Ammonia and nitrite oxidation in the Eastern Tropical North Pacific

Xuefeng Peng1, Clara A. Fuchsman2, Amal Jayakumar1, Sergey Oleynik1, William Martens-Habbena3, Allan H. Devol2, and Bess B. Ward1

1Department of Geosciences, Princeton University, Princeton, New Jersey, USA, 2School of Oceanography, University of Washington, Seattle, Washington, USA, 3Department of Civil and Environmental Engineering, University of Washington, Seattle, Washington, USA

Abstract
Nitrification plays a key role in the marine nitrogen (N) cycle, including in oceanic oxygen minimum zones (OMZs), which are hot spots for denitrification and anaerobic ammonia oxidation (anammox). Recent evidence suggests that nitrification links the source (remineralized organic matter) and sink (denitrification and anammox) of fixed N directly in the steep oxycline in the OMZs. We performed shipboard incubations with 15N tracers to characterize the depth distribution of nitrification in the Eastern Tropical North Pacific (ETNP). Additional experiments were conducted to investigate photoinhibition. Allylthiourea (ATU) was used to distinguish the contribution of archaeal and bacterial ammonia oxidation. The abundance of archaeal and β-proteobacterial ammonia monooxygenase gene subunit A (amoA) was determined by quantitative polymerase chain reaction. The rates of ammonia and nitrite oxidation showed distinct subsurface maxima, with the latter slightly deeper than the former. The ammonia oxidation maximum coincided with the primary nitrite concentration maximum, archaeal amoA gene maximum, and the subsurface nitrous oxide maximum. Negligible rates of ammonia oxidation were found at anoxic depths, where high rates of nitrite oxidation were measured. Archaeal amoA gene abundance was generally 1 to 2 orders of magnitude higher than bacterial amoA gene abundance, and inhibition of ammonia-oxidizing bacteria with 10 μM ATU did not affect ammonia oxidation rates, indicating the dominance of archaea in ammonia oxidation. These results depict highly dynamic activities of ammonia and nitrite oxidation in the oxycline of the ETNP OMZ.

1. Introduction
Nitrification links the source and sink of fixed nitrogen (N) by oxidizing ammonium (NH4+), the initial product of organic matter mineralization, to nitrite (NO2−) and then nitrate (NO3−), which can be reduced back to dinitrogen gas (N2) by denitrification and anaerobic ammonia oxidation (anammox). This link is usually considered indirect and to occur on a long time and space scale, because nitrification is aerobic and thus restricted to oxic environments whereas denitrification and anammox are anaerobic processes. These different relationships to oxygen imply spatial segregation in the classic view of the N cycle and preclude a direct link between nitrification and the N sink processes that determine the oceanic fixed N budget. However, there is emerging evidence of direct coupling between remineralization, nitrification, and N loss processes in the oxygen minimum zones (OMZs) of the ocean [e.g., Jensen et al., 2008; Fernandez et al., 2011; Dalsgaard et al., 2014].

OMZs are responsible for removing up to 30–50% of fixed N from the ocean, despite their tiny volume (~0.1% of total ocean volume [DeVries et al., 2012, 2013]). While N loss processes have historically been thought to be inhibited by the presence of oxygen [Tiedje et al., 1982; Strous et al., 1997; Zumft, 1997], it appears that anammox has a relatively wide range of oxygen sensitivity and can be active up to 10–20 μM oxygen [Dalsgaard et al., 2014; Jensen et al., 2008; Kalvelage et al., 2011]. Given that both ammonia and nitrite oxidizers have been shown to be active under low-oxygen conditions [Bristow et al., 2013; Lucker et al., 2013], they could potentially interact with denitrification and anammox in several ways: (1) nitrifiers may supply NO2− and NO3− to denitrification and anammox, (2) nitrifiers could compete for NH4+ and NO2− with anammox and denitrifying bacteria, and (3) oxygen consumption by nitrifiers in the oxycline could lower the oxygen levels to a range that is less inhibitory for N removal processes. Indeed, Lam et al. [2009] suggested that nitrification could supply >65% of the NO3− required by anammox in the eastern tropical South Pacific (ETSP) OMZ, and a recent metaproteomics study detected proteins involved in the nitrification,
denitrification, and anammox pathways in the oxycline of Saainch Inlet [Hawley et al., 2014]. It should be noted, however, that the interactions between nitrification and N loss processes are probably restricted to a narrow depth interval of the oxycline, near the oxic-anoxic interface above the anoxic layer. Here the terms “oxycline” and “oxic-anoxic interface” refer to those features above the anoxic layer in the OMZ rather than the oxycline below the anoxic layer in the OMZ, except when explicitly mentioned. “Anoxic” refers to depths where oxygen was undetectable by the STOX sensor (1–10 nM [Revsebech et al., 2009]).

Since the discovery of ammonia-oxidizing archaea (AOA) [Könneke et al., 2005], evidence for their dominant contribution to ammonia oxidation over their bacterial counterparts has been accumulating [e.g., Santoro et al., 2010; Beman et al., 2012]. However, the argument that AOA account for most of ammonia oxidation in marine environments is based mainly on the fact that AOA outnumber ammonia-oxidizing bacteria (AOB) in the environment [Prosser and Nicol, 2008] rather than on direct measurements of the relative contribution of each group to the measured rates. A recent study used an inhibitor specifically inhibiting AOA [2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide, PTIO] to investigate the proportion of archaeal and bacterial ammonia oxidation [Martens-Habbena et al., 2014], and they found that AOA dominated nitrification in the North Pacific open ocean and a coastal environment. PTIO is a nitric oxide (NO) scavenger that has been shown to inhibit AOA, presumably because NO is an intermediate of archaeal but not bacterial ammonia oxidation [Akaike et al., 1993; Martens-Habbena et al., 2014]. Furthermore, the same study demonstrated that 10 μM of allylthiourea (ATU) inhibited the six β-proteobacterial AOB tested, but none of the cultured marine AOA. Therefore, PTIO and ATU can be used to distinguish the relative proportion of archaeal and bacterial ammonia oxidation.

