All about Nitrite: Exploring Nitrite Sources and Sinks in the Eastern Tropical North Pacific Oxygen Minimum Zone

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Abstract

Oxygen minimum zones (OMZs), due to their large volumes of perennially deoxygenated waters, are critical regions for understanding how the interplay between anaerobic and aerobic nitrogen (N) cycling microbial pathways affects the marine N budget. Here we present a suite of measurements of the most significant OMZ N cycling rates, which all involve nitrite (NO_2^-) as a product, reactant, or intermediate, in the Eastern Tropical North Pacific (ETNP) OMZ. These measurements and comparisons to data from previously published OMZ cruises present additional evidence that NO_3^- reduction is the predominant OMZ N flux, followed by NO_2^- oxidation back to NO_3^-. The combined rates of both of these N recycling processes were observed to be much greater (up to nearly 200x) than the combined rates of the N loss processes of anammox and denitrification, especially in waters near the anoxic / oxic interface. We also show that NO_2^- oxidation can occur when O_2 is maintained near 1 nM by a continuous purge system, NO_2^- oxidation and O_2 measurements that further strengthen the case for truly anaerobic NO_2^- oxidation. We also evaluate the possibility that NO_2^- dismutation provides the oxidative power for anaerobic NO_2^- oxidation. The partitioning of N loss between anammox and denitrification differed widely from stoichiometric predictions of at most 29% anammox; in fact, N loss rates at many depths were entirely due to anammox. Our new NO_3^- reduction, NO_2^- oxidation, dismutation, and N loss data shed light on many open questions in OMZ N cycling research, especially the possibility of truly anaerobic NO_2^- oxidation.

1. Introduction

Nitrogen (N) is essential for life because of its prominent role in DNA, RNA, and protein chemistry. As a result, N limits biological productivity in many marine environments. The

dissimilatory biological N loss and recycling pathways are traditionally understood to be strictly separated by O_2 tolerance. The N loss processes of denitrification, the stepwise reduction of NO_3^- to N_2, and anaerobic ammonium oxidation (anammox), the oxidation of NH_4^+ with NO_2^- to make N_2, require low O_2 while the N recycling pathways of NH_4^+ oxidation to NO_2^- and NO_2^- oxidation to NO_3^- are viewed as obligately aerobic. Importantly, NO_2^- is a product, reactant, or intermediate in all these pathways. Therefore, developing an understanding of NO_2^- sources and sinks is essential for a complete understanding of marine N biogeochemistry.

Oxygen minimum zones (OMZs) and sediments are the two main marine environments where N loss occurs. There are three major OMZs, the Eastern Tropical North Pacific (ETNP), the Eastern Tropical South Pacific (ETSP), and the Arabian Sea, which occupy 0.1 - 1% of total ocean volume, depending on the O_2 threshold used (Codispoti and Richards, 1976; Naqvi, 1987; Bange et al., 2000; Codispoti et al., 2005; Lam and Kuypers, 2011). Importantly, the OMZ water column is not completely deoxygenated from top to bottom; OMZs are characterized by an oxygenated surface, a depth interval of steeply declining O_2 around the mixed layer depth, called the oxycline, an oxygen deficient zone (ODZ) spanning several hundred meters where O_2 declines below the detection limit of common shipboard CTD O_2 sensors, and then a second, gradual, oxycline that transitions to oxygenated deep water. Despite OMZ regions' small size, they are responsible for 30-50 % of total marine N loss (DeVries et al., 2013), a magnitude significant for the global marine N budget. In this work, in order to answer several open questions about OMZs and marine N cycling, we conducted a suite of ^{15}N stable isotope measurements of the most important N cycling microbial pathways in OMZs. We report the N loss rates of anammox and denitrification, as well as the N recycling rates of NO_3^- reduction, NO_2^- oxidation, and NH_4^+ oxidation, all of which involve NO_2^-.

A distinctive feature of OMZs is a secondary nitrite maximum (SNM) (Codispoti et al., 2001; Brandhorst, 1959; Codispoti and Packard, 1980). The highest nitrite concentrations within the SNM can reach 10 μM, much higher than the peak values found in the primary nitrite maximum at the base of the photic zone, which average ~100 nM globally (Lomas and Lipschultz, 2006). Several recent works have shown or argued that the SNM's NO_2^- is supplied via high rates of the first step of denitrification, NO_3^- reduction to NO_2^- (Lam et al., 2009; Lam and Kuypers, 2011; Kalvelage et al., 2013; Babbin et al., 2017, 2020). NO_3^- reduction has been proposed (Anderson et al., 1982) to be one-half of a rapid loop where NO_3^- and NO_2^- are recycled through simultaneously occurring NO_3^- reduction and NO_2^- oxidation. This loop has been supported through experimental measurements of both rates (Babbin et al., 2017, 2020; Kalvelage et al., 2013; Lipschultz et al., 1990). In this view, elevated NO_3^- reduction also generates NH_4^+, via organic matter (OM) remineralization, which enhances anammox at the expense of denitrification in oxycline and upper ODZ waters (Babbin et al., 2020). In this study, we conducted tests to further document this rapid loop's existence and role in enhancing anammox.

Recent measurements of NO_2^- oxidation have returned significant rates from both the oxycline and the ODZ, findings that challenge the paradigm that NO_2^- oxidation is an obligately aerobic process. Evidence for high, widespread NO_2^- oxidation rates in low O_2 waters has accumulated from direct rate measurements via ^{15}N tracers (Füssel et al., 2011; Lipschultz et al., 1990; Peng et al., 2015, 2016; Ward et al., 1989; Kalvelage et al., 2013; Tsementzi et al., 2016; Sun et al., 2017, 2021a; Babbin et al., 2017, 2020), models (Buchwald et al., 2015), and ^{15}N natural abundance measurements (Casciotti et al., 2013). Many explanations have been proposed including microaerophilic nitrite oxidizing bacteria (NOB) adapted to low but non-zero

98 O_2 conditions (Penn et al., 2016; Bristow et al., 2016; Tsementzi et al., 2016; Bristow et al.,
99 2017) where the O_2 for these NOB is transiently supplied to previously deoxygenated waters by
100 (1) vertical or horizontal mixing of the ocean surface or nearby oxic water (Casciotti et al., 2013;
101 Tiano et al., 2014; Bristow et al., 2016; Ulloa et al., 2012), even into the anoxic ODZ
102 (Margolskee et al., 2019), or (2) a cryptic O_2 cycle where low-light adapted phototrophs produce
103 O_2 that is consumed by NOB (Garcia-Robledo et al., 2017; Fuchsman et al., 2019).
104 Despite the power of these explanations, they do not preclude the possibility of
105 widespread NOB capable of truly anaerobic NO_2^- oxidation, especially in waters from the deep,
106 dark, and deoxygenated ODZ core. This possibility is bolstered by sequencing data that show
107 the presence of an NOB metagenome assembled genome (MAG) with a preference for the
108 deoxygenated ODZ core in the ETSP (Sun et al., 2019) and ODZ core kinetics experiments
109 where O_2 concentrations above 5 μM inhibit NO_2^- oxidation (Sun et al., 2021a). Here we build
110 on past stable isotope experimental results by performing additional depth profile experiments
111 with purged waters from the ODZ *and* O_2 manipulation ^{15}N tracer experiments across a gradient
112 of O_2 concentrations from 1 nM to 10 μM. Our O_2 manipulation experiments, unlike previous
113 studies, were conducted in vessels that were continuously purged throughout each incubation
114 with a precisely calibrated mixture of N_2, O_2, and CO_2. This experimental design allowed us to
115 continuously maintain low O_2 conditions. In addition, our oxygen concentrations in these assays
116 were verified via a LUMOS sensor, a sensor class with a detection limit of 0.5 nM O_2 (Lehner et
117 al., 2015). Together, these method improvements convincingly show that the O_2 contamination
118 observed to occur in Niskin sampling (Garcia-Robledo et al., 2016, 2021) is removed and that
119 vanishingly low O_2 is maintained throughout the experiment.

Anaerobic NO_2^- oxidation would require an alternative oxidant other than O_2. Many candidates have been proposed (Sun et al., 2023) for this oxidant including IO_3^- (Babbin et al., 2017), Mn^{4+}, Fe^{3+} (Sun et al., 2021a), the anammox core metabolism (Sun et al., 2021a), the observed reversibility of the nitrite oxidoreductase enzyme (Wunderlich et al., 2013; Kemeny et al., 2016; Koch et al., 2015; Buchwald and Wankel, 2022), and NO_2^- dismutation (Babbin et al., 2020; Füssel et al., 2011; Sun et al., 2021a). Due to multiple considerations such as very low IO_3^- in the ODZ core (Moriyasu et al., 2020), low favorability of Mn^{4+} or Fe^{3+} mediated NO_2^- oxidation at marine pH values (Luther, 2010), low anammox rates that do not explain the observed stoichiometry of NO_2^- oxidation to anammox (Kalvelage et al., 2013; Babbin et al., 2020; Sun et al., 2021a), and the inability of the enzyme hypothesis to account for structural and phylogenetic differences in the NXRs of the four NOB genera (Buchwald and Wankel, 2022; Sun et al., 2019), we conducted experiments to test the remaining most plausible hypothesis: NO_2^- dismutation.

NO_2^- dismutation (Eq. (R4)) is energetically favorable (Strohm et al., 2007; Van de Leemput et al., 2011) although it has not been detected in nature. The reaction is proposed to occur in three steps (Eq. (R1-3)) (Babbin et al., 2020) and DNA sequences that encode possible enzymes for steps two and three (Eqs. (R2, R3)) have been found in ODZ core metagenomic reads and MAGs (Padilla et al., 2016; Babbin et al., 2020). While these sequences were not classified as NOB, they do indicate that parts of the pathway could occur in OMZs. If discovered in OMZs, NO_2^- dismutation would be another N loss pathway, albeit one indistinguishable from denitrification since the ^{15}N atoms in $^{30}N_2$ come from $^{15}NO_2^-$ in both pathways. Here we evaluate the hypothesis that NO_2^- dismutation is a significant mechanism for NO_2^- oxidation under low O_2, by searching for product inhibition, the inhibition of both NO_2^-

oxidation and $^{30}N_2$ production (i.e. denitrification) in response to addition of NO_3^-, substrate stimulation (increases in both $^{30}N_2$ production and NO_2^- oxidation in response to addition of $^{15}NO_2^-$), and by comparing the NO_2^- oxidation to the produced $^{30}N_2$ ratio. A ratio near the 3:1 stoichiometry of dismutation (3 NO_3^-: 1 N_2, Eq. (R4)) would indicate that dismutation could explain the NO_2^- oxidation measured in the ODZ core.

$$3NO_2^- + 2H^+ \rightarrow NO_3^- + 2NO + H_2O \quad \text{(R1)}$$

$$2NO \rightarrow N_2 + O_2 \quad \text{(R2)}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad \text{(R3)}$$

$$5NO_2^- + 2H^+ \rightarrow N_2 + 3NO_3^- + H_2O \quad \text{(R4)}$$

A final area of OMZ biogeochemistry that we investigate is the relative balance between anammox and denitrification and these pathways' relationships to the rapid NO_2^- oxidation / NO_3^- reduction loop. After the discovery of anammox, many OMZ studies (Kalvelage et al., 2013; Kuypers et al., 2005; Hamersley et al., 2007; Jensen et al., 2011; Thamdrup et al., 2006; Lam et al., 2009), but not all (Ward et al., 2009; Bulow et al., 2010; Dalsgaard et al., 2012) have reported that anammox is the dominant N loss flux in OMZs, a surprising difference from the stoichiometric based prediction that OMZ N loss should be at most 29% anammox (Dalsgaard et al., 2003). While the first wave of these studies did not realize that vial septa were introducing O_2 into the incubations, many studies after this discovery observed the same result (Kalvelage et al., 2013; Jensen et al., 2011; Babbin et al., 2020). The prediction of a 29% anammox partition assumes that all NH_4^+ for anammox was derived from remineralization of OM with a mean marine C:N ratio through complete denitrification of NO_3^- to N_2 (Dalsgaard et al., 2003, 2012). Anammox rates exceeding 29% of total N loss would therefore require an additional source of NH_4^+ beyond current observations of denitrification and the resulting NH_4^+ remineralization.

