is mediated by ammonia-oxidizing archaea (AOA) and bacteria (AOB); NO$_2^-$ oxidation is mediated by nitrite-oxidizing bacteria (NOB). These groups are all obligate aerobes but are apparently microaerophilic and function well at very low oxygen concentrations. Natural assemblages of ammonia oxidizers [Horak et al., 2013; Newell et al., 2013] and pure cultures of marine AOA [Martens-Habbema et al., 2009]...
have demonstrated high affinity for NH$_4^+$ oxidation. The ubiquitous AOA are considered predominantly responsible for NH$_4^+$ oxidation in the ocean [Beman et al., 2012; Newell et al., 2013; Santoro et al., 2010].

The ultimate source of NH$_4^+$ for nitrification in the water column is remineralization of organic matter produced in the euphotic zone, which exerts a first-order control on nitrification rates [Kalvelage et al., 2013; Newell et al., 2011; Ward and Zafiriou, 1988]. OMZs are characterized by their high productivity in the euphotic zone, which in turn fuels remineralization, creating a sharp oxycline reaching oxygen levels below detection. A poorly ventilated anoxic layer underlies the upper oxycline. The strong vertical oxygen gradient in OMZs should be another key control of aerobic NH$_4^+$ oxidation, for which molecular oxygen is required. The physiological control by oxygen on NH$_4^+$ oxidation has been studied both in culture and in the environment. The cultivated AOA N. maritimus demonstrated a low half-saturation concentration for oxygen ($K_m = 3.9$ $\mu$M) [Martens-Habbema et al., 2009], and a natural assemblage collected from the ETSP displayed an even higher affinity for oxygen ($K_m = 330$ nM) [Bristow et al., 2013]. Such high affinity for oxygen by ammonia oxidizers suggests that NH$_4^+$ oxidation rates are only sensitive to oxygen concentrations at very low levels.

The subunit A of the ammonia monooxygenase enzyme (encoded by the gene amoA), which functions to insert an oxygen atom into ammonia during the first step of NH$_4^+$ oxidation, is found in both AOA and AOB. The archaeal and bacterial amoA genes are commonly used as a molecular marker to enumerate AOA and AOB in the environment. While a few previous studies in marine water columns have found a positive correlation between the abundance of AOA and NH$_4^+$ oxidation rates [Beman et al., 2008; Smith et al., 2014a], the correlation was not ubiquitous. For example, $> 10^5$ copies of archaeal amoA gene per mL seawater have been reported at anoxic depths of the major OMZs, where NH$_4^+$ oxidation rates were undetectable or negligible [Lam et al., 2009; Newell et al., 2011]. We measured the archaeal and bacterial amoA gene abundances at two stations at relatively high depth resolution in order to resolve the relationship between rates and abundances in both oxic and anoxic zones.

Like NH$_4^+$ oxidation, the distribution of NO$_2^-$ oxidation in OMZs should also be controlled by oxygen concentrations. A Michaelis-Menten relationship between NO$_2^-$ oxidation rates and oxygen concentration in both the ETSP ($K_m = 0.78$ $\mu$M) [Bristow et al., 2013] and the Namibian OMZs ($K_m = \sim 4$ $\mu$M) [Fussel et al., 2012] was reported previously. Both studies determined NO$_2^-$ oxidation rates using incubations with $^{15}$NO$_2^-$ and manipulated the oxygen level using additions of oxygen-saturated seawater. However, high rates of NO$_2^-$ oxidation have been measured at the anoxic depths of OMZs, where no alternative electron acceptors are known [Beman et al., 2013; Kalvelage et al., 2013; Lipschultz et al., 1990; Peng et al., 2015]. No satisfactory explanations have been provided for this puzzle of how high NO$_2^-$ oxidation rates can occur at essentially zero oxygen concentrations.

The main goal of this study was to determine the depth distribution of NH$_4^+$ and NO$_2^-$ oxidation rates over chemical gradients in the ETSP OMZ. Additional experiments were performed to investigate the substrate dependence of NH$_4^+$ and NO$_2^-$ oxidation and to examine the effects of metal additions and photoinhibition on nitrification. Nitrification as a source of NO$_3^-$ in the euphotic zone is often ignored because nitrification is assumed to be inhibited by light. The photoinhibition of nitrification is not complete, however, indicating that both in situ nitrification and physical processes provide NO$_3^-$ for uptake by phytoplankton [Dore et al., 1996; Ward et al., 1989]. Our data, along with nitrification rates measured in other OMZs, demonstrate that NH$_4^+$ and oxygen concentrations and light levels are critical controls of NH$_4^+$ and NO$_2^-$ oxidation rates in the upper oxycline of OMZs.

2. Methods

2.1. Site Description and Physicochemical Data Collection

Nitrification incubations were performed at 8 stations in the ETSP in June and July 2013 on board the R/V Nathaniel B. Palmer (Figure 1). Two of the stations (BB2 and 21) were coastal (<50 km offshore, bottom depth = 1625 and 2207 m, respectively), characterized by higher surface chlorophyll levels than the rest of the stations, which were offshore (>200 km offshore, bottom depths >3500 m). Stations BB1 and BB2 were sampled with greater resolution than the other stations. Concentrations of NH$_4^+$, NO$_2^-$, and NO$_3^-$ were determined by standard spectrophotometric methods onboard, with detection limits of 0.06 $\mu$M for NH$_4^+$, 0.03 $\mu$M for NO$_2^-$, and 0.10 $\mu$M for NO$_3^-$ [UNESCO, 1994]. Dissolved oxygen concentration was determined
using the SBE 43 dissolved oxygen sensor attached to a SBE 911+ Conductivity, Temperature and Depth (CTD) system. For some of the casts, a STOX sensor was also deployed with the CTD to measure oxygen concentration (Revsbech et al., 2009). The main objective of using the STOX sensor was to define the depth range and region of anoxia in the OMZ to aid in selecting sample depths, which is possible because the STOX sensor has an extremely low detection limit (Revsbech et al., 2009). Thus the STOX sensor, when available, was used to select the sample depths, but the values reported here are from the SBE 43 sensor, because it provided a complete coverage of all stations sampled on this cruise.

2.2. N$_2$O Measurements

Dissolved N$_2$O concentrations were measured using a modified version of the sampling and analytical methods developed for the measurements of the dissolved transient tracers - chlorofluorocarbons 11 and 12 and sulfur hexafluoride by Bullister and Wisegarver [2008]. Water samples were collected into 250 cm$^3$ ground glass syringes through a plastic three-way stopcock inserted directly into the Niskin bottle petcock. These samples were stored at 3–5$^\circ$C until 30–45 min. before analysis. They were then heated to approximately 35$^\circ$C prior to analysis.