Both archaeal and bacterial ammonia oxidizers are capable of producing nitrous oxide (N₂O), either as a by-product of ammonia oxidation, or via nitrifier denitrification [Goreau et al., 1980; Santoro et al., 2011]. If ammonia oxidation is active at the low oxygen depths in the oxycline of OMZs, it will potentially be an important pathway of N₂O production, because N₂O yield as a percentage of ammonia oxidation increases as ammonia oxidation is active at the low oxygen depths in the oxycline of OMZs, it will potentially be an important pathway of N₂O production, because N₂O yield as a percentage of ammonia oxidation increases with decreasing oxygen [Cohen and Gordon, 1979; Goreau et al., 1980; Löschker et al., 2012]. While a subsurface maximum of N₂O concentration has been observed in the oxycline of OMZs [Naqvi and Noronha, 1991], it remains unclear whether ammonia oxidation was directly responsible for the N₂O production in the oxycline. N₂O consumption rates were measured on the same cruise as this study, and the N₂O production rates calculated using a 1-D model suggest that both ammonia oxidation and denitrification contributed toward N₂O production at the oxic-anoxic interface [Babbin et al., 2015].

While there are numerous studies of denitrification and anammox in OMZs, nitrification in this environment has received less attention. This study aims to determine the distribution of ammonia and NO₂⁻ oxidation in relation to oxygen and dissolved inorganic nitrogen (DIN) distributions in the OMZ. We performed shipboard incubations using 15N tracers to measure nitrification rates in the Eastern Tropical North Pacific (ETNP) OMZ, targeting the surface mixed layer, the primary NO₃⁻ maximum, the oxycline, the anoxic layer, the secondary NO₂⁻ maximum (SNM), and the deep oxycline below the anoxic layer. The abundance of the functional gene for both archaeal and bacterial ammonia oxidation, amoA, was measured using quantitative polymerase chain reaction (qPCR). Additional experiments were performed to directly distinguish the contribution to ammonia oxidation by AOA and AOB, using the inhibitors ATU and PTIO in parallel incubations.

2. Methods
2.1. Site Description and Physicochemical Data Collection
Nitrification incubations were performed at six stations in the ETNP in March and April 2012 on board the R/V Thomas G. Thompson (Figure 1). One of the stations (BB1) was coastal (<50 km offshore, bottom depth = 2207 m), characterized by higher surface chlorophyll levels than the rest of the stations, which were offshore (>200 km offshore, bottom depths >3000 m). Stations BB1 and BB2 were sampled with greater resolution than the other stations. Nutrient samples were filtered (GF/F glass fiber) before analysis, and all water samples were analyzed using the U.S. Joint Global Ocean Flux Study protocols [United Nations Educational, Scientific and Cultural Organization, 1994]. Concentrations of NH₄⁺, NO₂⁻, and NO₃⁻ were determined by standard spectrophotometric methods on board, with detection limits of 0.12 μM for NH₄⁺, 0.02 μM for NO₂⁻, and 0.15 μM for NO₃⁻. Dissolved oxygen concentration was determined using the SBE
43 dissolved oxygen sensor attached to the conductivity, temperature, and depth (CTD) rosette. For some of the sampling 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 anoxic depth interval in the OMZ, because the STOX sensor has an extremely low detection limit (1–10 nM [Revsbech et al., 2009; Tiano et al., 2014]). However, the values reported in this paper are from the SBE 43 sensor because it provided a complete coverage of all sampling stations on this cruise. Seawater samples for dissolved N2O concentration were collected from Niskin bottles into 160 mL serum bottles over flowing three times and crimp sealed. N2O was extracted using sequential head-space equilibration of equal volumes (25 mL) of helium and seawater [McAuliffe, 1971; Naqvi et al., 1998]. Each extraction (0.5 mL) was injected into a Shimadzu GC Model GC-8A gas chromatograph with an electron capture detector and a 2 m by 2.2 mm inner diameter Hayesep D column (80/100 mesh). The column was set at 30°C, while the injection port and detector were maintained at 50 and 300°C, respectively. The carrier gas was ultra-high-purity nitrogen. Analyses were standardized using ambient air and a 1 ppm N2O standard (Scott Gas).

2.2. Onboard Incubation Experiments

Samples were collected using 10 L Niskin bottles on a CTD rosette. As soon as the CTD rosette arrived on deck the bottles from the anoxic depths were sampled first. Approximately 450 mL of seawater were incubated in duplicate with 15NH4+ or 15NO2- in opaque, metal-free, gas-tight, and tri-laminate bags, which had been triple flushed with nitrogen gas half an hour prior to filling. The bags were filled directly from the Niskin bottles avoiding seawater contact with the atmosphere, and the tracer solutions were injected during the filling process to ensure complete mixing. The final concentration of 15N substrates reached ~ 400 nM, which should be above the substrate saturation concentration for nitrification [Horak et al., 2013; Newell et al., 2013; Qin et al., 2014].

In an attempt to distinguish archaeal and bacterial ammonia oxidation, parallel incubations with 15NH4+ and allylthiourea (ATU) or 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO) were included. ATU was intended to inhibit β-proteobacterial AOB, with minimal effect on AOA at the concentration in our incubations (~10 μM [Martens-Habbema et al., 2014]). PTIO was intended to inhibit AOA but not AOB at the concentrations in our incubations (~80 μM). Both 15N tracers and inhibitors were degassed under vacuum for 30 min to remove dissolved oxygen before they were added to bags. The bags were incubated at a temperature close to the in situ temperature (within 0–6°C of in situ temperature). After 12 h of incubation, a 45 mL aliquot of the sample was transferred to a 50 mL centrifuge tube and stored at ~80°C.

2.3. Measurement of Ammonia Oxidation

NO2−, the product of ammonia oxidation, was converted to N2O following the azide method of McIlvin and Altabet [2005]. In brief, a fresh 1:1 mixture of 2 M sodium azide and 20% acetic acid was prepared daily and purged with helium gas at 40 mL min−1 for 20 min to remove any preexisting N2O. For samples with NO2− concentration lower than 0.5 μM, 4 mL of 1 μM NaNO2 was added as a carrier. Samples were purged with helium gas at 40 mL min−1 for 10 min in a gas-tight vial, and then 0.4 mL of the sodium azide/acetic acid mixture was added to each sample. After incubating for 15 min at room temperature, the pH of samples was raised to greater than 12 via the addition of 0.3 ml of 10 M sodium hydroxide (NaOH).

Because no 14NO2− carrier was added with the 15NH4+ tracer during the shipboard incubation, a portion of the 15NO2− produced by ammonia oxidation could have been subsequently oxidized to 15NO3− during the incubation, especially in samples with low NO2− concentration. To evaluate whether the azide method resulted in an underestimation of ammonia oxidation rates when no 14NO2− carrier was included during
the incubation, the denitrifier method [Sigman et al., 2001], which converts both NO$_2^-$ and NO$_3^-$ to N$_2$O, was used to measure the production of $^{15}$NO$_2^-$ plus $^{15}$NO$_3^-$ for samples from stations BB1 and BB2.