166 The best supported explanations for elevated anammox are that (1) denitrification is the
167 NH_4^+ source, but that complete denitrification peaks episodically in response to OM quality
168 while anammox occurs at a slow, consistent, low rate (Ward et al., 2008; Thamdrup et al., 2006;
169 Babbin et al., 2014; Dalsgaard et al., 2012). The snapshots afforded by isotopic incubations on
170 cruises could therefore easily miss episodes of high complete denitrification. (2) Denitrifiers
171 have a strong preference for particles (Ganesh et al., 2013, 2015; Fuchsman et al., 2017) and
172 CTD samples do not capture marine particles very well (Suter et al., 2017). As a result,
173 differences from the expected percent N loss partition in water column samples are due to
174 missing denitrifiers. (3) The rapid loop between NO_3^- and NO_2^- described previously functions
175 as an "engine" to generate NH_4^+ for anammox at the expense of denitrification. The observed
176 magnitudes of NO_3^- reduction and NO_2^- oxidation and these processes' ability to produce NH_4^+
177 from the remineralization of OM with standard C:N ratios without complete denitrification make
178 this an additional logical hypothesis.
179 The third hypothesis, the NO_2^-/ NO_3^- loop, is supported by several pieces of evidence.
180 Firstly, 'omics studies have revealed widespread modular denitrification in OMZs (Sun et al.,
181 2021b; Fuchsman et al., 2017). Furthermore, experimental studies have shown that as NO_3^-
182 reduction increases near the coast, anammox rates also increase (Kalvelage et al., 2013). Our
183 study's considerable number of data points, as well as our ability to compare results to rate
184 measurements obtained from identical methods on previous cruises offers a unique chance to test
185 both the variable denitrification and rapid loop hypotheses for elevated anammox rates.
186 OMZs are essential regions for the marine N cycle; however, the biogeochemistry of
187 OMZs may currently be in flux due to anthropogenic pressures. Observational studies have
188 reported decreases in O_2 across the Pacific (Ito et al., 2017) and the expansion of denitrification

and anoxia in the ETNP (Horak et al., 2016). Modeling studies suggest that OMZ volume will continue to grow in the near future, with uncertain impacts (Stramma et al., 2008; Keeling et al., 2010; Busecke et al., 2022). As a result, it is important to develop a thorough understanding of OMZ N cycling to be able to predict any changes in marine productivity as deoxygenated regions grow. This study contributes towards this goal through examining four open research questions in OMZ biogeochemistry:

(1) Is the rapid cycle hypothesis correct, i.e., that NO_3^- reduction and NO_2^- oxidation rates are much greater than N loss rates, especially in the oxycline and ODZ top?

(2) Does truly anaerobic NO_2^- oxidation occur in OMZ regions?

(3) If yes, is NO_2^- dismutation the mechanism by which it occurs?

(4) Is anammox the dominant N loss flux? If yes, what is the explanation?

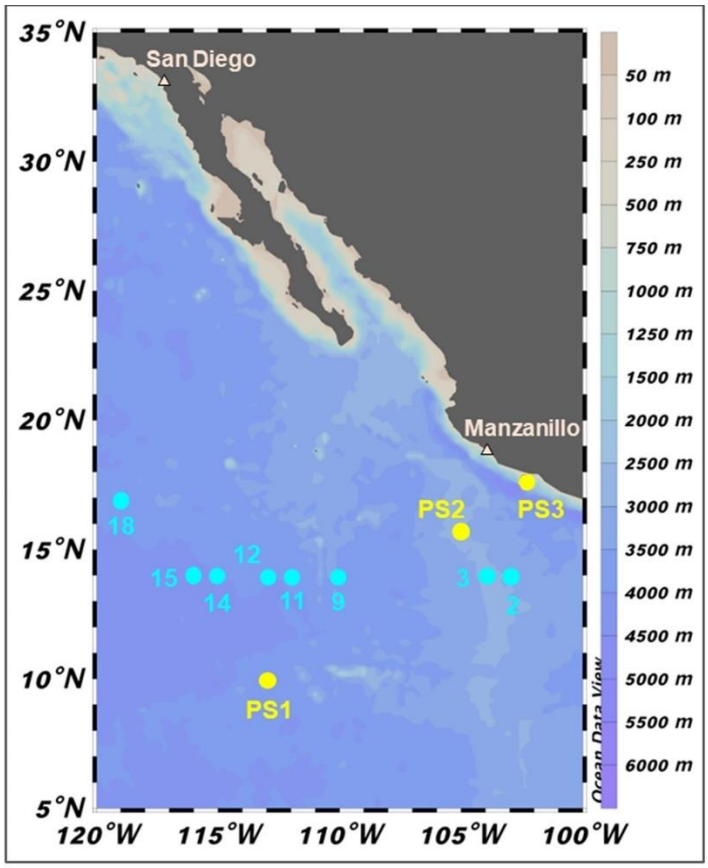


Figure 1: Sampling locations during 2018 cruises to the ETNP OMZ. SR1805 stations (spring 2018) are shown in yellow while FK180624 (summer 2018) stations are shown in cyan. Stations PS1 and 18 are located in more oxic environments on the boundary of the OMZ region. The remaining FK180624 stations occur along a gradient towards the center of the OMZ region, represented by stations PS2 and FK180624 stations 2 and 3. These three stations are referred to as OMZ core stations. Station PS3 (referred to as coastal) represents a final biogeochemical subregion due to its proximity to the coast.

2. Methods

2.1 NO_2^-, NO_3^-, and NH_4^+ concentration measurements

Nutrient measurements on all cruises were conducted as follows. Ambient NO_2^- concentrations were measured on each vessel using the sulfanilimide and NED colorimetric technique with a spectrophotometer (Strickland and Parsons, 1972). NO_3^- profile samples were frozen onboard each ship, then thawed and measured immediately using the chemilumenscence method upon return to the Ward laboratory (Braman and Hendrix, 1989). Ambient NH_4^+ concentrations were measured on each ship using the OPA method (Holmes et al., 1999; Taylor et al., 2007; ASTM International, 2006). In some cases, NO_2^- and NH_4^+ were measured on different casts than those of the rate measurements. In these cases, figures and calculations use interpolated nutrient values based on the potential density of nutrient sampling and rate measurement depths. Interpolations were performed with the Matlab pchip function.

2.2 NH_4^+ oxidation and NO_3^- reduction rates

Incubation experiments were performed on board the R/V *Sally Ride* in March and April 2018 (SR1805). NH_4^+ oxidation and NO_3^- reduction rates were measured at three stations: PS1 (open ocean OMZ boundary), PS2 (open ocean, OMZ), and PS3 (coastal OMZ) (Fig. 1). Rates were measured throughout the water column at ten depths per station (see supplemental Table S1 for depths). Water was directly sampled from the CTD into 60 mL serum vials. After overflowing three times, bottles were sealed with a rubber stopper and crimped with an

aluminum seal. After this, a 3 mL headspace of He was introduced and samples from below the oxygenated surface depths were purged for 15 min with He at a flow rate of 0.4 L min^{-1}. This flow rate exchanged the volume of each bottle one hundred times. Immediately after this, 0.1 mL of tracer solution was added to all bottles. $^{15}NH_4^+$ and $^{15}NO_3^-$ tracers were added to reach final concentrations of 0.5 µM and 3 µM, respectively. Five bottles were incubated per time course and incubations were ended at 0 (one bottle), 12, and 24 hours (two bottles each) via addition of 0.2 mL of saturated $ZnCl_2$. Samples were analyzed at the University of Basel using a custom-built gas bench connected by a Conflow IV interface to a Delta V plus IRMS (Thermo Fisher Scientific). Five mL of the sample were used to convert NO_2^- to N_2O using the azide method (McIlvin and Altabet, 2005). A linear increase of $^{15}N\text{-}NO_2^-$ over time, along with a standard curve to convert from peak area units to nmol N was used to calculate the NO_2^- production rates according to Eq. (5) and (6) below,

$$\text{Ammonium oxidation rate} = \frac{d\ ^{15}NO_2^-}{dt\ (F_{NH_4^+})} \quad (5)$$

$$\text{Nitrate reduction rate} = \frac{d\ ^{15}NO_2^-}{dt\ (F_{NO_3^-})} \quad (6)$$

where:

$\frac{d\ ^{15}NO_2^-}{dt}$ is the slope of $^{15}NO_2^-$ produced over time and

$F_{NH_4^+}$ and $F_{NO_3^-}$ are the fraction of the NO_3^- and NH_4^+ pools that are labelled with ^{15}N.

The significance of the rates was evaluated using a Student's t test with a significance level of 0.05. The reported error bars are the standard error of the regression. The NH_4^+ oxidation rates reported here were previously published and the experimental method used is more thoroughly described in this previous publication (Frey et al., 2022).