Concentrations of N$_2$O were measured by shipboard electron capture gas chromatography (EC-GC). The gas was introduced to the EC-GC via a purge-and-trap technique developed by D. P. Wisegarver and J. L. Bullister (personal communication, 2013). Approximately 200 mL of water sample were purged with nitrogen and the compounds of interest were trapped on a Porapak Q/Carboxen 1000/Molecular Sieve 5A trap cooled by an immersion bath to $-60^\circ$C. The major modifications to the analytical system described by Bullister and Wisegarver [2008] are the additions of a second precolumn (13 cm of 80/100 mesh molecular sieve 5A) to separate the N$_2$O from the CFCs and an analytical column for N$_2$O (30 cm of molecular sieve 5A) in a 220$^\circ$C oven. Instrumental grade P-5 gas (95% Ar/5% CH$_4$) was directed onto the second precolumn and into the third column for the N$_2$O analyses.

The analytical system was calibrated frequently using a standard gas of known composition in gas sample loops of known volume at measured temperatures and pressures. The procedures used to transfer the standard gas to the trap, precolumns, main chromatographic columns and EC detectors were similar to those used for analyzing water samples. Full-range calibration curves were run at the beginning and end of the cruise, and they were supplemented with occasional injections of multiple aliquots of the standard gas at more frequent time intervals.
On this expedition, based on the analysis of 15 duplicate samples, we estimate precisions (1 standard deviation) as the larger of 1.9 nmol kg$^{-1}$ or 3.5% for dissolved N$_2$O.

2.3. Onboard Incubation Experiments
Approximately 450 mL of seawater was transferred from Niskin (10 L) bottles into opaque, metal-free, gastight, tri-laminate bags avoiding contact with the atmosphere. The bottles from anoxic depths were sampled first as soon as the CTD system arrived on deck. Degassed 15N tracer solutions (in a vacuum chamber for >30 min prior to the incubation to remove dissolved oxygen) were injected during the filling process to ensure complete mixing. 15NH$_4^+$ + 14NO$_3^-$ were added for NH$_4^+$ oxidation incubations, and 15NO$_2^-$ was added for NO$_2^-$ oxidation incubations. The final concentration of 15N substrates and the 15N carrier reached a reasonable range. Sensitivity tests demonstrated that fluctuations of the natural abundance Altabet [2005], which converts NO

\[ \frac{d}{d} \]

The rate of NH$_4^+$ oxidation was calculated following the equation:

\[ V_{\text{NH}_4^+} = \frac{\Delta [15NO_2^-]}{\text{f}_{\text{15NO_2^-}} \cdot T} \]

where \( \Delta [15NO_2^-] \) is the change in concentration of 15NO$_2^-$ between the start and the end of the incubation as a result of NH$_4^+$ oxidation, \( \text{f}_{\text{15NO_2^-}} \) is the fraction of NH$_4^+$ that was labeled with 15N at the start of the incubation, and T is the length of incubation. The concentration of 15NO$_2^-$ at the start of the incubation ([15NO$_2^-$]) was calculated from the measured ambient NO$_2^-$ concentration assuming natural abundance 15N-NO$_2^-$. The actual value of natural abundance 15N-NO$_2^-$ for each sample was taken from a depth profile at the closest station in the ETSP reported by Casciotti et al. [2013]. For samples collected from depths shallower than the anoxic layer, the [15NO$_2^-$] was calculated with the natural abundance 15N-NO$_2^-$ reported for the depth that had the same oxygen concentration as the published depth profile [Casciotti et al., 2013]. For samples collected from the anoxic depths, the [15NO$_2^-$] was calculated with the natural abundance 15N-NO$_2^-$ reported for depths with the same NO$_2^-$ concentration corresponding to their relationship to the secondary nitrite maximum (i.e., above or below the SNM) in the published depth profile [Casciotti et al., 2013]. For the two samples collected from 1000 m (deeper than the anoxic layer), the [15NO$_2^-$] was calculated assuming a 15N-NO$_2^-$ of 0%$_{\text{hor}}$. Rates were not particularly sensitive to variability of initial 15N-NO$_2^-$ in a reasonable range. Sensitivity tests demonstrated that fluctuations of the natural abundance 15N-NO$_2^-$ by ±5%$_{\text{hor}}$ would result in negligible differences in calculated ammonia oxidation rates (supporting information Figure S1).

2.5. Measurement of NO$_2^-$ Oxidation
The 15N-NO$_2^-$ was measured using the denitrifier method [McIlvin and Casciotti, 2011; Sigman et al., 2001]. Samples were first treated with 15 mM sulfamic acid (final concentration) for 30 min to remove any preexisting NO$_2^-$, and the pH was raised to ~7 with NaOH [Granger and Sigman, 2009]. The efficiency of NO$_2^-$ removal was >97.5%, and the trace amount of contaminating 15NO$_3^-$ was accounted for as in Peng et al. [2015]. Three NO$_3^-$ international reference materials (IAEA-N3, USGS 34, and USGS 32) were used to calibrate the 15N-NO$_3^-$. 

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The rate of NO$_2^-$ oxidation was calculated following the equation:

$$V_{NO_2^-} = \frac{\Delta^{[15]NO_3^-}}{f_{NO_2^-} \times T}$$

where $\Delta^{[15]NO_3^-}$ is the change in concentration of $^{15}$NO$_3^-$ between the start and the end of the incubation as a result of NO$_2^-$ oxidation, $f_{NO_2^-}$ is the fraction of NO$_2^-$ that was labeled with $^{15}$N at the start of the incubation, and $T$ is the length of incubation. The $^{[15]NO_3^-}$ at the start of the incubation ($^{[15]NO_3^-}_0$) was calculated from the measured ambient NO$_3^-$ concentration assuming natural abundance $\delta^{15}$N-NO$_3^-$. The actual value of natural abundance $\delta^{15}$N-NO$_3^-$ for each sample was taken from a depth profile at the closest station in the ETSP reported by Casciotti et al. (2013). For samples collected from depths shallower than the anoxic layer, the $^{[15]NO_3^-}_0$ was calculated with the natural abundance $\delta^{15}$N-NO$_3^-$ reported for the depth that had the same oxygen concentration on the published depth profile [Casciotti et al., 2013]. For samples collected from the anoxic depths, the $^{[15]NO_3^-}_0$ was calculated with the natural abundance $\delta^{15}$N-NO$_3^-$ for depths with the same NO$_2^-$ concentration corresponding to their relationship to the SNM (i.e., above or below the SNM) in the published depth profile [Casciotti et al., 2013]. For the two samples collected from 1100 m (deeper than the anoxic layer), the $^{[15]NO_3^-}_0$ was calculated assuming a $\delta^{15}$N-NO$_3^-$ of 5‰. We did not account for isotope dilution by regeneration of the $^{15}$N-labeled N substrate, so the calculated NH$_4^+$ and NO$_2^-$ oxidation rates calculated may be underestimations.