The concentration of N$_2$O and ratio of $^{45}$N$_2$O/$^{44}$N$_2$O were determined on a Delta V Plus isotope ratio mass spectrometer coupled to a purge-and-trap front end. The detection limit was 0.2 nmol of N, and the precision of $\delta^{15}$N was 0.2‰. $\delta^{15}$N = ($R^{15}N_{sample}/R^{15}N_{reference}$ − 1) × 1000‰, where $R^{15}N$ is ratio of $^{15}$N to $^{14}$N molecules in the sample or reference material, and atmospheric N$_2$ is used as a reference here.) Fresh NO$_2^-$ standards were prepared on the same day as the samples were processed for determination of sample NO$_2^-$ concentration. To calibrate the high $\delta^{15}$N-NO$_2^-$, seven NO$_2^-$ stable isotope standards (Table S1 in the supporting information) were made by combining different quantities of 98% Na$^{15}$NO$_2$ (Cambridge Isotopes) and NaNO$_2$ (Reagent A.C.S., Fisher). The $\delta^{15}$N of NaNO$_2$ was calibrated against a NO$_2^-$ stable isotope working standard ($\delta^{15}$N = 0.45‰). The true sample $\delta^{15}$N-NO$_2^-$ was calculated from the isotope signature of the N$_2$O produced from azide conversion with a standard curve (Figure S1 in the supporting information), and because the azide (from which one of the N atoms in the produced N$_2$O derives) had a $\delta^{15}$N close to zero, the calculated $\delta^{15}$N-NO$_2^-$ is about twice the measured $\delta^{15}$N-N$_2$O.

The rate of ammonia oxidation was calculated following the equation:

$$V_{NH_4^+} = \frac{\Delta[15NO_2^-]}{f_{NH_4^+} \times T}$$

where $\Delta[15NO_2^-]$ is the change in concentration of $^{15}$NO$_2^-$ between the start and the end of the incubation as a result of ammonia oxidation, $f_{NH_4^+}$ is the fraction of NH$_4^+$ that was labeled with $^{15}$N at the start of the incubation, and $T$ is the length of incubation. The concentration of $^{15}$NO$_2^-$ at the start of the incubation ($[15NO_2^-]_0$) was assumed to be natural abundance. The actual value of natural abundance $\delta^{15}$N-NO$_2^-$ for each sample was taken from a depth profile in the ETNP reported by Casciotti and McIlvin [2007]. For samples collected from depths shallower than the anoxic layer, the $[15NO_2^-]_0$ was calculated with the natural abundance $\delta^{15}$N-NO$_2^-$ reported for the depth that had the same oxygen concentration on the published depth profile [Casciotti and McIlvin, 2007]. For samples collected from the anoxic depths, the $[15NO_2^-]_0$ was calculated with the natural abundance $\delta^{15}$N-NO$_2^-$ reported 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 and McIlvin, 2007]. For the two samples collected from 1100 m (deeper than the anoxic layer), we calculated the $[15NO_2^-]_0$ assuming a $\delta^{15}$N-NO$_2^-$ of 0‰. Rates were not particularly sensitive to variability of initial $\delta^{15}$N-NO$_2^-$ in a reasonable range. Sensitivity tests demonstrated that fluctuations of the natural abundance $\delta^{15}$N-NO$_2^-$ by ±5‰ would result in negligible differences in calculated ammonia oxidation rates (Figure S2).

### 2.4. Measurement of NO$_2^-$ Oxidation

The $\delta^{15}$N-NO$_3^-$ was measured using the denitrifier method [Sigman et al., 2001; McIlvin and Casciotti, 2011]. Samples were first treated with 15 mM sulfamic acid (final concentration) for 1 h to remove any preexisting NO$_2^-$, and the pH was raised to ~7 with NaOH [Granger and Sigman, 2009]. Three NO$_3^-$ international reference materials (IAEA-N3, USGS 34, and USGS 32) were used to calibrate the $\delta^{15}$N-NO$_3^-$.

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

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

where $\Delta[15NO_3^-]$ 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 $[15NO_3^-]$ at the start of the incubation ($[15NO_3^-]_0$) was assumed to be natural abundance. The actual value of natural abundance $\delta^{15}$N-NO$_3^-$ for each sample was taken from a depth profile in the ETNP reported by Casciotti and McIlvin [2007], in the same way as done for the natural abundance of $\delta^{15}$N-NO$_2^-$ for samples collected from depths shallower than the anoxic layer and from the anoxic depths. For the two samples collected from 1100 m (deeper than the anoxic layer), we calculated the $[15NO_3^-]_0$ 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$_4^+$/NO$_2^-$ oxidation rates calculated may be underestimations.
In the laboratory the efficiency of sulfamic acid in removing NO$_2^-$ was tested on mixtures of 15NO$_2^-$/C$_0$ and 14NO$_3^-$/C$_0$, with a final concentration of 9–10 μM of 15NO$_2^-$ and 30 μM of 14NO$_3^-$. Sulfamic acid was added to the mixture reaching a final concentration of 15 mM and incubated at room temperature for 1 h or 24 h. Two control groups were included: one was de-ionized water, and the other contained only 30 μM of 14NO$_3^-$. After incubation with sulfamic acid, the mixture was neutralized with NaOH before performing the denitrifier method to measure the N isotopic composition of the treated mixture.

There was apparently a small fraction (1.5%) of contaminating 15NO$_3^-$ ("15Ncontam") in the initial 15NO$_2^-$ tracer solution used in the experiments that were performed to test the efficiency of NO$_2^-$ removal with sulfamic acid. It was critical to subtract "15Ncontam" from the measured Δ 15NO$_3^-$ when calculating NO$_2^-$ oxidation rates in order to avoid overestimation. We calculated the "15Ncontam" as the minimum value of Δ 15NO$_3^-$ for each sampling cast (see Text S1 in the supporting information for detailed description of the method). This approach will result in only a slight underestimation in NO$_2^-$ oxidation rates.