2.3 Anammox and denitrification rates depth profiles

Incubation experiments were performed during SR1805 in March and April 2018 and on the R/V *Falkor* (FK180624) during June and July 2018. As above, rates were measured at PS1, PS2, and PS3 at ten depths per station (see Supplementary Table S1 for sampling depths) during SR1805. On FK180624, rates were measured at eight stations that spanned a gradient from the core of the OMZ region to its edges (see Supplementary Table S2 for sampling depths). At all stations and depths water was directly sampled from the CTD into 320 mL borosilicate ground glass stoppered bottles. After overflowing three times, bottles were stoppered with precision ground glass caps specifically produced to prevent gas flow. The bottles were transferred to a glove bag and amended with the following treatments: 3 μM each of $^{15}NO_2^-$ and $^{14}NH_4^+$ (denitrification and anammox) and 3 μM each of $^{15}NH_4^+$ and $^{14}NO_2^-$ (anammox) on SR1805. 2 μM amendments of $^{15}NO_2^-$ and $^{14}NH_4^+$ were used on FK180624. It should be noted that at many depths our tracer additions were far above in situ values. Due to this, all anammox and denitrification rates with high changes from baseline nutrient concentrations represent potential rates. Eight mL of tracer amended seawater was aliquoted into 12 mL exetainers (Labco). Exetainers were sealed in a glove bag with butyl septa and plastic screw caps that had been stored under helium for at least one month, removed and then purged for 5 min at 3 psi with helium gas to remove any O_2 that accumulated during sampling and processing. This step is another reason our anammox and denitrification rates sourced from partially or fully oxygenated waters should be regarded as potential rates.

Rates for each sampled depth were calculated using a five-timepoint time course with three replicates at each point. Incubations were ended by injecting 50 μL saturated $ZnCl_2$ and

vials were stored upside down to prevent the headspace from leaking through the vial cap during storage and transit. Six months after the cruise, samples were analyzed using a Europa 22-20 IRMS (Sercon). Raw data values were corrected for instrument drift due to run position and total N_2 mass. Drift corrected values and standard curves to convert from peak area units to nmol N_2 were used to calculate rates according to the equations below (Thamdrup et al., 2006; Thamdrup and Dalsgaard, 2000, 2002) (for more details see supplemental material),

Denitrification (from $^{15}NO_2^-$)

$$\text{Denitrification Rate} = \frac{d\,^{30}N_2}{dt(F_{NO_2^-})^2} \quad (7)$$

Anammox (from $^{15}NO_2^-$)

$$\text{Anammox Rate} = \frac{d\,^{29}N_2}{dt\,F_{NO_2^-}} - 2D\left(1 - F_{NO_2^-}\right) \quad (8)$$

Anammox (from $^{15}NH_4^+$)

$$\text{Anammox Rate} = \frac{d\,^{29}N_2}{dt\,F_{NH_4^+}} \quad (9)$$

where:

$\frac{d\,^{30\text{ or }29}N_2}{dt}$ is the slope of the regression of the amount of $^{30\text{ or }29}N_2$ vs. time,

$F_{NO_2^-}$ and $F_{NH_4^+}$ are the fraction of the NO_2^- and NH_4^+ pools labelled as ^{15}N, and

D is the denitrification rate calculated according to Eq. (7).

A Student's t test with a significance level of 0.05 was used to evaluate all rates. The reported error bars are the standard error of the regression. Since the anammox rates measured via both tracers on the SR1805 cruise were similar in magnitude (Supplementary Table S3), anammox values reported in Figs. 2, 3, 6, 7, 8, and 9 are based on a combination of these values (see

supplementary material for more information). Previously published (Babbin et al., 2020) anammox and denitrification rates are sourced from four stations occupied during the R/V *Thomas G. Thompson*'s March and April 2012 cruise to the ETNP (TN278) and the RVIB *Nathaniel B. Palmer*'s June and July 2013 ETSP cruise (NBP1305) and were conducted in the same manner as the SR1805 and FK180624 incubations. Crucially, the same mass spectrometer was used to measure N loss rates across the 2012, 2013, and 2018 cruises. Station locations for the 2012 and 2013 cruises were as follows: TN278 ETNP coastal (20° 00′ N, 106° 00′ W), ETNP offshore (16° 31′ N, 107° 06′ W) and NBP1305 ETSP coastal (20° 40′ S, 70° 41′ W), ETSP offshore (13° 57′ S, 81° 14′ W).

2.4 SR1805 NO_2^- oxidation depth profiles

Nitrite oxidation depth profiles were measured in the same exetainers used to measure anammox and denitrification depth profiles ($^{15}NO_2^-$ treatment only). The rate of NO_2^- oxidation was determined by converting the NO_3^- produced during the incubations to N_2O using the denitrifier method (Weigand et al., 2016; Granger, J., & Sigman, 2009) (see supplemental material for methods details). The samples were stored at room temperature in the dark until analysis on a Delta V (Thermo Fisher Scientific) mass spectrometer that measures the isotopic content of N in N_2O (Weigand et al., 2016). Samples were corrected for instrument drift due to run position and total N_2 mass (for more details see supplemental materials). Drift corrected $\delta^{15}N$ values and a standard curve were then used to calculate the rate as follows,

$$\frac{^{15}N}{^{14}N} = \frac{\left[{}^{\delta^{15}N}/_{1000} + 1\right] \times 0.003667}{1 - 0.003667} \quad (10)$$

$$NO_2^- \text{ ox. rate} = \frac{d\left[{}^{44}N_2O_{area} \times {}^{15}N/{}^{14}N\right]}{dt\, F_{NO_2^-}} \quad (11)$$

where Eq. (10) is a rearrangement of the definition of $\delta^{15}N$:

$$\delta^{15}N = \left[\frac{\frac{{}^{15}N}{{}^{14}N_{sample}}}{\frac{{}^{15}N}{{}^{14}N_{air}}} - 1\right] \times 1000 \quad (12)$$

and ${}^{44}N_2O_{area}$ is the amount of ${}^{44}N_2O$ measured as sample peak area in V · sec. 0.003667 is the natural abundance of ^{15}N in air. A Student's t test with a significance level of 0.05 was used to evaluate all rates. Reported error bars are the standard error of the regression. Previously published (Babbin et al., 2020) NO_2^- oxidation rates are from the previously mentioned TN278 and NBP1305 cruises and were conducted at the same four stations where N loss rates were measured. These NO_2^- oxidation rate measurements were conducted according to the same procedures used for the SR1805 depth profiles.

2.5 NO_2^- oxidation and O_2 manipulation experiments

Experiments were conducted during cruises SR1805 and FK180624 in spring and summer 2018. Wide-mouthed Pyrex round media bottles (800 mL total volume, 500 mL working volume; Corning, USA; product code 1397-500) were used for all incubations. These bottles were modified to include three stainless steel bulkhead fittings (Swagelok, USA) secured to the interior of the lid with a Viton rubber gasket and stainless-steel washer between the lid and the sealing nut. The three ports consisted of two one-eighth inch fluidic ports (inflow and outflow) and one one-quarter inch sampling port. The fluidic ports were fitted with one-eighth inch nylon tubing, with the inflow line penetrating to the base of the bottle. The one-quarter inch

sampling port had a butyl rubber septum between the Swagelok stem and nut. This setup permitted *continuous* gas purging of the bottles while maintaining an otherwise closed system.

For each depth and O_2 treatment, three bottles were filled to 500 mL with sample water from a Niskin bottle and closed. Sample water for all experiments except station 18 on the FK180624 cruise was drawn from water below 2.2 µM O_2 (See Table S4 for all ambient O_2 values). Highly precise digital mass flow controllers (Alicat) were then used to establish the desired O_2 concentrations in each bottle. Mixing ratios were calculated to create a range of O_2 concentrations spanning 1 nM, 10 nM, 100 nM, 1 µM, and 10 µM. The gas mixture modified by the mass flow controllers was a zero-air gas mixture (Airgas) consisting of 21% O_2 and 79% N_2 and 1000 ppm pCO_2 (the approximate in situ value). Initial gas flow was 1 L min^{-1} for 1 hour to equilibrate the seawater followed by 100 mL min^{-1} for the remainder of the experiment. Bottles were daisy-chained together to maintain the same flow rate among them (two bottles on SR1805, six on FK180624). As in the depth profile experiments, 3 µM $^{15}NO_2^-$ amendments were added prior to purging. Incubations were conducted in the dark at 12ºC in a cold room (SR1805) or beverage cooler (FK180624). At the beginning of the experiments, after purging for one hour, O_2 was checked with a LUMOS optode with a detection limit of 0.5 nM (Lehner et al., 2015) and CO_2 was checked by measuring pH using the colorimetric meta-cresol purple method. The LUMOS optode confirmed that O_2 concentrations were within a few nM of the calculated values. While our use of high precision digital mass flow controllers and this qualitative O_2 check provide confidence that our O_2 concentrations are accurate, due to the fact that O_2 was not continuously monitored through the time course, we refer to each O_2 concentration as a "putative" concentration for the remainder of this manuscript. Samples (50 mL) were withdrawn every 12 hours for two days with a four inch hypodermic needle attached to a 60 mL disposable

plastic syringe. Samples were ejected into acid-cleaned HDPE bottles pre-amended with 200 µL of saturated $ZnCl_2$ solution. Bottles were screwed closed and wrapped with parafilm. Samples from each of the three initially collected bottles were collected to create triplicates at each time point.

2.6 NO_2^- dismutation experiments

Nitrite dismutation experiments were performed during SR1805 at Station PS3 (coastal waters) at two deoxygenated depths: 60 m and 160 m. Incubations were performed in the same manner as the above anammox, denitrification, and NO_2^- oxidation experiments where all three rates were measured in the same exetainers. Experiments consisted of eight total treatments: four varying $^{15}NO_2^-$ tracer concentrations (1.125, 5.25, 10.5, and 20.25 µM for 160 m and 0.75, 1.5, 3.75, and 7.5 µM for 60 m) and two $^{14}NO_3^-$ treatments (0 or 20 µM). As above, both $^{30}N_2$ and NO_3^- production via the denitrifier method (Weigand et al., 2016) were measured. In order to test our hypothesis that, if dismutation is occurring, the unexplained NO_2^- oxidation rate (the difference between the measured NO_2^- oxidation and the NO_2^- oxidation due to anammox) and the denitrification rate (i.e. the $^{30}N_2$ production rate) should have a 3:1 ratio, a previously published anammox stoichiometry (Eq. (4) (Kuenen, 2008)) was used to calculate the NO_2^- oxidation due to anammox. The anammox rates used for this calculation are included in the supplementary material (Fig. S4).