2.6. Detection Limit of Rate Measurements
The detection limit was determined for every individual incubation following Santoro et al. (2013), and depends on the fraction of the substrate labeled with $^{15}$N at the beginning of the incubation as well as the concentration of the product pool. For samples from anoxic depths, the azide method (used for NH$_4^+$ oxidation measurements) had a detection limit of 0.01–0.10 nM d$^{-1}$, and the denitrifier method (used for NO$_2^-$ oxidation measurements) of 0.20–4.26 nM d$^{-1}$. For the rest of the samples, the azide method had a detection limit of 0.003–0.065 nM d$^{-1}$, and the denitrifier method of 0.01–0.51 nM d$^{-1}$. The azide method had a higher sensitivity than the denitrifier method, mainly because the NO$_2^-$ concentrations were lower than the NO$_3^-$ concentrations. The samples from anoxic depths had relatively higher detection limits due to the presence of high NO$_2^-$ concentrations, which results in a higher concentration product pool for the NH$_4^+$ oxidation measurements, and a lower substrate fraction labeled with $^{15}$N for the NO$_2^-$ oxidation measurements.

2.7. Kinetics Experiments
The dependence of NH$_4^+$ oxidation on NH$_4^+$ availability was tested by incubating seawater samples at five different levels of $^{15}$NH$_4^+$ addition (20, 50, 100, 200, and 500 nM), at both stations BB1 and BB2. Seawater was collected from 75 m at station BB1 and 30 m at station BB2, depths which were intended to target the primary nitrite maximum (PNM). However, NO$_2^-$ concentration measurements showed that 75 m at station BB1 was slightly (by ~10 m) below the PNM, and 30 m at station BB2 was slightly (by ~10 m) above the PNM. The dependence of NO$_2^-$ oxidation on NO$_3^-$ availability was tested by incubating seawater samples at five different levels of $^{15}$NO$_2^-$ addition (20, 50, 100, 200, and 500 nM) at station BB2. Seawater was collected from 70 m to target the depth of a NO$_2^-$ concentration minimum between the PNM and the secondary nitrite maximum (SNM).

2.8. Light/Dark Experiments on Nitrification
At stations BB1 and BB2, an additional experiment was performed to investigate photoinhibition of NH$_4^+$ and NO$_2^-$ oxidation. Seawater from 20 m was sampled in the early morning and the incubations were performed in 150 mL clear polycarbonate bottles, which were placed in a seawater incubator installed on deck with mesh screen to reduce the light to ~10% of surface irradiance. A parallel set of samples was incubated in the dark in a seawater incubator. The light level in the incubators was measured throughout the day using an underwater spherical quantum sensor (LI-COR, Lincoln, NE). The incubation was terminated just before sunset.

2.9. Quantification of Archaeal and Bacterial amoA Genes
At the process stations (BB1 and BB2) particulate material was collected by filtering 2.5 - 10 liters of seawater through 0.22 µm Sterivex filters with a peristaltic pump. Nucleic acids were extracted as described previously [Peng et al., 2013]. Archaeal and β-proteobacterial amoA gene copies were enumerated using qPCR in triplicate as described previously, using the QuantiTect SYBR Green PCR Kit [Newell et al., 2011].
Primers Arch-amoAF (5′-STAATGGTCTGGCTTAGACG-3′) and Arch-amoAR (5′-GGGGTTTCTACTGTGTTGATGT-3′) [Francis et al., 2005] were used for archaeal amoA gene quantification, and primers amoA-1F (5′-GGGGTTTCTACTGTGTTGATGT-3′) and amoA-2R (5′-CCCCCTCGSAAAGCCTCTTC-3′) [Rotthauwe et al., 1997] for β-proteobacterial amoA gene quantification. The detection limit for both archaeal and β-proteobacterial amoA gene qPCR assays was approximately 100 copies per assay. Therefore the sensitivity depends on the amount of DNA extracted from different volumes at different depths, and would translate to approximately 5–10 gene copies per mL of seawater.

2.10. Alternative Electron Acceptor Experiment

Additional experiments were performed to test the response of NH$_4^+$ oxidation at anoxic depths to the addition of oxygen and alternative electron acceptors (iron and manganese). At stations 9 and BB2, seawater from the deep chlorophyll maximum (DCM) and the SNM was collected to fill 12 mL exetainers (overflowing the exetainers by 3 volumes), which were sealed immediately to minimize oxygen contamination. Exetainers were covered in aluminum foil to reduce exposure to light. $^{15}$NH$_4^+$ + $^{15}$NO$_2^-$ (final concentration 600 nM) was added to four groups of parallel incubations. The first group served as a control without anything else added; the second group was supplemented with 0.5 mL of oxygen-saturated seawater to reach a final oxygen concentration of 9.6 μM; the third group was supplemented with Fe$_2$O$_3$ (final concentration 6.1 nM); and the fourth group was supplemented with MnO$_2$ (final concentration 12.7 nM). Immediately after adding the $^{15}$N substrate and Fe$_2$O$_3$/MnO$_2$, the exetainers were purged with Ultra High Purity Helium for 5 min to remove any oxygen that could have been introduced. The incubations supplemented with oxygen were purged before adding the oxygen-saturated seawater. The incubations were performed in the dark for 24 h. At the end of the incubation, the entire incubation volume was transferred to a 15 mL centrifuge tube and stored at −80°C.

3. Results

3.1. Chemical Characteristics of the ETSP OMZ

The degree of anoxia and the distribution of associated chemical concentrations in the water column varied widely among stations, which could be divided into three regimes: coastal OMZs, offshore OMZs, and OMZ margins. Coastal OMZs include stations BB2 and 21 (Figure 1), which are characterized by a thick anoxic layer (> 300 m) and high NO$_2^-$ accumulation (up to 8 μM) in the anoxic layer (Figures 2a and 2b). N$_2$O concentration at coastal OMZs showed two peaks, which were located in the upper and lower oxygenic layers near the oxic-anoxic interfaces (Figure 2d). The N$_2$O concentration peak in the upper oxycline covered a smaller depth range (< 50 m) than the one in the lower oxycline (> 600 m). NH$_4^+$ concentration at coastal OMZs decreased rapidly with depth, and a subsurface maximum (> 1.5 μM) was detected at station BB2 (Figure 2a).