2.5. Detection Limit of Rate Measurements

The detection limit was determined for every single incubation following Santoro et al. [2013], and depends on the fraction of the substrate labeled with 15N 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 ammonia oxidation measurements) had a detection limit of 0.01–0.12 nM d$^{-1}$, and the denitrifier method (used for NO$_2^-$ oxidation measurements) of 0.39–4.48 nM d$^{-1}$. For the rest of the samples, the azide method had a detection limit of 0.001–0.034 nM d$^{-1}$, and the denitrifier method of 0.07–2.55 nM d$^{-1}$. The azide method had a lower detection limit than the denitrifier method, mainly because NO$_2^-$ concentrations were lower than NO$_3^-$ concentrations. The samples from anoxic depths had relatively higher detection limits due to the presence of high NO$_3^-$ concentrations, which results in a higher concentration product pool for the ammonia oxidation measurements, and a lower substrate fraction labeled with 15N for the NO$_2^-$ oxidation measurements. Rates are reported only if the final amount of 15NO$_2^-$ in the two replicate incubations was significantly (p < 0.05) greater than the initial 15NO$_2^-$.

2.6. Quantification of Archaeal and Bacterial amoA Genes

At the process stations (BB1 and BB2) particulate material was collected by filtering 2.5–10 L of seawater through 0.22 μm Sterivex filters with a peristaltic pump. Nucleic acids were extracted as described previously.
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′-GCGGCCATCCATCTGTATGT-3′) [Francis et al., 2005] were used for archaeal amoA gene quantification, and primers amoA-1F (5′-GGGGTTTCTACTGGTGGT-3′) and amoA-2R (5′-CCCCTCKGSAAAGCCTTCTTC-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 to 10 gene copies per mL of seawater.

3. Results

3.1. Chemical Profiles

All chemical profiles at stations BB1 and BB2 were plotted against depth, as well as sigma-t (σt). Because data from multiple casts are presented for these two stations, σt profiles clearly display the juxtaposition of some of the key features better than depth profiles, although the depth profiles more clearly display the resolution of sampling. All stations were characterized by a strong oxycline in the upper water column (Figures 2–6), below which oxygen concentration was below the detection limit of the STOX sensor.

Figure 3. Rates, chemical, and amoA gene abundance profiles at the offshore station BB2. Panels and symbols are the same as in Figure 2. Samples from 30, 70, 90, 150, and 500 m were collected at 12:00, and the others at 18:00.

Figure 4. Rates and chemical profiles at the offshore station 135. Panels and symbols are the same as in Figure 2. No N2O concentration or amoA gene abundance were measured at this station. Samples were collected at 12:20.
The oxycline depth was deeper at the offshore stations than at the coastal station. Below the anoxic layer the oxygen concentration increased gradually, and at 1100 m there was still less than 15 μM of oxygen (Figures 2c and 3c). The NH₄⁺ concentration was very low or below the detection limit throughout most of the water column but showed a narrow distinct maximum in the oxycline region above the anoxic layer. The NH₄⁺ concentration maximum was not detected at station 135, although it may have been missed as a result of the sampling resolution (Figure 4d). The highest NH₄⁺ concentration, 1.2 μM, was observed at a single depth of the subsurface maximum at the coastal station (Figure 2d). At all stations, a primary NO₂⁻/C₀ maximum (PNM, NO₂⁻ concentration up to ~2 μM) was observed in the oxycline above the anoxic layer. Within the anoxic layer we observed a secondary NO₂⁻ maximum (SNM), with NO₂⁻ concentration of at least 2.3 μM and reaching as high as 8 μM at the coastal station. Between the two NO₂⁻ maxima, a NO₂⁻ minimum, with concentrations often below detection, corresponded to the depth of the oxic-anoxic interface. A pronounced N₂O peak was present in the lower part of the oxycline, where the oxygen concentration was below 20 μM (Figures 2g and 3g) [Babbin et al., 2015].

3.2. Sensitivity of the Rate Measurement Methods

For samples with relatively high rates of ammonia oxidation (>10 nM d⁻¹), the denitrifier method yielded rates that were very close to the azide method, suggesting that the azide method did not result in underestimation even though no ¹⁴NO₂⁻ carrier was used (Table S2). This argument is consistent with a recent study that employed the same two methods [Santoro et al., 2013]. However, for samples with low ambient NO₂⁻ concentration (<0.1 μM), the azide method consistently resulted in lower measured rates than the denitrifier method (Table S2). We suggest that the denitrifier method should be preferred over the azide method when ambient NO₂⁻ is low, and no ¹⁴NO₂⁻ carrier is used during the incubation. Ammonia oxidation rates reported in the following sections were measured using the azide method, which might be slight underestimates.
3.3. Ammonia Oxidation Rates

Ammonia oxidation rates were minimal in the surface water (<1 nM d\(^{-1}\) except coastal station BB1). The maximum rate of ammonia oxidation (8.55 nM d\(^{-1}\)) at the coastal station was at 50 m, where oxygen concentration was below the detection limit of the STOX sensor (Figure 2). At offshore stations, a subsurface maximum (26.4–36.3 nM d\(^{-1}\)) was measured in the oxycline, and it was usually at the same depth as the PNM and the N\(_2\)O concentration maximum (Figures 3–6). Within the anoxic layer, ammonia oxidation rates were either undetectable or very low (0.5–2.0 nM d\(^{-1}\)), except at the coastal station.

Ammonia oxidation rates were significantly correlated with N\(_2\)O concentration (\(p < 0.001\); Figure 7), but not with NO\(_2\)^– concentration (\(p = 0.27\)), NH\(_4\)^+ concentration (\(p = 0.63\)), or oxygen concentration (\(p = 0.92\)). A Michaelis-Menten-like relationship was observed between ammonia oxidation rates and in situ oxygen concentrations in low oxygen samples (oxygen < 50 μM; Figure 8a), with an estimated half-saturation concentration for oxygen of 3.6 ± 0.6 μM and a maximum ammonia oxidation rate of 37.1 ± 1.2 nM d\(^{-1}\) (Monte Carlo simulation \(N = 10000\)). The ammonia oxidation rates at offshore stations decreased exponentially with depth (Figure 9).

3.4. The Effect of Inhibitors on Ammonia Oxidation

Rates of ammonia oxidation in samples treated with 10 μM ATU were generally ~10–20% lower than those in the control (Table 1). The exception was the sample from 70 m at station BB2, where the addition of 10 μM ATU appeared to have stimulated ammonia oxidation rates. None of the differences, however, were statistically significant. In the treatments with 80 μM PTIO, ammonia oxidation rates were not different than those in the control, except for one case (Table 1). In the sample from 15 m at station BB1, 80 μM PTIO inhibited the ammonia oxidation activity by ~40%.

**Figure 7.** The positive correlation between N\(_2\)O concentration and ammonia oxidation rates. Theses 15 samples were from stations BB1 and BB2. Error bars represent the standard deviation of two replicates. When the error bars are not visible, they are covered by the symbol.