2.7 Calculation of N loss from NH_4^+ oxidation

The calculation of the maximum possible N loss from NH_4^+ oxidation via NO disproportionation by ammonium oxidizing archaea (AOA) in Supplementary Table S5 was

379 carried out by dividing the measured NH_4^+ oxidation rate by two in accordance with the
380 stoichiometry of NH_4^+ oxidation and NO disproportionation proposed in a previous study (Kraft
381 et al., 2022). It should be noted that this operation represents the extreme case where all $^{15}NO_2^-$
382 produced in NH_4^+ oxidation is converted to N_2. We acknowledge this as an unrealistic
383 assumption used to evaluate the extreme limits of the amount of total N loss attributible to NH_4^+
384 oxidation. This operation was carried out for all depths where NH_4^+ oxidation, anammox, and
385 denitrification rates were measured, irrespective of O_2 concentration.
386
387 **2.8 Redundacy analysis (RDA), Principle component analysis (PCA), and statistics**
388 All RDA, PCA, redundancy, and correlation analyses were performed with the available
389 packages in R (v4.2.1 "Funny-Looking Kid") (R: A language and environment for statistical
390 computing). All data were first normalized around zero before calculating the Pearson's
391 correlation coefficient. Gene abundances (*nirS* and *amoA*) from qPCR analyses used for the
392 RDA and correlation analyses were measured as previously described (Peng et al., 2015;
393 Jayakumar et al., 2009; Tang et al., 2022).
394
395 **2.9 Definition of shallow boundary and ODZ core nomenclature**
396 In the results and discussion sections, results are classified as shallow boundary or ODZ
397 core waters according to a previously published threshold (Babbin et al., 2020) where shallow
398 boundary samples have an in situ potential density < 26.4. This method is based on a global
399 profile of OMZ waters meant to delineate shallow boundary samples as waters that are oxic or
400 may be influenced by O_2 intrusions (the surface, the oxycline, and the ODZ top) from those that
401 are not normally influenced by O_2 instrusions (ODZ core). Due to the fact that the potential

density threshold is based on a global average, a few depths that are clearly in the deep oxycline based on the SR1805 O_2 depth profiles are classified as ODZ core ($\sigma_\theta > 26.4$) by the potential density threshold. Despite this caveat we used this naming scheme throughout the remainder of the manuscript to enable comparisons to previous literature (Babbin et al., 2020).

Depth	σ_θ	OMZ features	O_2 intrusions?
Shallow boundary waters	< 26.4	Surface, oxycline, ODZ top	Yes
ODZ core	> 26.4	ODZ core	No

Table 1: Explanation of shallow boundary waters and ODZ core potential density based nomenclature (Babbin et al., 2020).

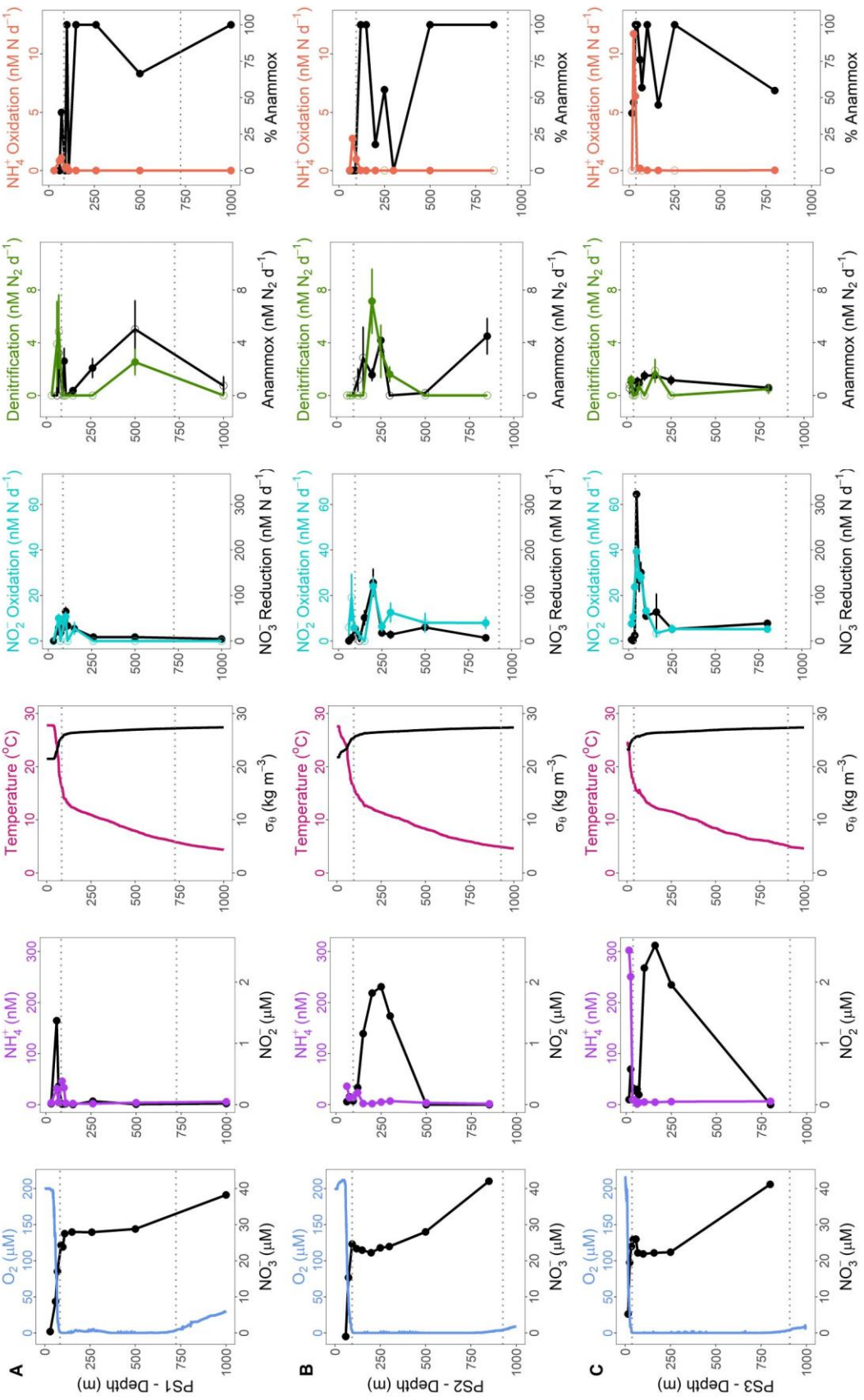
3. Results

3.1 2018 depth profiles of all N cycling rates

N cycling depth profile experiments were conducted on two cruises (SR1805 and FK180624) during spring and summer 2018. These two cruises sampled stations along a gradient from the edge of the OMZ region to near the coast. Physical and chemical conditions varied among stations PS1, PS2, and PS3 on the SR1805 cruise (spring 2018) and across all FK180624 stations (summer 2018) (Fig. 2, Fig. S1). Broadly speaking, the vertical span of the ODZ increased and the top of the ODZ shoaled as distance to shore decreased. Deep SNM were observed at almost all stations with the only exceptions being the furthest offshore stations, stations 11 and 18 from the FK180624 cruise (Fig. S1) and station PS1 from SR1805 (Fig. 2A). Peak NO_2^- values for all SNM were on the lower side of the range of previous ETNP observations (Horak et al., 2016), between 1.4 – 2.6 μM.

Of the five N cycling processes measured on the SR1805 cruise, NO_3^- reduction rates had the greatest magnitude at most depths. This trend was most pronounced within the upper ODZ, where NO_3^- reduction rates peaked at station PS2, and the oxycline where NO_3^- reduction rates

peaked at stations PS1 and PS3 (Fig. 2). Rates of NO_2^- oxidation closely tracked NO_3^- reduction in distribution; in fact, peak NO_2^- oxidation rates co-occurred with peak NO_3^- reduction rates at all three SR1805 stations, reaching maxima of ~40 (NO_2^- oxidation) and ~300 (NO_3^- reduction) nM N d^{-1} at PS3. However, the magnitudes of NO_2^- oxidation rates were usually lower than NO_3^- reduction rates, sometimes by as much as eightfold. The third N recycling process, NH_4^+ oxidation, peaked at or above the oxycline, with peaks of 10 nM N d^{-1} or less. NH_4^+ oxidation was consistently measured to be zero or near-zero throughout the rest of the water column.


A
B
C
PS1 - Depth (m)
PS2 - Depth (m)
PS3 - Depth (m)
O2 (µM)
NO3− (µM)
NH4+ (nM)
NO2− (µM)
Temperature (°C)
σθ (kg m−3)
NO2− Oxidation (nM N d−1)
NO3− Reduction (nM N d−1)
Denitrification (nM N2 d−1)
Anammox (nM N2 d−1)
NH4+ Oxidation (nM N d−1)
% Anammox

433

Figure 2: SR1805 depth profiles of physical parameters and N cycling rates. **(A)** From left to right, O_2 (μM) and NO_3^- (μM) respectively in blue and black, NH_4^+ (nM) and NO_2^- (μM) respectively in purple and black, temperature (°C) and σ_θ (kg m^{-3}) respectively in pink and black, NO_2^- oxidation and NO_3^- reduction rates (nM N d^{-1}) respectively in cyan and black, anammox and denitrification rates (nM N_2 d^{-1}) respectively in black and green, NH_4^+ oxidation rates (nM N d^{-1}), and percent anammox respectively in coral and black for station PS1 (offshore). **(B)** As above but for station PS2 (OMZ). **(C)** As above but for station PS3 (coastal). Rates that are significantly different from zero are shown as filled circles, open circles signify rates not significantly different from zero. Error bars are the standard error of the regression. Grey dotted lines indicate upper and lower ODZ boundaries at the time of sampling.

Across all SR1805 and FK180624 stations, the magnitude of the N loss processes of anammox and denitrification was almost always less than 10 nM N_2 d^{-1}, a much lower magnitude than the N recycling processes of NO_3^- reduction and NO_2^- oxidation. Like NO_3^- reduction and NO_2^- oxidation, the two N loss rates peaked in the upper ODZ or right at the oxycline in all three SR1805 stations, although a deep peak (850 m) in anammox was observed at station PS2 (Fig. 2B). This peak occurred near the bottom of the ODZ at an O_2 concentration of 1.5 μM. N loss rates also peaked near the oxycline in the three FK180624 stations with broad coverage of the ODZ water column, stations 2, 9 (6 July sampling), and 9 (9 July sampling) (Fig. S1). The relative balance between the two N loss processes as measured by percent anammox varied widely across the water column but largely deviated from the expected partitioning of at most 29% anammox (Dalsgaard et al., 2003, 2012). A striking example of this is that 100% anammox values were observed in both ODZ core and shallow boundary (see Table 1 for definitions) samples at many of the SR1805 and FK180624 stations (Fig. 2, Fig. S1).

3.2 Anaerobic NO_2^- oxidation and O_2 manipulation experiments

Significant NO_2^- oxidation rates were detected in depth profiles across a range of suboxic O_2 concentrations (1 – 5 μM) (definition from (Berg et al., 2022)) across all SR1805 stations, often at the same depths and in the same vials where the obligately anaerobic processes of

anammox and denitrification were occuring (Fig. 2, Fig. 3A-C, Fig. S2). In order to contextualize our observations, we compared our results to previously published measurements from the TN278 and NBP1305 cruises performed with identical procedures (Babbin et al., 2020). The highest rates were observed in shallow boundary waters across all three cruises (Fig. 3A-C, Fig. S2). Since low but significant levels of O_2 can still support aerobic NO_2^- oxidation, a series of O_2 manipulation experiments was carried out on both the SR1805 (spring) and FK180624 (summer) 2018 cruises (Fig. 4A-F and Fig. S3). In these experiments, anoxic conditions were checked using a LUMOS O_2 optode with a detection limit of 0.5 nM (Lehner et al., 2015). We observed significant NO_2^- oxidation, as well as NO_3^- reduction at putative concentrations as low as 1 nM. Notably, compared to previous experiments, gas flushing was constant, with a refresh time of 8 min, so as to maintain O_2 levels within the incubation even while organisms were respiring. At 1nM, O_2 is so scarce that such waters are usually classified as functionally anoxic, for example, a recent review paper defined 3 nM as the threshold below which O_2 cannot play biological or biogeochemical roles (Berg et al., 2022). As a result, these experiments present convincing additional evidence for the occurrence of NO_2^- oxidation up to ~100 nM N d^{-1} at O_2 concentrations too low to support aerobic metabolisms.