Offshore OMZs include stations BB1, 3, and 4 (Figure 1), which are also defined by an anoxic layer (< 300 m thick), but the SNM was thinner compared to coastal OMZs (Figures 3a and 3b). The depth profile of N$_2$O concentration at station BB1 was similar to that at coastal OMZs (Figure 3d; N$_2$O concentration was not measured at all stations). NH$_4^+$ concentration at offshore OMZ stations decreased rapidly with depth (from > 0.4 μM to below detection), except at station 3 where the NH$_4^+$ concentration near the surface was not higher than at depth (Figure 3a).

OMZ margins include stations 10 and 20, where no obvious SNM was detected, the anoxic layer was thinner (Figures 4a and 4b) and a distinct primary NO$_2^-$ maximum was present. At station 20, there was a subsurface maximum of N$_2$O concentration in the upper oxycline, but no measurements were made to define the depth profile below 200 m (Figure 4d). N$_2$O concentration at station 10 increased with depth but no subsurface maximum in the upper water column was detected. NH$_4^+$ concentration decreased rapidly with depth, and a subsurface maximum was evident at station 10 (Figure 4a).

3.2. NH$_4^+$ Oxidation Rates

NH$_4^+$ oxidation rates were low or undetectable in the surface waters, but reached a subsurface maximum in the upper oxycline at all stations. The maximum rate of NH$_4^+$ oxidation at coastal OMZs was higher than that at the offshore OMZs and OMZ margins. At coastal station BB2, we captured a single maximum NH$_4^+$ oxidation rate of 77 nM d$^{-1}$ (Figure 2c). At most of the other stations, NH$_4^+$ oxidation rates reached a
The maximum between 25 and 35 nM d⁻¹. Relatively low NH₄⁺ oxidation rates (< 8 nM d⁻¹) were measured at stations 3 (offshore OMZ, Figure 3c) and 10 (OMZ margin, Figure 4c). At all stations with N₂O concentration measurements except station 10 (OMZ margin), the depth of the subsurface maximum of NH₄⁺ oxidation rate was very similar to that of the maximum N₂O concentration in the upper oxycline. NH₄⁺ oxidation rates were either below detection or negligible (0.35–0.44 nM d⁻¹) in the anoxic layer and detectable but very low in the lower oxycline (0.44–2.68 nM d⁻¹) at all stations.

NH₄⁺ oxidation rates under 10% surface irradiance (up to 170 μE m⁻² s⁻¹, see supporting information Figure S2) were partially inhibited to a level about a third of the uninhibited rates in the dark (Table 1). In contrast, NO₂⁻ oxidation rates were not significantly different between the two light conditions.

The addition of oxygen, Fe₂O₃, and MnO₂ to samples collected at the SNM and the DCM did not increase NH₄⁺ oxidation rates significantly (t-test p > 0.05) over rates detected in the control experiments, except at the deep chlorophyll maximum at station BB2 (65 m). In this sample, the addition of 9.6 μM of oxygen stimulated the NH₄⁺ oxidation rate by eight-fold (Figure 5). The addition of 6.1 nM Fe₂O₃ also increased the NH₄⁺ oxidation rate in one but not the other of the two replicate incubations, so the result was not statistically significant (t-test p > 0.05).

3.3. NO₂⁻ Oxidation Rates

High rates of NO₂⁻ oxidation (up to ~100 nM d⁻¹) were found at the oxic-anoxic interface in the upper oxycline and in the anoxic layer at all stations (Figures 2c–4c). NO₂⁻ oxidation rates were generally much higher
than NH$_4^+$ oxidation rates, especially in the anoxic layer, where NH$_4^+$ oxidation rates were negligible but NO$_2^-$ oxidation rates were high. NO$_2^-$ oxidation rates were never detected in the shallowest samples (within the euphotic zone above the oxycline) at each station. At depths greater than 400 m, no NO$_2^-$ oxidation was detected except at station 20, at the low rate of 6.2 nM d$^{-1}$. At station 10 (OMZ margin), NO$_2^-$ oxidation rates were low or undetectable overall, except at the oxic-anoxic interface, where there is a small amount of NO$_2^-$ accumulation (0.04 µM).

### 3.4. Abundance of amoA Genes

The depth profile of archaeal amoA gene abundances at stations BB1 and BB2 resembled that of NH$_4^+$ oxidation rates, in that both showed subsurface maxima in the upper oxycline (Figures 2e and 3e). The archaean amoA gene abundances at the shallowest depths (2.5 – 30 m) were a few thousand copies mL$^{-1}$. The subsurface maximum of archaeal amoA gene abundance (1.6 $\times$ 10$^4$ copies mL$^{-1}$ at offshore station BB1 and 2.9 $\times$ 10$^5$ copies mL$^{-1}$ at coastal station BB2) coincided with the subsurface maximum of N$_2$O.
concentration. Substantial numbers of archaeal amoA genes (thousands of copies mL\(^{-1}\)) were enumerated at the anoxic depths and the lower oxycline, where NH\(_4^+\) oxidation rates were either negligible or very low. The abundance of β-proteobacterial amoA genes was overall 1 - 2 orders of magnitude lower than that of archaeal amoA genes (Figures 2e and 3e). The maximum β-proteobacterial amoA gene abundance (~250 copies mL\(^{-1}\)) was found in the surface layer at both stations BB1 and BB2. Unlike the archaeal amoA gene abundance, β-proteobacterial amoA gene abundance remained low at all depths below the subsurface maximum.

### 3.5. Nitrification Kinetics

NH\(_4^+\) oxidation rates displayed a classic Michaelis-Menten dependence on NH\(_4^+\) concentration in the kinetics experiment at the PNM at station BB1, with a half-saturation concentration of 27.4 ± 4.4 nM (Figure 6). The maximum NH\(_4^+\) oxidation rate of this water sample (24.9 ± 1.3 nM d\(^{-1}\)) was reached around 125 nM NH\(_4^+\). By contrast, no apparent response was observed to the different levels of \(^{15}\)N substrate addition at station BB2 (supporting information Figure S3), probably due to the high ambient concentrations of NH\(_4^+\) (1.48 mM at 30 m) and NO\(_2^-\) (2.79 mM at 70 m).

Grouping all measurements by their vertical position in the ETSP revealed different relationships or lack thereof between NH\(_4^+\) oxidation rates and NH\(_4^+\) concentrations (Figure 7a). There was a robust positive linear correlation between NH\(_4^+\) oxidation rates and NH\(_4^+\) concentrations in the upper oxycline (\(R^2 = 0.82, p < 0.0001\)). No clear relationships between NH\(_4^+\) oxidation rates and NH\(_4^+\) concentrations were observed in the euphotic zone. In the anoxic layer and depths below it, NH\(_4^+\) concentration was very low and was above detection limit (0.06 μM).