**Figure 8.** The relationship between oxygen concentration and ammonia oxidation rates (a) and NO\(_2\)^– oxidation rates (b) at oxygen levels lower than 50 μM (as measured by the Seabird sensor in situ). All samples are from the oxycline above the anoxic layer at offshore stations. The solid line in Figure 8a is a model fitted based on the Michaelis-Menten equation, where \(K_m\) is the half-saturation constant, and \(V_{max}\) is the maximum rate of ammonia oxidation. Standard deviations in Figure 8a were calculated with Monte Carlo simulation (\(N = 10000\)). Error bars in Figure 8b represent the standard deviation of two replicates. When the error bars are not visible, they are covered by the symbol.
3.5. NO$_2^-$ Oxidation Rates

The depth distribution of NO$_2^-$ oxidation rates was similar to that of ammonia oxidation rates in that both exhibited subsurface maxima. The maximum NO$_2^-$ oxidation rate occurred at a slightly deeper isopycnal than the ammonia oxidation rate maximum, where oxygen level was low (<10 μM), and NO$_2$ concentration was at its minimum between the PNM and the SNM (Figures 2–6, panel e). NO$_2^-$ oxidation rates were generally higher than ammonia oxidation rates, sometimes by several fold (Figures 2–6). At the coastal station, the highest NO$_2^-$ oxidation rate was at the same anoxic depth (50 m) where ammonia oxidation was maximal, and it was the highest NO$_2^-$ oxidation rate (536 nM d$^{-1}$) observed overall (Figure 2b). The highest NO$_2^-$ oxidation rate offshore (312 nM d$^{-1}$) was also within the anoxic layer (station BB2; Figure 3, panels b and c). In the surface mixed layer, NO$_2^-$ oxidation was not detected at stations 135 and BB2, but at the other stations NO$_2^-$ oxidation was higher than ammonia oxidation. At the PNM at stations BB2, 164 and 180, no NO$_2^-$ oxidation was detected. NO$_2^-$ oxidation and oxygen concentration demonstrated an inverse nonlinear relationship at oxygen levels <50 μM (Figure 8b).

3.6. Abundance of amoA Genes

The depth distributions of archaeal amoA gene abundance were very similar to that of ammonia oxidation rates, with a subsurface maximum (Figures 2, 3, and S3). The range of archaeal amoA gene abundance (12–7470 copies mL$^{-1}$) was much greater than that of β-proteobacterial amoA gene abundance (92–481 copies mL$^{-1}$). Archaeal amoA genes were approximately an order of magnitude more abundant than β-proteobacterial amoA genes, except for at the well-lit depths (surface at BB1 and down to 50 m at BB2). The subsurface maximum of the β-proteobacterial amoA gene was shallower than that of the archaeal amoA gene at both stations BB1 and BB2.

At the coastal station, archaeal amoA gene abundance reached a maximum of 7470 copies mL$^{-1}$ at 40 m where the oxygen level was <2 μM (Figure 2h). In the anoxic layer, archaeal amoA genes were an order of magnitude less abundant compared to the subsurface maximum. At 1100 m (oxygen ~15 μM), the archaeal amoA gene abundance (5590 copies mL$^{-1}$) was on the same order of magnitude as in the subsurface maximum. Beta-proteobacterial amoA gene abundance remained low below the anoxic layer.

At the offshore station BB2, the highest abundance of archaeal amoA gene (14180 copies mL$^{-1}$) was also found at the depth of the oxic-anoxic interface (<1 μM at 100 m), and it was very near the depth of the N$_2$O maximum (Figure 3h). Archaeal amoA gene abundance was very low in the surface mixed layer, where it was even lower than the β-proteobacterial amoA gene abundance. Substantial abundances of both archaeal and β-proteobacterial amoA genes were found in the anoxic layer. Below the anoxic layer, there was high archaeal amoA gene abundance at 1100 m (5930 copies mL$^{-1}$), which was comparable to the archaeal amoA abundance at 1100 m at the coastal station (Figure 3h).

Table 1. The Ratio (Shown as %) of Ammonia Oxidation Activity in Duplicate Incubations With ATU and PTIO to the Activity in Parallel Incubations Without Inhibitors

<table>
<thead>
<tr>
<th>Station and depth</th>
<th>10 μM ATU</th>
<th>80 μM PTIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB1 - 15 m</td>
<td>85%, 79%</td>
<td>61%, 62%</td>
</tr>
<tr>
<td>BB1 - 20 m</td>
<td>84%, 93%</td>
<td>83%, 109%</td>
</tr>
<tr>
<td>BB2 - 70 m</td>
<td>131%, 98%</td>
<td>103%, 65%</td>
</tr>
<tr>
<td>BB2 - 100 m</td>
<td>65%, 82%</td>
<td>73%, 86%</td>
</tr>
</tbody>
</table>
4. Discussion

4.1. Chemical Profiles

The dissolved oxygen and inorganic N concentration depth profiles are typical of oceanic OMZs, where the upper part of the water column is characterized by a sharp oxycline, underlain by an anoxic layer. The NO$_2^-$ concentration in the secondary NO$_2^-$ maximum observed during this cruise was greater in magnitude than previous reports in the ETNP [Ward and Zafririou, 1988; Beman et al., 2013]. The chemical profiles at the coastal station were compressed relative to those at offshore stations (e.g., oxygen was depleted at ~30 m at station BB1, compared to 100–150 m at the offshore stations; Figures 2–6, panel c). No primary NO$_2^-$ maximum was distinguished at the coastal station likely due to the compact structure of the chemical profiles. The depth profiles of dissolved oxygen and inorganic nitrogen at stations BB1 and BB2 included measurements from multiple casts at the same station (28 casts for BB1 and 24 for BB2). Although there is some variability among the measurements made on different casts, overall they delineate the oxygen and DIN profiles with a high depth resolution when plotted against $\sigma_t$ (Figures 2 and 3, bottom plots). For example, it is clear that the maximum NH$_4^+$ concentration was restricted to a very narrow depth interval. At the stations with lower sampling resolution, we have likely missed sampling the depth interval of maximum NH$_4^+$ concentration (e.g., station 135, Figure 4).