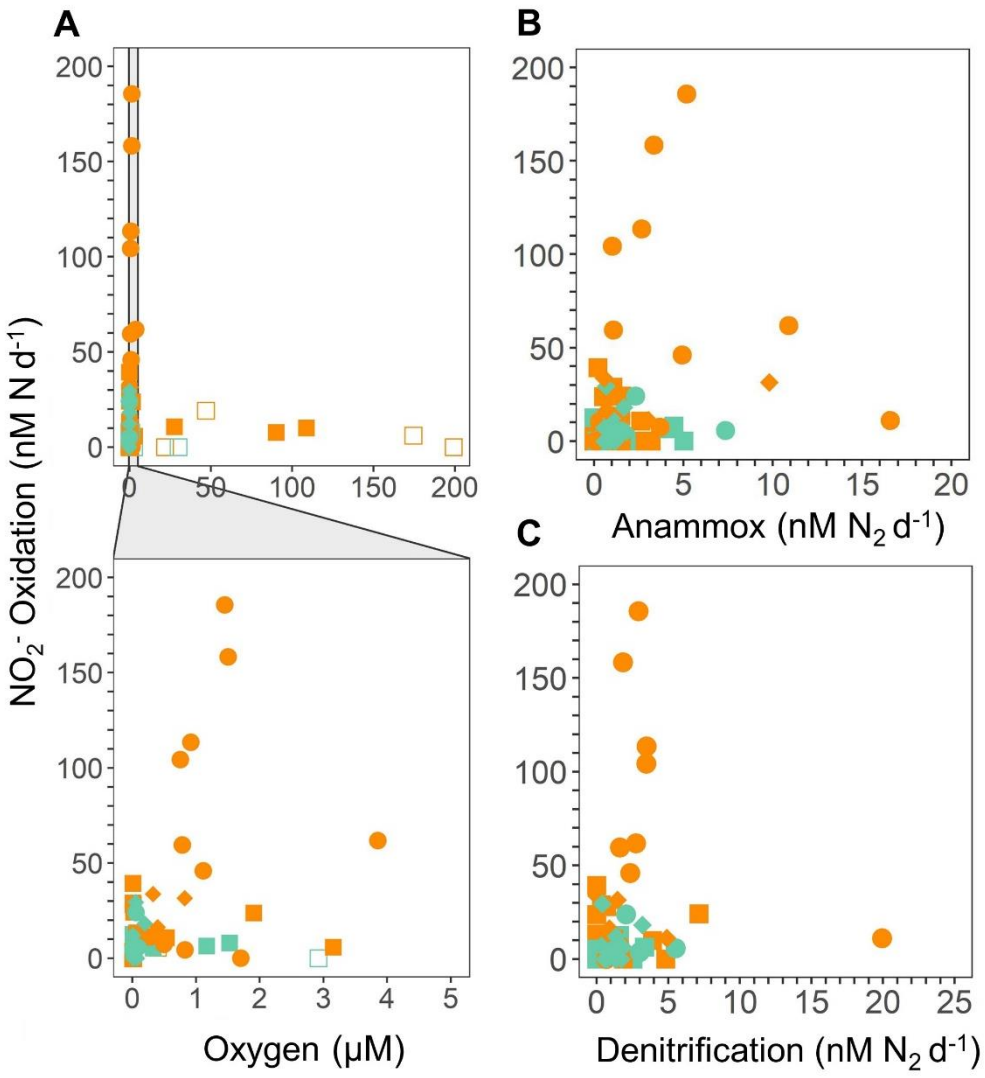


Figure 3: NO_2^- oxidation rates (nM N d^{-1}) from the 2018 SR1805 (squares), 2012 ETNP TN278 (circles), and 2013 ETSP NBP1305 (diamonds) cruises vs. **(A)** O_2 concentration (µM) from shipboard CTD sensors, **(B)** anammox rates (nM N_2 d^{-1}), and **(C)** denitrification rates (nM N_2 d^{-1}). O_2 concentrations were normalized across cruises. In A, rates that are significantly different from zero as assessed via a Student T-test (p value < 0.05) are displayed as filled symbols, while insignificant NO_2^- oxidation rates are shown as open symbols. Rates measured in shallow boundary waters are colored orange while rates from the ODZ core and below are colored teal. 2012 and 2013 data are republished (Babbin et al., 2020).

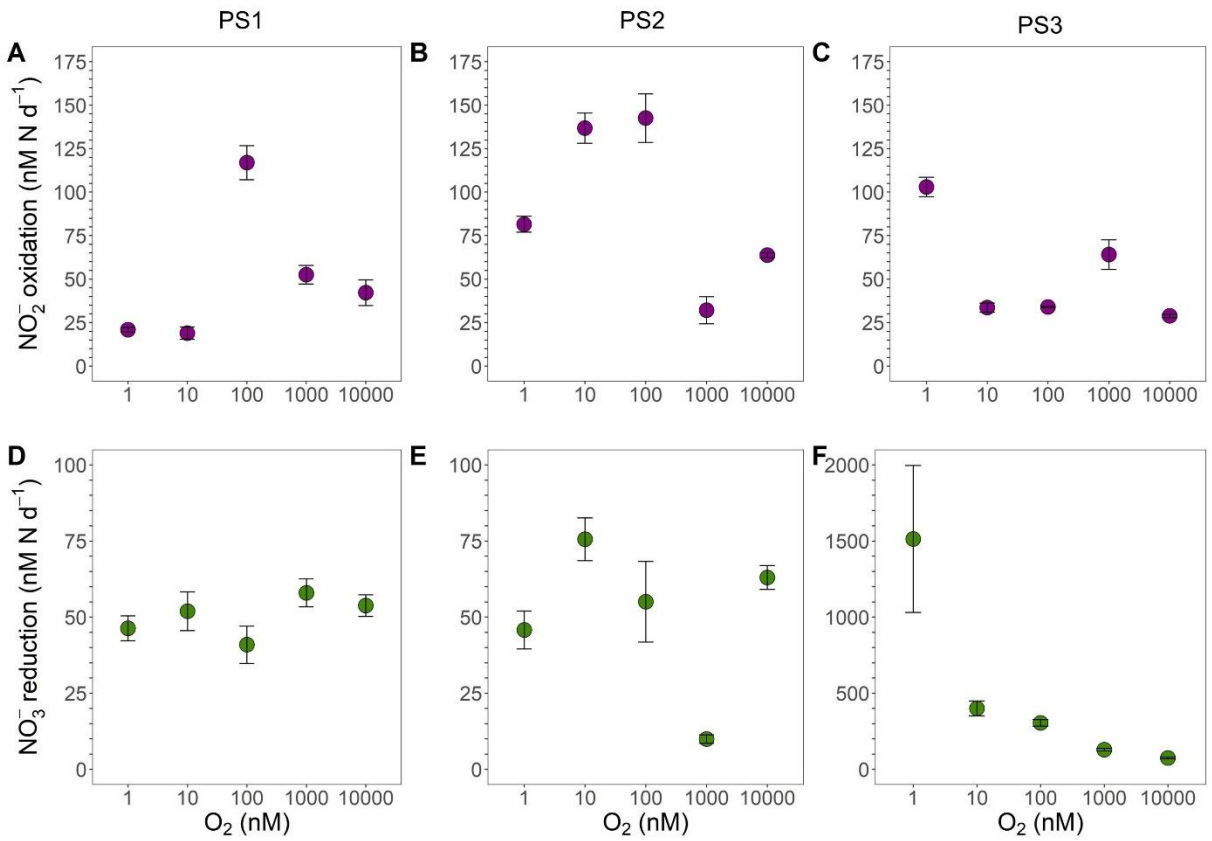


Figure 4: Oxygen manipulation experiments that show NO_2^- oxidation (purple) **(A-C)** and NO_3^- reduction (green) **(D-F)** rates (nM N d^{-1}) measured across putative O_2 concetrations from 1 to 10,000 nM during the SR1805 cruise. Experiments were conducted with waters from the ODZ top: 93 – 110m (PS1) **(A, D)**, 113 – 130m (PS2) **(B, E)**, and 45 – 60m (PS3) **(C, F)**. Error bars are the standard error of the regression. All rates were significantly different from zero.

3.3 NO_2^- dismutation

In order to investigate the mechanism for the observed anaerobic NO_2^- oxidation, experiments were conducted to search for evidence of NO_2^- dismutation. If NO_2^- dismutation is the dominant explanation for the observed anaerobic NO_2^- oxidation, we hypothesized that (1) adding NO_3^- should suppress both $^{30}N_2$ and NO_3^- production by LeChatelier's principle, (2) increasing $^{15}NO_2^-$ concentration should increase both denitrification (the $^{30}N_2$ production rate) and NO_2^- oxidation especially when no additional NO_3^- was added, and (3) that the ratio between the "unexplained NO_2^- oxidation," i.e., the difference between the observed NO_2^-

oxidation and the NO_2^- oxidation due to anammox, and the observed denitrification ($^{30}N_2$ production) rate should be close to 3:1. In experiments with He-purged water from two deoxygenated depths (60 and 160 m at station PS3, see table S5 for O_2 values) during the SR1805 cruise we observed that adding 20 μM NO_3^- suppressed NO_2^- oxidation across nearly all pairs of 0 and 20 μM NO_3^- experiments where the NO_2^- concentration was identical (Fig. 5). However, we did not observe a simultaneous suppression of N_2 production due to the fact that the measured denitrification rate was low and insignificantly different from zero in most of our 16 treatments (Fig. 5). The lack of an observed response in N_2 production could be due to already elevated ambient NO_3^- concentrations, 26 μM and 22.2 μM at 60 m and 160 m respectively. Roughly doubling the amount of NO_3^- would have little effect on the denitrification rate if the relevant enzymes were already saturated, as is plausible at those concentrations. As a result of our inability to observe denitrification, our first hypothesis yielded little evidence of dismutation.