### Figure 4

Rates and chemical profiles at OMZ margin stations 10 and 20. Plots and symbols are the same as in Figure 2. No amoA gene abundance was measured at these stations.

### Table 1

A Comparison of NH\(_4^+\) and NO\(_2^-\) Oxidation Rates (Average ± 1 Standard Deviation, \(n = 3\)) Measured at 10% Surface Irradiance and in the Dark

```latex
<table>
<thead>
<tr>
<th>Station</th>
<th>NH(_4^+) Oxidation Rate (nM d(^{-1}))</th>
<th>NO(_2^-) Oxidation Rate (nM d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB1 10% Light</td>
<td>0.94 ± 0.07</td>
<td>12.52 ± 1.71</td>
</tr>
<tr>
<td>BB1 Dark</td>
<td>3.27 ± 0.19</td>
<td>8.85 ± 6.64</td>
</tr>
<tr>
<td>BB2 10% Light</td>
<td>0.35 ± 0.01</td>
<td>4.68 ± 4.35</td>
</tr>
<tr>
<td>BB2 Dark</td>
<td>1.00 ± 0.02</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
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\(^a\)Samples were taken from 20 m in the early morning at stations BB1 (offshore) and BB2 (coastal).
only two samples (250 m at station 3 and 1000 m at station BB2). In the upper oxycline, NO$_2^-$ oxidation rates appeared to be positively related to NO$_2^-$ concentration (Figure 7b). In particular, at NO$_2^-$ concentrations < 0.1 μM, there was a positive linear correlation between NO$_2^-$ oxidation rates and NO$_2^-$ concentration ($R^2 = 0.68$, $p < 0.01$).

When results from all stations were combined, NH$_4^+$ oxidation rates in the upper oxycline showed a Michaelis-Menten-like relationship ($p < 0.05$) with oxygen concentration (Figure 8a). In contrast, NO$_2^-$ oxidation rates displayed a nonlinear inverse relationship with oxygen concentration (Figure 8b). For the six samples in which both NH$_4^+$ oxidation rates and amoA gene abundance were measured, per cell NH$_4^+$ oxidation rates were calculated assuming each ammonia oxidizer carries one copy of either archaeal or bacterial amoA gene (Figure 9). The per cell NH$_4^+$ oxidation rates demonstrated a Michaelis-Menten relationship with in situ oxygen concentrations ($p < 0.01$), with a half-saturation concentration of 2.0 ± 1.2 μM and a maximum per cell NH$_4^+$ oxidation rate of 1.14 ± 0.15 fmol cell$^{-1}$ d$^{-1}$.

4. Discussion

4.1. Key Chemical Features of the ETSP OMZ
The degree of anoxia and NO$_2^-$ accumulation in the anoxic layer in the region likely reflects the amount of organic matter input from the surface layer, because organic matter remineralization in the water column...
4.2. NH$_4^+$ Oxidation and Its Determinants

NH$_4^+$ oxidation rates displayed subsurface maxima in the oxycline at all stations, and decreased with depth below the maximum (Figures 2c–4c). This pattern directly reflects the magnitude of organic matter flux in the water column, which is the source of NH$_4^+$ [Ward and Zafiriou, 1988]. The NH$_4^+$ oxidation rates measured in this study could be potential rates, because final NH$_4^+$ concentration (400–1600 nM) in the incubations often exceeded ambient NH$_4^+$ levels. The results of the kinetics experiment indicate that NH$_4^+$ oxidation rates should be saturated at these concentrations. Additionally, incubations were performed in the dark, which may have allowed samples taken from the euphotic zone to recover from photoinhibition, and reduced competition for NH$_4^+$ with phytoplankton [Eppley et al., 1971]. On the other hand, NH$_4^+$ regeneration, which was not accounted for in our calculation, would dilute the $^{15}$N-labeled NH$_4^+$ pool, and hence lead to underestimation of NH$_4^+$ oxidation rates. Both isotope dilution and competition with phytoplankton are probably minimal at this level of NH$_4^+$ addition. In the following discussion, we take the measured rates at face value and consider the three factors (NH$_4^+$ concentration, oxygen concentration, and light level) likely to control NH$_4^+$ oxidation.

NH$_4^+$ availability is one of the controls of NH$_4^+$ oxidation rates in our experiments. The classic Michaelis-Menten response of NH$_4^+$ oxidation rates to NH$_4^+$ concentration in the kinetics experiment demonstrated that the natural assemblage of ammonia oxidizers had extremely high affinity for NH$_4^+$ (27.2 ± 4.4 nM, Figure 6). The half-saturation concentration for NH$_4^+$ of this particular sample, collected from the PNM at the offshore station BB1, was the lowest among all published values determined in both culture and field studies. The half-saturation concentration for NH$_4^+$ of Nitrosopumilus maritimus determined in culture was 133 nM [Martens-Habbena et al., 2009]. The half-saturation concentration for NH$_4^+$ for the natural assemblage was 65 ± 41 nM at the PNM in the Sargasso Sea [Newell et al., 2013], and 98 ± 14 nM below the euphotic part of the water column of Hood Canal, Puget Sound, WA, USA [Horak et al., 2013]. The Sargasso Sea at least is as oligotrophic as the ETSP offshore OMZ, and the $K_m$’s for NH$_4^+$ by the natural assemblages of ammonia oxidizers in these two regions were not significantly different. $N. maritimus$ was isolated from a nutrient-rich environment and its $K_m$ is higher than all of the reported natural assemblages but still in the nM range. The very low $K_m$ for NH$_4^+$ by the natural assemblages of ammonia oxidizers in the ETSP indicates that they have adapted to the oligotrophic environment and are strong competitors for NH$_4^+$.

On the other hand, the linear relationship between NH$_4^+$ oxidation rates and in situ NH$_4^+$ concentrations measured in the upper oxycline at all stations (Figure 7a) reflects the population size of ammonia oxidizers. This is because all incubations in these experiments were performed at NH$_4^+$ concentrations (400–1600 nM) above saturation NH$_4^+$ concentrations (Figure 6), and therefore measured the potential of NH$_4^+$ oxidation.

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Figure 6. The dependence of NH$_4^+$ oxidation rate on NH$_4^+$ concentration at 75 m at offshore station BB1. The rates are plotted against the sum of ambient NH$_4^+$ and $^{15}$NH$_4^+$ added. The solid line is fitted based on the Michaelis-Menten equation, where $K_m$ is the half-saturation constant, and $V_{max}$ is the maximum rate of NH$_4^+$ oxidation. Standard deviations were calculated with Monte Carlo simulation (N = 10000).