The close association of the PNM and subsurface maximum of ammonia oxidation at stations 164 and 180 strongly suggests that ammonia oxidation was at least partially responsible for the formation of the PNM in the oxycline at these two stations (Figures 5 and 6). Such a relationship is less clear at stations BB2 and 135 because the PNM was 10 m shallower than the subsurface maximum of ammonia oxidation (Figures 3 and 4). At station BB1, the correlation between the PNM and the subsurface maximum of ammonia oxidation could not be resolved because the sampling resolution in the thin oxyx layer (<30 m) was not high enough (Figure 2). It has been debated whether phytoplankton exudation or ammonia oxidation contributes more to the formation of the PNM in stratified water columns [Lomas and Lipschultz, 2006]. Because we do not have data on phytoplankton release of NO$_2^-$, the proportion that ammonia oxidation contributes to form the PNM cannot be computed. However, if ammonia oxidation is assumed to be the only source of NO$_2^-$, then the turnover of NO$_2^-$ at the PNM would be on the order of tens of days (69 days at station BB2, 40 days at station 135, 53 days at station 164, and 16 days at station 180), indicating that the PNM is highly dynamic.

4.2. Ammonia Oxidation

Like most environmental rate measurements, ammonia oxidation rates measured in this study are likely potential rather than in situ rates. First, the incubations were performed at a final NH$_4^+$ concentration (300–550 nM) higher than in situ NH$_4^+$ concentrations (typically <150 nM). Ammonia oxidation rates were likely at their maximum at this concentration of NH$_4^+$, based on previous studies on substrate dependence of ammonia-oxidizing cultures [Martens-Habbena et al., 2009] and estimates of the substrate dependence of natural assemblages [Horak et al., 2013; Newell et al., 2013]. Additionally, measured ammonia oxidation rates from the euphotic zone were likely higher than in situ rates, because incubations were performed in the dark, which may have allowed the ammonia oxidizers to recover from photoinhibition, and reduced competition for NH$_4^+$ with phytoplankton [Epplley et al., 1971]. However, phytoplankton competition may not have been an issue at the level of substrate addition. 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 ammonia oxidation rates, but this effect was probably also minimal at these substrate levels. Because rates may depend on instantaneous supply of NH$_4^+$, rather than simply the measured NH$_4^+$ concentration, it is not clear how these measured rates relate to in situ rates. In the following discussion, we assume the measured rates are accurate.

Although our study was the first to use modern methods to measure nitrification rates in the region of the ETNP where the OMZ is at full extent, the ammonia oxidation rates in this study were similar to those measured in the ETNP previously [Ward and Zafririou, 1988; Sutka et al., 2004; Beman et al., 2012]. The integrated ammonia oxidation rate from 100 to 1100 m at offshore stations in this study (Figure 9; 1475 $\mu$mol m$^{-2}$ d$^{-1}$) was on the same order of magnitude as those found by Ward and Zafririou [1988] (1134–2756 $\mu$mol m$^{-2}$ d$^{-1}$), and Beman et al. [2012] (1380–7720 $\mu$mol m$^{-2}$ d$^{-1}$). Ammonia oxidation rates measured using $^{15}$NH$_4^+$ as a tracer have been reported in the other two major OMZs, the Arabian Sea
[Newell et al., 2011], and the ETSP [Ward et al., 1989; Lipschultz et al., 1990; Lam et al., 2009]. Some of these previous studies reported rates on the same order of magnitude (e.g., 0–21 nM d\(^{-1}\) in the Arabian Sea), and others reported much higher rates (0–4900 nM d\(^{-1}\) in the ETSP [Lam et al., 2009]).

We hypothesize that the different ammonia oxidation rates captured in different regions and/or at different times are a result of variation in organic matter flux from the surface layer of the ocean, because the NH\(_4^+\) required for ammonia oxidation is derived from remineralization of organic matter. With higher supply of nutrients, coastal areas are generally more productive than the offshore areas of the OMZs, and therefore should support higher rates of ammonia oxidation. However, the maximum ammonia oxidation rate found at the coastal station (8.55 nM d\(^{-1}\)) was lower than rates at offshore stations (around 30 nM d\(^{-1}\)). It is possible that an ammonia oxidation peak greater than 8.55 nM d\(^{-1}\) was missed by the sample interval at the coastal station. On the other hand, it is also possible that ammonia oxidation rates at the coastal station were actually lower than the rates at the offshore stations, perhaps due to stronger competition for NH\(_4^+\) with phytoplankton at the coastal station. Smith et al. [2014b] used a 3 day competition experiment to demonstrate that phytoplankton exert a strong control on ammonia oxidation in the euphotic zone off the California coast.

Oxygen concentration appeared to play a significant role in determining the ammonia oxidation rates in the oxycline above the anoxic waters, where light was unlikely an inhibiting factor (Figure 8). If the measured rates represent substrate-saturated rates, then oxygen could be the limiting factor in the incubations. It was remarkable to find a half-saturation constant for oxygen (\(K_m = 3.6 \mu M\)) that was so similar to that determined for Nitrosopumilus maritimus (\(K_m = 3.9 \mu M\) [Martens-Habbena et al., 2009]), and other AOA in culture [Jung et al., 2011], especially considering that our field measurements were made on samples from stations located hundreds of kilometers apart from each other. This suggests that the high affinity for oxygen of AOA determined in the laboratory manifests in OMZs. However, it should be noted that the observed Michaelis-Menten-like relationship between in situ oxygen concentration and ammonia oxidation rates could be fortuitous, because the number of ammonia-oxidizing cells in the eight samples from the five stations are not necessarily the same, and higher number of microorganisms should lead to higher observed metabolic activity. For the same reason, the apparent maximum rate of ammonia oxidation (\(V_{max}\)) calculated from these eight low-oxygen samples cannot be compared to the usual Michaelis-Menten \(V_{max}\) which is a specific rate. We cannot calculate specific rates for these eight low-oxygen samples because we do not have qPCR data for most of them. Another approach to assess the control of oxygen on the ammonia oxidation activity of a certain natural assemblage is to perform incubations at a range of oxygen concentration with aliquots of the same water sample. Using this method, very low \(K_m\) for oxygen (0.33 \(\mu M\)) was measured in the ETSP [Bristow et al., 2013]. Work in the Namibian and ETSP OMZs, however, showed that ammonia oxidation was not sensitive to oxygen concentrations ranging from 0.6 to 24.9 \(\mu M\) [Kalvelage et al., 2011].

Ammonia oxidation adapted to such low oxygen concentrations could directly supply NO\(_2^-\) for denitrification and anammox at the oxic-anoxic interface (100 m) of the offshore station BB2. Shipboard incubations with \(^{15}\)N tracers during the same cruise measured rates of denitrification and anammox that were an order of magnitude lower than the ammonia oxidation rate at the same depth and station [Babbin et al., 2014].