Across all four 60 m 0 μM added NO_3^- treatments (Fig. 5A), adding NO_2^- did increase NO_2^- oxidation; however, we did not observe an increase in denitrification. Surprisingly, across the four 60 m 20 μM added NO_3^- treatments, adding NO_2^- decreased NO_2^- oxidation, the reverse of our hypothesis (Fig. 5). Across all four 160 m 0 μM added NO_3^- treatments, we also observed an increase in NO_2^- oxidation at higher NO_2^- concentrations but did not observe an increase in the measured denitrification rate (Fig. 5B). In the four 160 m 20 μM added NO_3^- treatments, NO_2^- oxidation and denitrification did not increase with NO_2^- concentration (Fig. 5B). Due to the consistently low and insignificant denitrification rates our test of the NO_2^- addition hypothesis also yielded little evidence for dismutation.

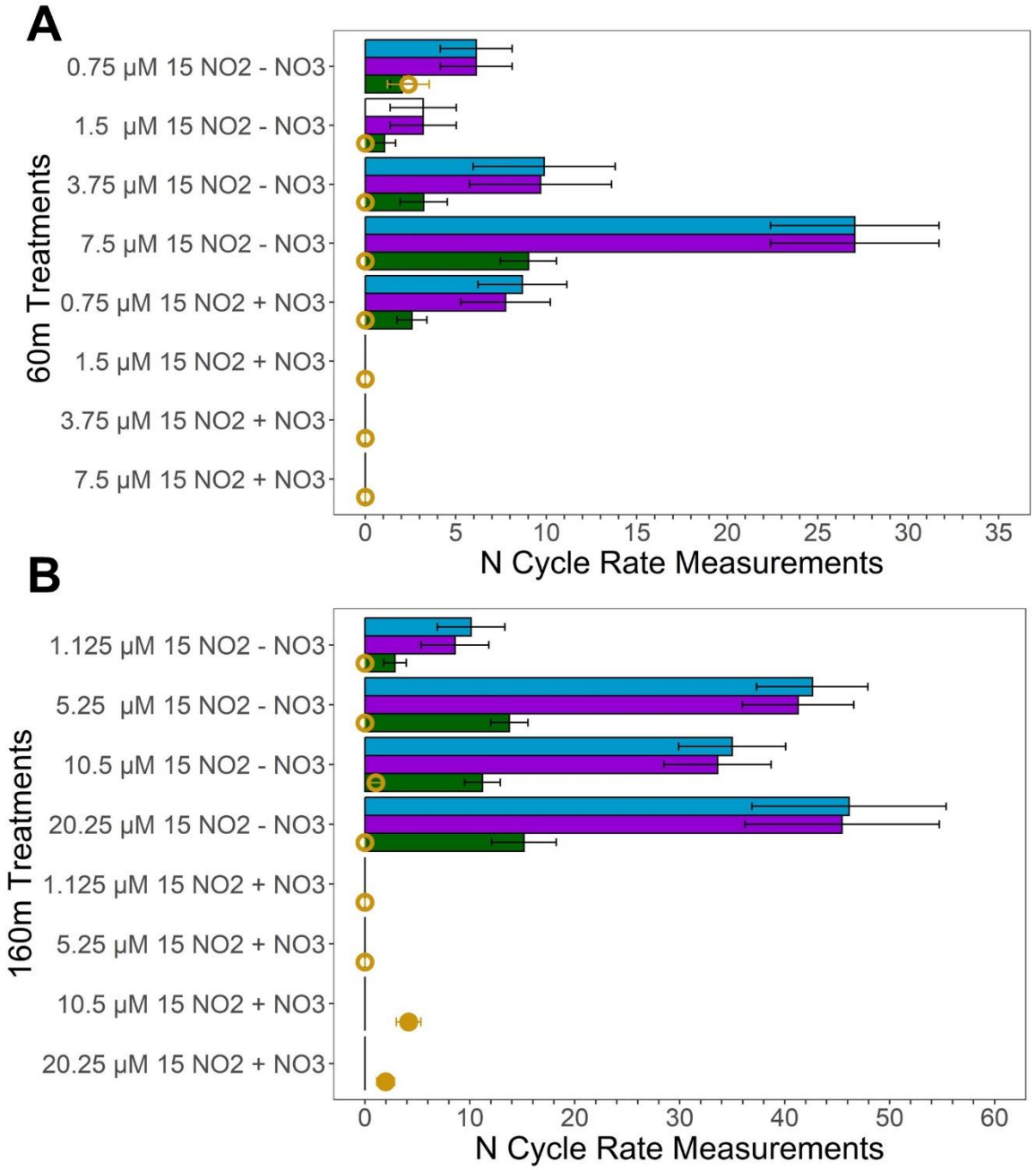


Figure 5: NO_2^- dismutation tests conducted in deoxygenated waters from 60m **(A)** and 160m **(B)** at station PS3 during the SR1805 cruise. Measured NO_2^- oxidation rates (nM N d^{-1}) are displayed in blue, unexplained NO_2^- oxidation rates, the difference between the measured NO_2^- oxidation and the NO_2^- oxidation due to anammox (nM N d^{-1}), are shown in purple. The predicted denitrification (nM $^{30}N_2$ d^{-1}) if all the unexplained NO_2^- oxidation was due to NO_2^- dismutation is shown in green. The measured denitrification rate (nM $^{30}N_2$ d^{-1}) is shown in yellow where filled circles indicate significant rates and open circles indicate rates that are not significantly different from zero. All bars filled with colors indicate significant rates (i.e. the white bar for the 60 m 1.5 µM $^{15}NO_2^-$, 0 µM NO_3^- treatment NO_2^- oxidation rate denotes an

539 insignificant rate). Error bars are the standard error of the regression for NO_2^- oxidation, or are
540 calculated based on the rules of error propagation from the standard error of the regressions for
541 the NO_2^- oxidation and anammox rates. (+) NO_3^- treatments received 20 μM $^{14}NO_3^-$ additions
542 while the (-) NO_3^- treatments received no addition. Anammox rates used to calculate the
543 unexplained NO_2^- oxidation rate are shown in the supplementary material.
544
545 We were also unable to observe evidence for the ratio hypothesis due to the paucity of
546 significant denitrification ($^{30}N_2$ production) rates (Fig. 5). Since denitrification rates were
547 consistently low or insignificantly different from zero, the ratio of NO_2^- oxidation to
548 denitrification deviated from the 3:1 ratio expected if NO_2^- dismutation accounts for most of the
549 observed NO_2^- oxidation. The only slight exception to this is the 60 m treatment with 0.75 μM
550 $^{15}NO_2^-$ and 0 μM added NO_3^-, the treatment closest to in situ conditions. In this treatment, the
551 measured denitrification rate, while insignificantly different from zero on the basis of the p value
552 of the regression, agrees with the predicted denitrification rate based on the 3:1 stoichiometry of
553 dismutation. While our dismutation experiments as a whole suggest that NO_2^- dismutation is not
554 a likely explanation for observed anaerobic NO_2^- oxidation, results from the 60 m 0.75 μM
555 $^{15}NO_2^-$, 0 μM NO_3^- treatment provide slight justification to continue tests of this hypothesis.
556
557 **4. Discussion**
558 **4.1 Rapid NO_2^- / NO_3^- cycle**
559 Depth profiles of N transformation rates obtained on the SR1805 cruise show that the
560 rates of NO_2^- oxidation and NO_3^- reduction are far greater than rates of the N loss processes of
561 anammox and dentrification, especially in shallow boundary (see Table 1 for definition) waters
562 (Fig. 2, Fig. 6A – B). In fact, when the combined N recycling pathways of NO_2^- oxidation and
563 NO_3^- reduction are compared to the total N loss, the N recycling pathways are 3.2 – 192.8 times
564 larger than the total N loss. That the minimum ratio is ~3 strongly emphasizes the

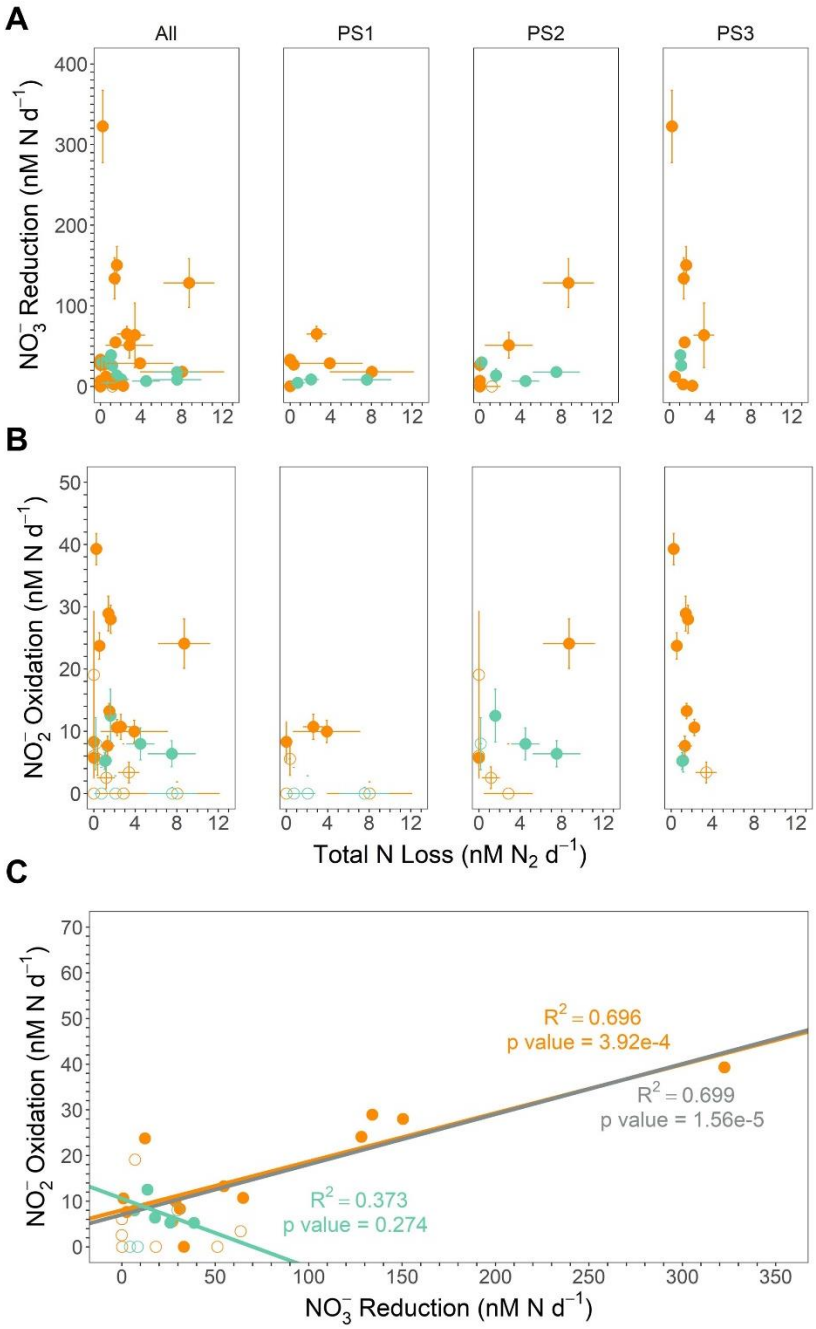
preponderance of NO_2^- oxidation and NO_3^- reduction above N loss processes. As expected due to the oligotrophic nature of the offshore ETNP (Fuchsman et al., 2019) and as previously found in an ETSP N cycling study (Kalvelage et al., 2013), NO_2^- oxidation and NO_3^- reduction generally increased from the offshore station (PS1) towards the coast. We observed NO_3^- reduction rates of a similar magnitude to previously reported ETSP studies (Kalvelage et al., 2013; Babbin et al., 2017), a finding that generalizes the predominance of NO_3^- reduction to NO_2^- to the ETNP. Thus, our work supports several recent studies (Babbin et al., 2020, 2017; Peters et al., 2016) suggesting that most nitrogen within OMZ regions is continously recycled between NO_2^- and NO_3^- by rapid NO_2^- oxidation and NO_3^- reduction, especially in shallow boundary waters.