Previous studies have also found a linear relationship between NH$_4^+$ oxidation rates and in situ NH$_4^+$ concentrations. During a strong upwelling event in the Washington coastal waters, NH$_4^+$ oxidation rates increased with in situ NH$_4^+$ concentrations, and did not saturate at NH$_4^+$ concentrations as high as 3 μM [Ward, 1985]. Similarly, in the California coastal waters which are also under the influence of upwelling, nitrification rates displayed a linear correlation with in situ NH$_4^+$ concentrations up to 0.9 μM [Smith et al., 2014b].

Indeed, the population size of ammonia oxidizers, measured as the archaeal amoA gene abundance, showed a robust positive linear correlation with NH$_4^+$ oxidation rates in the upper oxycline (Figure 10). This indicates that when no other environmental variables are limiting ammonia-oxidizing assemblages, their population size would directly respond to NH$_4^+$ availability, which is indicated by NH$_4^+$ concentrations. Such a linear correlation has also been identified in previous studies. In the Gulf of California, both the archaeal amoA gene abundance of Water Column Group A and marine Crenarchaeota 16S rRNA gene copies were positively correlated with NH$_4^+$ oxidation rates [Beman et al., 2008]. Similarly, nitrification rates were found to be positively correlated with the archaeal amoA gene abundance of Water Column Group A in the surface waters off the California coast [Smith et al., 2014a].

Ammonia oxidizers responded to higher NH$_4^+$ availability not only at the population level, but also at the cellular level. The average per cell NH$_4^+$ oxidation rate in this study (0.81 fmol cell$^{-1}$ d$^{-1}$), as revealed by the slope of the linear regression line in Figure 10, was 7 times lower than that reported from the California coastal water (6.48 fmol cell$^{-1}$ d$^{-1}$) [Smith et al., 2014a], where the NH$_4^+$ supply was higher than in the ETSP. We also calculated per cell NH$_4^+$ oxidation rates for each individual sample for which both NH$_4^+$ oxidation rates and amoA gene abundance data were available. Per cell NH$_4^+$ oxidation rate ranged from 0.1 to 2.1 fmol cell$^{-1}$ d$^{-1}$, and was similar to rates measured in other field experiments (0.2–15 fmol cell$^{-1}$ d$^{-1}$ in Santoro et al. [2010], 0.10–5.96 fmol cell$^{-1}$ d$^{-1}$ in Urakawa et al. [2014], 0.1–4.1 fmol cell$^{-1}$ d$^{-1}$ in Peng et al. [2015]), but on the lower range of those determined in culture (1.8–15.4 fmol cell$^{-1}$ d$^{-1}$ in Konneke et al. [2005]; 2–4 fmol cell$^{-1}$ d$^{-1}$ in Wuchter et al. [2006]).

The control of NH$_4^+$ availability on NH$_4^+$ oxidation rates is not only manifested on large spatial scales and molecular levels, but also reflected on temporal scales such as seasonal cycles. The NH$_4^+$ supply for nitrification in the ETSP originates from remineralization of organic matter produced in the euphotic zone.
Upwelling is one of the primary physical processes that transport nutrients to the euphotic zone and hence fuels primary production. As a result, primary productivity in the ETSP also displays a seasonal cycle [Berelson et al., 2015; Pennington et al., 2006]. NH\textsubscript{4}\textsuperscript{+} oxidation rates measured in this study fell into the low range of NH\textsubscript{4}\textsuperscript{+} oxidation rates reported from the ETSP OMZ previously (Table 2). This could be a result of the sampling time of austral winter (July 2013), when the productivity is relatively low [Berelson et al., 2015].

Besides NH\textsubscript{4}\textsuperscript{+}, oxygen is also a critical control on the rates of NH\textsubscript{4}\textsuperscript{+} oxidation. Per cell NH\textsubscript{4}\textsuperscript{+} oxidation rates from the upper oxycline of stations BB1 and BB2 showed a Michaelis-Menten relationship with oxygen concentration (Figure 9) with a half-saturation concentration (K\textsubscript{m}) of 2.0\textpm{}1.2 \textmu M, which is very close to the K\textsubscript{m} determined with Nitrosopumilus maritimus (3.9\textpm{}0.6 \textmu M) [Martens-Habbena et al., 2009]. This indicates that the oxygen level directly controls NH\textsubscript{4}\textsuperscript{+} oxidation rates on a molecular level. The oxygen concentration measurements used to calculate the K\textsubscript{m} were from the SBE oxygen sensor, which had a detection limit of 2.0 \textmu M. Hence the actual K\textsubscript{m} for oxygen is likely even lower than 2.0 \textmu M. In fact, a very low K\textsubscript{m} for oxygen (0.33\textpm{}0.13 \textmu M) has been measured by Bristow et al. [2013] in the ETSP, suggesting the ammonia-oxidizing assemblage in OMZs is adapted to extremely low oxygen concentrations. Such high affinity for oxygen by ammonia oxidizers implies that their activity is only affected by a narrow range of oxygen concentration, which is found at the oxic-anoxic interface of OMZs. At oxygen concentrations >2 \textmu M in the upper oxycline, oxygen is unlikely to limit NH\textsubscript{4}\textsuperscript{+} oxidation rates. Therefore, the Michaelis-Menten-like relationship between NH\textsubscript{4}\textsuperscript{+} oxidation rates and \textit{in situ} oxygen concentration in the upper oxycline (Figure 8a) probably does not reflect NH\textsubscript{4}\textsuperscript{+} oxidation rates controlled by oxygen concentrations, but rather by the NH\textsubscript{4}\textsuperscript{+} availability, which covaried with oxygen.

The relationships between NH\textsubscript{4}\textsuperscript{+} oxidation rates and \textit{in situ} NH\textsubscript{4}\textsuperscript{+} and oxygen concentrations also reveal that when neither substrate is limiting, light could inhibit NH\textsubscript{4}\textsuperscript{+} oxidation (Figures 7 and 8). NH\textsubscript{4}\textsuperscript{+} oxidation rates were slower in the euphotic zone than in the upper oxycline at the same NH\textsubscript{4}\textsuperscript{+} concentrations (Figure 7a), suggesting that light controls the population size, and therefore potential oxidation rates, in the euphotic zone even when samples are incubated in the dark. Light inhibits nitrifying activities by damaging the cytochromes in bacterial ammonia and nitrite oxidizers [Ward, 2011] but the mechanisms for light inhibition of AOA is unknown. These experiments demonstrated that photoinhibition of nitrification is not complete at 10%