Given overlapping ranges of oxygen concentration that allow both aerobic ammonia oxidation, anammox, and denitrification [Babbin et al., 2014; Dalsgaard et al., 2014], ammonia oxidation should play an important role in directly supplying NO\(_2^-\) for anammox and denitrification at the oxic-anoxic interface in OMZs [Lam et al., 2009]. On the other hand, Babbin et al. [2014] argued that in the ETNP OMZ, coupled nitrification-anammox is possible but unlikely to contribute significantly to the total N loss, due to the steepness of the oxycline and the small vertical extent of the oxic-anoxic interface. These examples highlight the importance of understanding the role of aerobic ammonia oxidation in low-oxygen environments, where it potentially cooccurs and hence interacts with anaerobic N cycling processes.

### 4.3. Ammonia Oxidation in Relation to N\(_2\)O

The positive correlation between ammonia oxidation and N\(_2\)O concentration at stations BB1 and BB2 suggests that ammonia oxidation significantly contributes to N\(_2\)O production in the ETNP. Marine AOA are capable of producing N\(_2\)O, and potentially responsible for most of the N\(_2\)O production in the ocean, based on the isotopic signature produced by a marine AOA enrichment culture [Santoro et al., 2011; Lösch et al., 2012]. Since N\(_2\)O yield as a percentage of ammonia oxidation increases with decreasing oxygen...
[Cohen and Gordon, 1979; Goreau et al., 1980; Lösch et al., 2012], and there is evidence that OMZs are expanding [Stramna et al., 2008; Keeling et al., 2010], it is possible that there will be greater \( \text{N}_2\text{O} \) production from ammonia oxidation from OMZs in the future. However, if our rates of ammonia oxidation at station BB2 are compared to modeled \( \text{N}_2\text{O} \) production rates from the same station [Babbin et al., 2015], the \( \text{N}_2\text{O} \) produced at the oxic-anoxic interface would have to be 34–48% of oxidized ammonia. This \( \text{N}_2\text{O} \) yield is much larger than previously reported [Goreau et al., 1980; Santoro et al., 2011; Lösch et al., 2012], implying additional \( \text{N}_2\text{O} \) production from denitrification at these depths. \( \text{N}_2\text{O} \) production by both ammonia oxidizers and denitrifiers in the ETNP oxycline is consistent with modeled results from Babbin et al. [2015].

4.4. The Contribution to Ammonia Oxidation by AOA Versus AOB

ATU has been used in both culture and field experiments to inhibit ammonia oxidation [e.g., Molina and Farias, 2009; Shen et al., 2013], because ATU effectively chelates copper, a presumptive cofactor for the ammonia monoxygenase [Walker et al., 2010]. It is generally used at a level that completely inhibits ammonia oxidation (usually 86 \( \mu \text{M} \) [e.g., Hall, 1984; Santoro and Casciotti, 2011]). Although ATU stimulated ammonia oxidation in a soil microcosm where it was applied at high concentrations (500 or 1000 nmol g \(^{-1}\) soil), the observed stimulation might be a result of ATU degradation which released ammonia [Lehtovirta-Morley et al., 2013]. In order to distinguish the contribution of archaea and bacteria to ammonia oxidation, we chose an ATU dosage that should inhibit only bacterial ammonia oxidizers (\( \sim 10 \mu \text{M} \); data from laboratory cultures in Martens-Habbena et al. [2014]). Further evidence that environmentally relevant AOA should not be affected by \( \sim 10 \mu \text{M} \) ATU during our 12 h incubation comes from the California Current, where the activity of AOA was not compromised at 86 \( \mu \text{M} \) of ATU for 24 h [Santoro et al., 2010]. Ammonia oxidation rates by marine ammonia-oxidizing assemblages (Hood Canal and Ocean Station Papa) were not inhibited at 10 \( \mu \text{M} \) ATU [Martens-Habbena et al., 2014]. Moreover, it was shown that a soil AOB strain was inhibited by ATU at \(<1 \mu \text{M} \), while a soil AOA strain was only slightly affected at 80 \( \mu \text{M} \) [Shen et al., 2013].

Results of incubations with 10 \( \mu \text{M} \) ATU (Table 1) indicate that bacterial ammonia oxidizers do not contribute significantly to ammonia oxidation in the oxycline above the anoxic layer of the ETNP. This is consistent with the fact that archaeal \( \text{amoA} \) gene abundance was generally about 1 order of magnitude higher than bacterial \( \text{amoA} \) gene abundance (Figure S4). Most previous studies that argued for the dominant role of AOA over AOB in ammonia oxidation were based on the higher abundance of archaeal than bacterial \( \text{amoA} \) genes [e.g., Beman et al., 2012; Horak et al., 2013]. This study substantiates this correlation with the use of specific inhibitors.

An additional attempt to quantify the bacterial contribution to ammonia oxidation was implemented with the use of PTIO. PTIO is a nitric oxide (NO) scavenger that has been shown to inhibit AOA, presumably because NO is an intermediate of archaeal ammonia oxidation [Akaiae et al., 1993; Martens-Habbena et al., 2014]. However, the level of PTIO applied in our incubations (70–90 \( \mu \text{M} \)) may not have been high enough to completely inhibit AOA [Martens-Habbena et al., 2014], except in the sample from 15 m at station BB1. Therefore, the ammonia oxidation rates from incubations with PTIO may represent a partial inhibition of the AOA community, but cannot be interpreted further to distinguish bacterial ammonia oxidation. PTIO at higher concentrations has been successfully used to distinguish the contribution to ammonia oxidation by AOA and AOB in a sequencing batch reactor at a level of 200 \( \mu \text{M} \) [Yan et al., 2012], and in both coastal and open ocean marine environments at 100–300 \( \mu \text{M} \) [Martens-Habbena et al., 2014].