A previous work (Babbin et al., 2017) predicted that NO_3^- reduction should follow a Martin curve (Martin et al., 1987) power law distribution across the water column due to its dependence on the OM flux from shallower waters. Such a distribution was observed at stations PS1 and PS3; however, NO_3^- reduction at station PS2 did not follow a classical Martin curve profile since the NO_3^- production peak is well below the oxycline. This exception could be due to zooplankton which have been observed to migrate into the ODZ on a daily basis (Bianchi et al., 2014). Due to the fact that migrating zooplankton funnel surface OM to the mesopelagic (Cram et al., 2022), such a transfer would move OM in a pattern not consistent with the Martin curve. The transferred OM could then support the observed peak in NO_3^- reduction (Fig. 2).

These results are consistent with the idea, also supported by many recent studies (Kalvelage et al., 2013; Lam and Kuypers, 2011; Lam et al., 2009; Babbin et al., 2020, 2017; Füssel et al., 2011; Lam et al., 2011), that the accumulated NO_2^- in the SNM usually results from an imbalance between NO_3^- reduction and other N cycling pathways. We further investigated

588 this hypothesis by constructing a net NO_2^- budget derived from the five microbial N cycling
589 metabolisms measured on the SR1805 cruise (Fig. 7). Summing the depth profiles of NO_2^-
590 consumption (anammox, denitrification, and NO_2^- oxidation) and production (NH_4^+ oxidation
591 and NO_3^- reduction) pathways revealed that net depth integrated NO_2^- production across the
592 sampled OMZ water column depths is on the order of tens of millimoles of NO_2^- per square
593 meter per day at all three stations (8.19 at PS1, 14.49 at PS2, and 28.97 mmol NO_2^- m^{-2} d^{-1} at
594 PS3). This excess NO_2^- is driven by NO_3^- reduction, which across all stations is of a much
595 greater magnitude than all other measured N cycling processes (Fig. 2 and Fig. 7).
596 These budget calculations take the reported rates at face value, ignoring the likelihood
597 that some of them are potential rates. For example, anammox might have been enhanced by the
598 addition of 3 μM NH_4^+. Denitrification is less likely to be stimulated by the addition of NO_2^-
599 because it is generally limited by organic matter availability (Ward et al. 2008, Babbin et al
600 2014). Thus, the relative importance of anammox and denitrification might be perturbed due to
601 differential responses of the two rates to tracer additions. Analogously, NO_2^- oxidation was
602 likely stimulated by addition of NO_2^- tracer (Sun et al. 2017), but NO_3^- reduction less so by
603 addition of NO_3^- tracer because the latter is a heterotrophic process, and as a component of the
604 complete denitrification pathway, likely limited by organic matter availability. These differential
605 limitations by substrate probably mean that the calculated budget of Figure 7 is not completely
606 accurate, but the relative importance of the processes is robust. If anything, the dominance of
607 anammox over denitrification is probably less than that observed, and the excess of NO_3^-
608 reduction over NO_2^- oxidation greater than observed. Overall, the dominance of the NO_3^- / NO_2^-
609 loop over the N loss pathways and the overwhelming importance of NO_3^- reduction are both
610 supported by these considerations.

611 Additional support that NO_3^- reduction supplies the accumulated NO_2^- in the SNM can
612 be found by comparing the net NO_2^- production rates with the measured NO_2^- concentrations
613 along the SR1805 cruise track from offshore station PS1 to coastal station PS3. As would be
614 expected if the SNM depended on NO_2^- derived from NO_3^- reduction, the peak net NO_2^-
615 production value across all depths at each station, the depth integrated NO_2^- production values
616 for each station, and the magnitude of the SNM peak NO_2^- concentrations all increase together
617 from offshore station PS1 to coastal station PS3. Importantly, we did not take into account water
618 column mixing in both vertical and horizontal directions that would carry away produced NO_2^-
619 or NO_2^- assimilation into OM, and we recommend follow up studies that include
620 parameterizations for these values in OMZ N Cycling modeling.



621

622 **Figure 6: (A)** NO_3^- reduction (nM N d^{-1}) vs. Total N loss (the sum of denitrification and
623 anammox in nM N_2 d^{-1}) from the SR1805 cruise. **(B)** NO_2^- oxidation (nM N d^{-1}) vs. Total N loss
624 from the SR1805 cruise. **(C)** NO_2^- oxidation vs. NO_3^- reduction. Regression lines and statistics
625 are shown for the significant rates from shallow boundary waters only (orange), ODZ core
626 waters only (teal), and all significant data (grey). All points from shallow boundary waters are
627 colored orange while all points from the ODZ core or below are colored teal. Open circles
628 indicate points where the NO_3^- reduction rate (A), NO_2^- oxidation rate (B), or in (C) either NO_3^-
629 reduction or NO_2^- oxidation rate is not significantly different from zero while filled circles
630 indicates rates significantly different from zero.

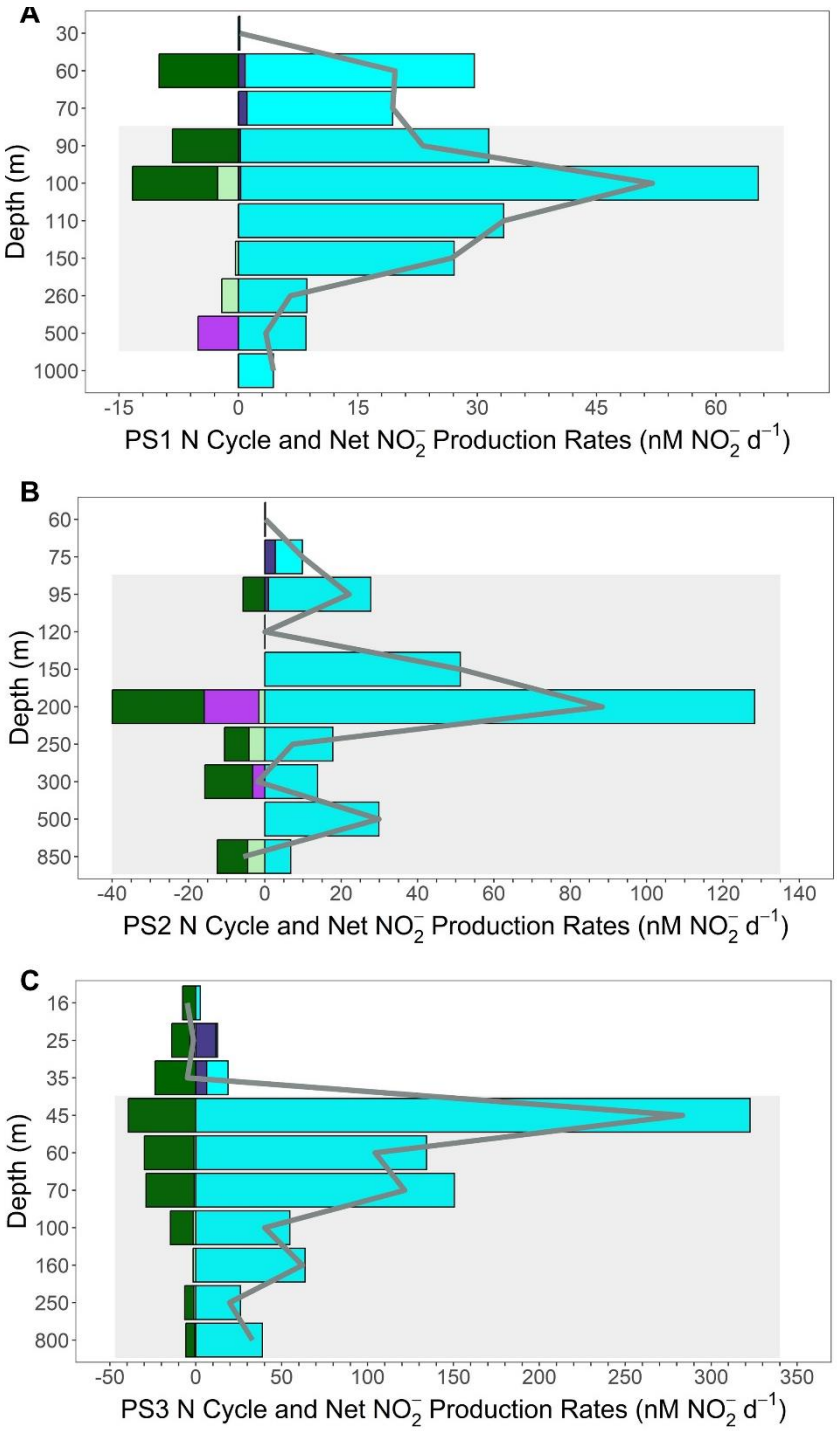
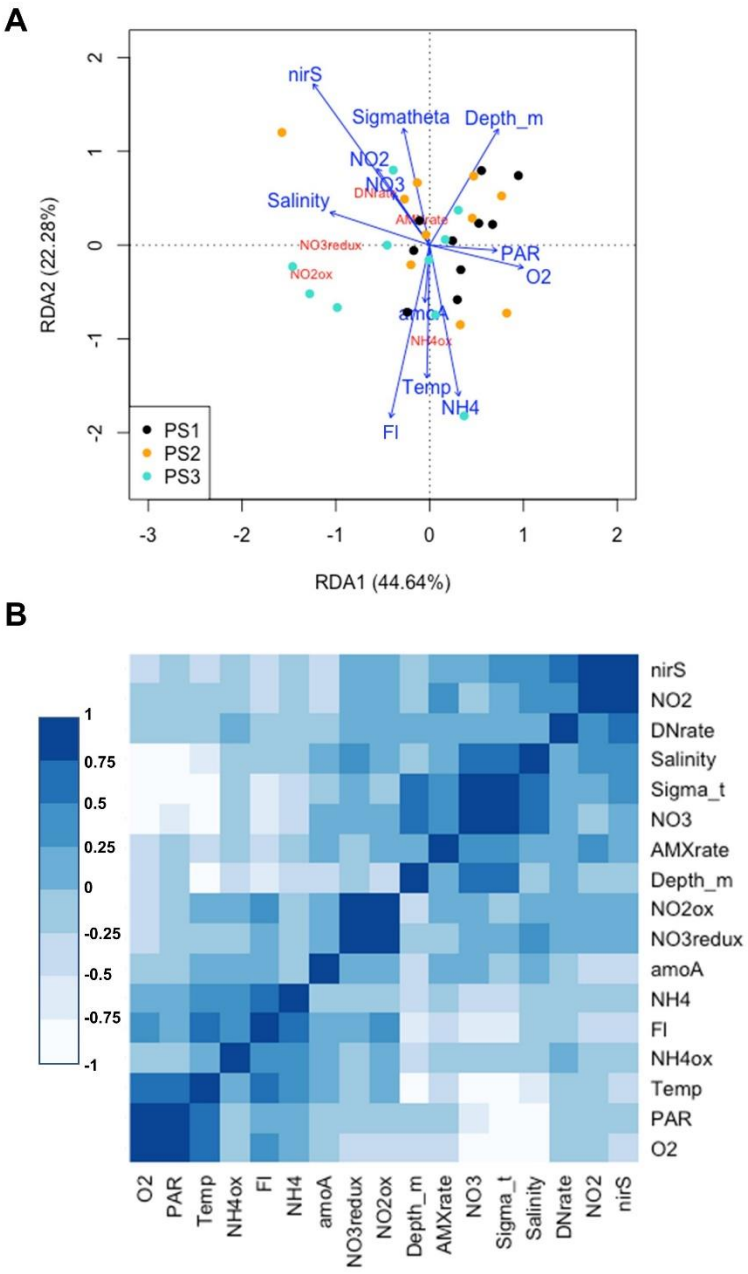