surface irradiance. The partial inhibition of NH$_4^+$ oxidation by light is consistent with a number of previous studies both in the field and in culture. Horrigan et al. [1981] found that ammonia oxidizers from sea surface films were partially inhibited under the 8:16 light-dark cycle at a light level (167 µE m$^{-2}$ s$^{-1}$) very similar to that in our incubations (167 µE m$^{-2}$ s$^{-1}$ at station BB1 and 127 µE m$^{-2}$ s$^{-1}$ at station BB2, supporting information Figure S2). Culture studies of AOA and AOB both demonstrated that photo-inhibition of nitrifying activity is only partial, and the degree of inhibition varies among species [Guerrero and Jones, 1996; Merbt et al., 2012; Qin et al., 2014]. Moreover, there is evidence suggesting that nitrification activity in the euphotic zone was inhibited during the day, but could recover at night [Horrigan et al., 1981; Qin et al., 2014].

A confounding factor to photoinhibition of nitrification in the euphotic zone is the competition between ammonia oxidizers and phytoplankton for NH$_4^+$. Because all of our incubations were performed in the dark, such competition for NH$_4^+$ is likely minimized. NH$_4^+$ uptake by phytoplankton has been shown to control nitrification in the euphotic zone of California coastal waters, where light did not have an inhibitory effect on nitrification [Smith et al., 2014b].

The lack of response of NH$_4^+$ oxidation to the addition of Fe$_2$O$_3$ and MnO$_2$ at the SNM suggests that the ammonia-oxidizing microorganisms are incapable of oxidizing NH$_4^+$ using metals as oxidants in place of oxygen. It is worth noting though, that at the deep chlorophyll maximum (DCM) at station BB2, the NH$_4^+$ oxidation rate was eight-fold higher with the addition of 9.6 µM of oxygen (Figure 5). Given their low $K_m$ for oxygen, these ammonia oxidizers should have reached the maximum NH$_4^+$ oxidation rate per cell. This suggests that the large population of AOA at the oxic-anoxic interface are metabolically ready for oxygen supply, either from diffusion/turbulent mixing from the upper oxycline, or from the oxygen production by the Prochlorococcus at the DCM. The same addition of oxygen to the SNM samples had no effect, suggesting that the AOA detected in the anoxic layer are not metabolically active with respect to aerobic pathways even when oxygen is supplied.

### 4.3. NH$_4^+$ Oxidation and N$_2$O Production

The proximity of the subsurface maxima of NH$_4^+$ oxidation rates and N$_2$O concentration implies that NH$_4^+$ oxidation is likely a major pathway of N$_2$O production in the upper oxycline. Farias et al. [2009] have attributed the N$_2$O production in the upper oxycline of the ETSP to both NH$_4^+$ oxidation and partial denitrification (the reduction of NO$_2^-$/NO$_3^-$ to N$_2$O). However, it is worth noting that bacterial ammonia oxidizers can also produce N$_2$O through denitrification [Casciotti and Ward, 2001]. The pathway by which N$_2$O is produced by AOA is still not clear. Nitrification (i.e., from added $^{15}$NH$_4^+$) was the only pathway of N$_2$O production at oxygen concentrations above 5 µM in the upper oxycline detected by $^{15}$N tracer experiments on the same cruise [Ji et al., 2015]. This result indicates that nitrification is responsible for the N$_2$O concentration maxima in the upper oxycline, which were found at oxygen concentration of 93.2 µM at station BB1 and 27.9 µM at station BB2. Importantly, the upper oxycline is the depth interval from which N$_2$O might be emitted to the atmosphere by mixing of surface waters.

### 4.4. NO$_2^-$ Oxidation Rates

NO$_2^-$ oxidation rates also displayed a subsurface maximum in the upper oxycline, but in the anoxic layer, high rates of NO$_2^-$ oxidation were measured. The subsurface maximum of NO$_2^-$ oxidation was deeper than that of NH$_4^+$. 

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**Figure 9.** The relationship between NH$_4^+$ oxidation rates normalized by amoA gene abundances and oxygen concentration. The samples were from the upper oxycline at stations BB1 and BB2. The solid line is fitted based on the Michaelis-Menten equation, with a half-saturation concentration for O$_2$ of 2.7 ± 0.8 µM (Monte Carlo simulation N = 10000).
oxidation. Some of the NO$_2^-$ oxidation rates measured in this study were likely potential rates for the same reasons mentioned earlier for NH$_4^+$ oxidation rates. Although the NO$_2^-$ oxidation rate did not show a clear response to the total NO$_2^-$ concentration (supporting information Figure S3), it is clear that the NO$_2^-$ oxidation rate has saturated at NO$_2^-$ concentrations greater than 3.2 μM. Hence at the SNM, where NO$_2^-$ concentrations were high, the measured NO$_2^-$ oxidation rates were not affected by substrate addition. The NO$_2^-$ oxidation rates measured in this study are comparable to previous reports from OMZs (Table 2).

The decrease of NO$_2^-$ oxidation rates with depth below 75 m, excluding the samples from the anoxic layer (supporting information Figure S4), suggests that NO$_2^-$ oxidation in the upper oxycline also depends on remineralization for substrate supply. The linear relationship between NO$_2^-$ oxidation rates and NO$_2^-$ concentration at <0.1 μM in the upper oxycline indicates that the substrate dependence of NO$_2^-$ oxidation is the strongest at low NO$_2^-$ concentrations (Figure 7b). Because NO$_2^-$ oxidation rates were generally higher than NH$_4^+$ oxidation rates, there was likely another source of NO$_2^-$. Previous studies in the ETSP showed that NO$_3^-$ reduction is another major source of NO$_2^-$ in the upper oxycline [Kalvelage et al., 2013; Lipschultz et al., 1990]. The rate of NO$_3^-$ reduction to NO$_2^-$ was generally higher at lower oxygen levels in the upper oxycline of the ETSP, and potentially supporting a large population of nitrite oxidizers at those depths. This might be one of the reasons for the observed deeper subsurface maximum of NO$_2^-$ oxidation rates than that of NH$_4^+$ oxidation.