4.5. Abundance of \textit{amoA} Genes

The higher abundance of AOA over AOB in this study is consistent with previous studies in the open ocean [e.g., Santoro et al., 2010; Newell et al., 2011; Beman et al., 2012; Bouskill et al., 2012]. In low-\( \text{NH}_4^+ \) environments like the oxycline of the OMZs, AOA may outcompete AOB because AOA have extremely high substrate affinity [Martens-Habbena et al., 2009]. This argument is not completely grounded, because the dominant marine AOB have never been cultured, so their half-saturation constant for \( \text{NH}_4^+ \) is unknown. At a few of the shallowest depths AOB outnumbered AOA. The shallower maximum of \( \beta \)-proteobacterial \( \text{amoA} \) gene abundance suggests that other environmental parameters such as light contributed to determine the depth distribution of AOA and AOB. In fact, it has been shown in culture that AOA were more light-sensitive than AOB [Merbt et al., 2012], but those results were not conclusive, as Qin et al. [2014] demonstrated opposite results.
Archaeal amoA gene abundance showed a generally positive correlation with ammonia oxidation rates at both coastal station BB1 and offshore station BB2 (Figures 2 and 3, panel h), suggesting that AOA were responsible for the ammonia oxidation and the production of N$_2$O. At station BB1, it is likely that there was a subsurface maximum of ammonia oxidation between 20 and 50 m, where no rate measurements were made. This conjecture is based on the observation of the maxima in archaeal amoA gene abundance and N$_2$O concentration between 20 and 50 m at station BB1. Higher depth resolution (i.e., ≤5 m) is required to delineate the processes in the presence of a sharp oxycline.

It remains unexplained what supports the AOA and AOB in the anoxic layer of the OMZ, where ammonia oxidation was either not detected or negligible, but amoA gene abundances were relatively high. It has been shown in the other two major OMZs (the Arabian Sea and the ETSP) that the community composition of the AOA in the anoxic layer of the OMZ did not differ from that of the AOA residing in the surface mixed layer [Peng et al., 2013]. This suggests that microorganisms that possess an archaeal amoA gene might be capable of alternative metabolisms in the anoxic layer of the OMZ. One prominent piece of evidence for AOA’s metabolic versatility is the obligate mixotrophy demonstrated by two recently isolated marine AOA strains, which require α-ketoglutaric acid for growth [Qin et al., 2014].

Ammonia oxidation rate per cell above and below the anoxic layer (0.1–4.1 fmol cell$^{-1}$ d$^{-1}$), assuming that each ammonia oxidizer carries one copy of the functional gene, was of the same order of magnitude as that determined in culture experiments (1.8–15.4 fmol cell$^{-1}$ d$^{-1}$ in Könneke et al. [2005]; 2–4 fmol cell$^{-1}$ d$^{-1}$ in Wuchter et al. [2006]). This range of estimated per cell ammonia oxidation rate was also similar to those measured in field experiments off the California coast (0.2–15 fmol cell$^{-1}$ d$^{-1}$ in Santoro et al. [2010; –6 fmol cell$^{-1}$ d$^{-1}$ for Water Column Group A in Smith et al. [2014a]).

### 4.6. NO$_2^-$ Oxidation

Some of the NO$_2^-$ oxidation rates measured in this study were likely potential rates for the same reasons mentioned earlier for ammonia oxidation rates. It is worth noting that there are no reports on the kinetics of marine NO$_2^-$ oxidation, but it is likely that in samples with low NO$_2^-$ concentration, the addition of $^{15}$NO$_2^-$ stimulated NO$_2^-$ oxidation rates. At the SNM, because the in situ NO$_2^-$ concentration was more than an order of magnitude higher than the concentration of added $^{15}$NO$_2^-$, the measured NO$_2^-$ oxidation rates were unlikely to be an overestimation. NO$_2^-$ oxidation has also been shown to be inhibited by light [e.g., Guerrero and Jones, 1996a, 1996b], so the measured rates in the euphotic zone, although generally very low, were likely higher than in situ rates. It is possible that NO$_2^-$ assimilation competes for NO$_2^-$ with NO$_2^-$ oxidation, but very little is known about the degree of such competition.

NO$_2^-$ oxidation rates were higher than ammonia oxidation rates in this study, as found previously in OMZs [Lipschultz et al., 1990; Fussel et al., 2012; Beman et al., 2013]. Although we could not explain why in some cases NO$_2^-$ oxidation rates were 2 orders of magnitude higher than ammonia oxidation rates, our results support the notion that ammonia oxidation is the limiting step of nitrification. Nonetheless, it is worth noting that at the depth of maximum ammonia oxidation at stations 164 and 180, NO$_2^-$ oxidation rates were below detection (Figures 5 and 6, panels a and b). The decoupling of ammonia oxidation and NO$_2^-$ oxidation in this case likely contributed to the formation of PNM, which was found at the same depth (Figure 5 and 6, panel e). The maximum NO$_2^-$ oxidation rates above the anoxic layer were positioned deeper than the maximum ammonia oxidation, even closer to the anoxic layer at the oxic-anoxic interface. It is probable that this NO$_2^-$ oxidation maximum is responsible for the depletion of NO$_2^-$ between the PNM and the SNM (except at station 135).

The depth profile of NO$_2^-$ oxidation suggests that these NO$_2^-$ oxidizers are adapted to low-oxygen environments, and this was also revealed by the genome of Nitrospina gracilis, a major marine NO$_2^-$ oxidizer [Lücker et al., 2013]. However, it remains unexplained why there were high rates of NO$_2^-$ oxidation at anoxic depths of the OMZ, given the absence of known electron acceptors. NO$_2^-$ dismutation that produces NO$_3^-$ and N$_2$ has been postulated as an energetically favorable mechanism [van de Leemput et al., 2011], but it has never been demonstrated experimentally. Iodate and manganese are theoretically viable electron acceptors to support NO$_2^-$ oxidation in the anoxic layer [Gaye et al., 2013], but there is no experimental evidence demonstrating this mechanism. More curiously, NO$_2^-$ oxidation rates were inversely correlated with oxygen concentration in low oxygen environments (Figure 8b), suggesting that NO$_2^-$ oxidation does not always...
require oxygen. This inverse relationship we found is the opposite of what would be expected from a Michaelis-Menten-like relationship between NO$_2^-$ oxidation and oxygen concentration, reported from an experiment performed in the ETSP, where oxygen level was manipulated at submicromolar levels [Bristow et al., 2012]. Such inconsistency suggests that there might be another process that produces NO$_3^-$ independent of oxygen concentration.

5. Conclusions

The oxycline of the ETNP OMZ was characterized by narrow subsurface maxima of NH$_4^+$ and NO$_2^-$ oxidation rates, with the latter slightly deeper than the former. Ammonia oxidation should at least partially account for the formation of the PNM. Both ammonia and NO$_2^-$ oxidation occurred at very low oxygen concentrations in the oxycline. NO$_2^-$ oxidation was also observed at truly anoxic depths, co-occurring with N$_2$ production processes. As determined by both qPCR and the inhibitor ATU, AOA were primarily responsible for ammonia oxidation, and likely the production of N$_2$O as well. It remains unknown what oxidant supports NO$_2^-$ oxidation at the anoxic layer of the OMZs.

References