Recent studies have found a Michaelis-Menten relationship between NO$_2^-$ oxidation rates and oxygen concentration in the in situ NO$_2^-$-oxidizing assemblage in both the Namibian and the ETSP OMZs [Bristow et al., 2013; Fussel et al., 2012]. The half-saturation concentration for oxygen ($K_m$) determined using incubations with $^{15}$NO$_2^-$ and manipulation of the oxygen level with additions of oxygen-saturated seawater was very low ($K_m = 0.78$ μM in the ETSP and ~4 μM in the Namibian OMZ). However, there was no clear relationship between

![Figure 10.](image)

**Table 2.** A Summary of NH$_4^+$ and NO$_2^-$ Oxidation Rates Measured in the Major Oceanic OMZs Using $^{15}$N Technique

<table>
<thead>
<tr>
<th>OMZ Region</th>
<th>Max/Median of Max NH$_4^+$ Oxidation (nM d$^{-1}$)</th>
<th>Max/Median of Max NO$_2^-$ Oxidation (nM d$^{-1}$)</th>
<th>Method</th>
<th>Sampling Date</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETSP</td>
<td>77/22</td>
<td>278/107</td>
<td>$^{15}$N</td>
<td>Jun/Jul 2013</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>288/85</td>
<td>605/202</td>
<td>$^{15}$N</td>
<td>Feb/Mar 1985</td>
<td>Lam et al. [2009]</td>
</tr>
<tr>
<td></td>
<td>4900/144</td>
<td>N.M.</td>
<td>$^{15}$N</td>
<td>Apr 2005</td>
<td>Lipshultz et al. [1990]</td>
</tr>
<tr>
<td></td>
<td>89/33</td>
<td>186/37</td>
<td>$^{15}$N</td>
<td>Dec 2008 to Feb 2009</td>
<td>Kalvelage et al. [2013]</td>
</tr>
<tr>
<td>ETNP</td>
<td>46/38</td>
<td>115/81</td>
<td>$^{15}$N</td>
<td>Nov 1982/ May 1983</td>
<td>Ward [1987]</td>
</tr>
<tr>
<td></td>
<td>22/12</td>
<td>N.M.</td>
<td>$^{15}$N</td>
<td>Nov 1983</td>
<td>Ward and Zafiriou [1988]</td>
</tr>
<tr>
<td>Arabian Sea</td>
<td>23/15</td>
<td>N.M.</td>
<td>$^{15}$N</td>
<td>May/ Jun 2000</td>
<td>Satka et al. [2004]</td>
</tr>
<tr>
<td></td>
<td>348/47</td>
<td>136/57</td>
<td>$^{15}$N</td>
<td>Jul/Aug 2008</td>
<td>Beman et al. [2013]</td>
</tr>
<tr>
<td></td>
<td>36/28</td>
<td>536/312</td>
<td>$^{15}$N</td>
<td>Mar/Apr 2012</td>
<td>Peng et al. [2015]</td>
</tr>
<tr>
<td></td>
<td>21/9</td>
<td>N.M.</td>
<td>$^{15}$N</td>
<td>Sep-Oct 2007</td>
<td>Newell et al. [2011]</td>
</tr>
<tr>
<td></td>
<td>4/4</td>
<td>N.M.</td>
<td>$^{15}$N</td>
<td>Sep-Oct 2007</td>
<td>Lam et al. [2011]</td>
</tr>
</tbody>
</table>

*Max: the maximum NH$_4^+$ and NO$_2^-$ rates found in each study. Median of Max: the median of maximum NH$_4^+$ and NO$_2^-$ rates measured at each sampling station within the study. N.M.: not measured.*
NO$_2^-$ oxidation rates and in situ oxygen concentration in the Namibian OMZ [Fussel et al., 2012]. Our incubation measurements did not reveal any clear relationship between NO$_2^-$ oxidation rates and in situ oxygen concentration across several stations (Figure 8b). This may be due to the different population size of NO$_2^-$ oxidizers in each sample. Nitrite oxidizers were not enumerated in this study because we have not optimized a qPCR protocol for an appropriate functional gene and the nitrite oxidizers are too diverse to allow quantification by one set of 16S rRNA primers [Daims et al., 2011]. High rates of NO$_2^-$ oxidation measured in the anoxic layer in the absence of molecular oxygen were also reported previously [Kalvelage et al., 2013; Lipschultz et al., 1990; Peng et al., 2015]. However, no alternative electron acceptors have been identified and these high rates remain unexplained. The physiology and biochemical potential of nitrite oxidizers is not well known and investigation of their anaerobic metabolism may yet provide some explanation for high anoxic oxidation rates.

NO$_2^-$ oxidation appeared to be more sensitive to light than NH$_4^+$ oxidation, because NO$_2^-$ oxidation was detected in only 6 out of the 12 samples collected from the euphotic zone in which NH$_4^+$ oxidation was detected. This is consistent with a culture study comparing the photoinhibition by AOB and NOB [Guerrero and Jones, 1996]. On the other hand, when NO$_2^-$ oxidation was not completely inhibited in the euphotic zone, it might also be colimited by NO$_2^-$ supply, because NH$_4^+$ oxidation rates in the euphotic zone were either very low or below detection. NO$_2^-$ oxidation rates measured in the light/dark experiment were too variable to be significantly different than zero (Table 1).

5. Conclusion

The NH$_4^+$ oxidation rates in the ETSP mainly displayed a depth distribution consistent with ultimate control by the vertical flux of organic material, i.e., NH$_4^+$ supply. In addition, NH$_4^+$ oxidation rates depended on NH$_4^+$ concentration, oxygen concentration, and light. In the euphotic zone, NH$_4^+$ oxidation was partially photoinhibited, and in the anoxic layer and the oxic-anoxic interface of OMZs, NH$_4^+$ oxidation was limited by oxygen concentration. NH$_3^+$ oxidation displayed extremely high affinity for both NH$_4^+$ and oxygen. The positive linear correlations between NH$_4^+$ oxidation rates and in situ NH$_4^+$ concentrations and amoA gene abundances in the upper oxycline indicate that natural assemblage of ammonia oxidizers responds to in situ NH$_4^+$ concentrations by adjusting their population size, which determines the NH$_4^+$ oxidation potential. The depth distribution of archaeal and bacterial amoA gene abundances and NO$_2^-$ concentration suggests that AOA were predominantly responsible for NH$_4^+$ oxidation, which was the primary source of N$_2$O production in the upper oxycline of the OMZ.

Besides the primary influence from the vertical organic matter flux, NO$_2^-$ oxidation was also controlled by substrate availability and light. The depth of the subsurface NO$_2^-$ oxidation maximum in the upper oxycline was greater than that of NH$_4^+$ oxidation maximum. At this depth, the NO$_2^-$ oxidation rates were higher than NH$_4^+$ oxidation rates, suggesting an additional source of NO$_2^-$ in the lower part of the upper oxycline near the oxic-anoxic interface. Considerable rates of NO$_2^-$ reduction to NO$_2^-$ at this depth have been measured in the ETSP previously [Kalvelage et al., 2013; Lipschultz et al., 1990], and might be a major source of NO$_2^-$ for NO$_2^-$ oxidation. The high NO$_2^-$ oxidation in the absence of molecular oxygen in the anoxic layer of OMZs remains unexplained.

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