Figure 7: NO_2^- budget profiles from the SR1805 cruise. Plots are a combination of the NO_2^- production pathways of NO_3^- reduction (cyan), NH_4^+ oxidation (dark purple) and the NO_2^- consumption pathways of anammox (light green), denitrification (bright purple), and NO_2^- oxidation (dark green). Consumption pathways are reported as negative numbers. All rates are reported in nM NO_2^- d^{-1}. The net NO_2^- production or consumption rate (nM NO_2^- d^{-1}) is represented as a grey line for each depth. Grey boxes indicate the completely deoxygenated ODZ region at each station at the time of sampling. **(A)** PS1, **(B)** PS2, and **(C)** PS3.

4.2 NO_2^- oxidation – distribution and magnitude in comparison to previous studies

The high rates of observed NO_3^- reduction provide sufficient NO_2^- to support NO_2^- oxidation both in the oxycline and in the ODZ, as previously proposed (Anderson et al., 1982), Our observations also further confirm isotopic studies that suggested high NO_2^- oxidation rates because rapid re-oxidation of NO_2^- back to NO_3^- was necessary to achieve isotopic mass balance (Buchwald et al., 2015; Casciotti et al., 2013; Granger and Wankel, 2016). Our results also align with previous experimental observations of high NO_2^- oxidation rates (Kalvelage et al., 2013; Babbin et al., 2020; Lipschultz et al., 1990). Support for a closely connected rapid cycle between the two processes can be seen in the strong correlation between NO_2^- oxidation and NO_3^- reduction observed in all SR1805 cruise samples, especially those from shallow boundary waters (Fig. 6C, Fig. 8). Similarly to some previous ETSP papers (Babbin et al., 2017, 2020; Frey et al., 2020) and two ETNP studies (Peng et al., 2015; Sun et al., 2017) we observed that rates of NO_2^- oxidation, like rates of NO_3^- reduction, peaked in the oxycline or in the ODZ top (Fig. 2) and then declined throughout the ODZ. Unlike some stations in these studies (Babbin et al., 2020, 2017) we did not observe a second peak in NO_2^- oxidation near the deep oxycline. In addition to observing a similar distribution, we also observed that NO_2^- oxidation occurs at a similar magnitude to some stations in previous ETSP studies (Babbin et al., 2020, 2017; Peng et al., 2016) and ETNP (Peng et al., 2015), although our highest rates (25 – 40 nM N d^{-1}) were much lower than the peaks measured at other stations in most of these reports (Babbin et al., 2020; Peng et al., 2015, 2016), which reached as high as ~600 nM N d^{-1} (Peng et al., 2015; Lipschultz et al., 1990).



661

662 **Figure 8: (A)** Redundancy analysis of all environmental variables and microbial rates measured
663 on the SR1805 cruise. Points are color-coded by station, black (PS1), yellow (PS2), and cyan
664 (PS3). Variables names and arrows are color coded so that environmental variables are blue and
665 rate measurements are red. **(B)** Correlation analysis for all environmental variables and
666 microbial N cycle rates from the SR1805 cruise. More positive correlations are shaded to
667 become bluer as significance grows while negative correlations are shaded to become whiter as
668 significance grows. Abbreviations used are as follows: O2 (oxygen concentration normalized
669 across different sensors), PAR (photosynthetically active radiation normalized across sensors),
670 NH4ox (NH_4^+ oxidation rate), Fl (chlorophyll fluorescense normalized across different sensors),
671 NH4 (NH_4^+ concentration), *amoA* (*amoA* abundance), NO3redux (NO_3^- reduction rate), NO2ox

672 (NO_2^- oxidation rate), AMXrate (anammox rate), NO3 (NO_3^- concentration), DNrate
673 (denitrification rate), NO2 (NO_2^- concentration), and *nirS* (*nirS* abundance).
674
675
676 **4.3 NO_2^- oxidation – can it occur anaerobically?**

677 NO_2^- oxidation depth profiles (Figs. 2, 3) and O_2 manipulation experiments (Fig. 4)
678 provide further evidence that NO_2^- oxidation can occur even when O_2 is as low as ~1 nM. .
679 While our O_2 manipulation experiments provide the most convincing evidence of anaerobic
680 NO_2^- oxidation, several factors argue that the NO_2^- oxidation observed in our depth profile
681 incubations may be O_2 independent. As argued previously (Babbin et al., 2020):
682 (1) The pre-incubation He purging step in our depth profile method removes more than 99% of
683 the N_2 present in exetainers (Babbin et al., 2020). If it is assumed that O_2 is removed at identical
684 efficiency, a reasonable proposition since O_2 equilibrates faster than N_2 (Wanninkhof, 1992), the
685 introduction during sample processing of as much as 1 μM O_2 would result in a ~10 nM
686 contamination. As a result, if NO_2 oxidation is observed in samples from the deoxygenated ODZ
687 core, contamination during sampling would be kept very small by our purging step. This
688 conclusion was further validated by direct O_2 measurements using Lumos sensors in exetainers.
689 These tests of our purging method showed that O_2 was reduced to less than 10 nM in 5 minutes
690 (Sun et al. unpublished data).
691 (2) Linear timecourses across all timepoints were observed in some of our experiments,
692 including many from deoxygenated depths at station PS3 (Supplemental Figs. S7-9). If NO_2^-
693 oxidation depended on O_2, an initial acceleration (due to O_2 contamination that sparked NO_2^-
694 oxidation) or later steep drop (due to the exhaustion of O_2 by aerobic NOB) in NO_2^- oxidation
695 would be expected, not a consistent linear slope.

(3) Metagenomic evidence has revealed distinct NOB communities in oxic surface waters, the oxycline and ODZ top, and the ODZ core in OMZ regions (Sun et al., 2019). In addition we observed decreasing NO_2^- oxidation rates with increasing in situ O_2 in the SR1805 incubations as well as the TN278 and NBP1305 incubations (Fig. 3A). These observations are consistent with the hypothesis that aerobic NOB from oxic depths are ill-equipped to oxidize NO_2^- in deoxygenated conditions but that the unique MAGs recently identified in draft genomes from the ODZ top and core (Sun et al., 2019), are adapted to perform anaerobic NO_2^- oxidation.

(4) Through plotting O_2 concentrations against the ratio between NO_3^- reduction and NO_2^- oxidation at all SR1805 depths with significant, positive NO_2^- oxidation rates, we observed that the known anaerobic process of NO_3^- reduction and NO_2^- oxidation did not exhibit differential regulation by O_2 as would be expected if NO_2^- oxidation was an obligately aerobic process (Fig. S5).

Previous studies have shown that O_2 additions to purged incubations of ODZ waters can inhibit NO_2^- oxidation (Sun et al., 2017, 2021a) and that NO_2^- oxidation can occur in the absence of O_2 consumption (Sun et al., 2021a). However, another kinetics study has reported O_2 stimulation of NO_2^- oxidation in OMZ waters (Bristow et al., 2016) and concluded that NO_2^- oxidation is fundamentally an aerobic process. This apparent contradiction might be explained by several details in the experimental process of that study (Bristow et al., 2016):

(1) The study site is at the farthest edge of the ETSP OMZ in a location that is only anoxic in the austral summer.

(2) The cruise was conducted as austral summer turned to fall (March 20 – 26th), a period where O_2 intrusions would be more likely.

718 (3) O_2 data from the study's cruise (Tiano et al., 2014) show that the depths from which NO_2^-
719 oxidation O_2 kinetics samples were sourced experienced O_2 concentrations of 2 μM (50 m), 10
720 μM (40 m), and > 60 μM (30m) either during sampling or a few days prior to sampling.
721 As a result, we argue that the observed stimulation of NO_2^- oxidation by O_2 (Bristow et al., 2016)
722 occurred not because all OMZ NOB are aerobic NO_2^- oxidizers, but instead because the location,
723 season, and levels of O_2 of the sampled station selected for aerobic NOB in the source water for
724 the purged incubations. Thus, as suggested by (Sun et al., 2017, 2021a), different NOB
725 populations with different historical exposures to O_2 and adaptations likely respond differently to
726 O_2 manipulations.
727 Here we built on the above previous tests of anaerobic NO_2^- oxidation by conducting a
728 series of incubations across an O_2 gradient from ~1 nM to 10 μM. Site waters for these
729 incubations were drawn from the ODZ top at each SR1805 station (Table S4). We did not
730 observe a clear inhibitory or stimulatory response of NO_2^- oxidation to O_2 within the SR1805 or
731 FK180624 stations; however, this lack of a clear response is in itself a revealing result - a lack of
732 consistent stimulation by O_2 implies at least some anaerobic NOB were present. In addition, we
733 consistently observed significant NO_2^- oxidation at all putative O_2 concentrations, including 1
734 nM, a concentration usually considered functionally anoxic. Since the O_2 in the incubations was
735 continuously supplied by a mass flow controller and subsequently checked via an extremely
736 sensitive O_2 sensor for all incubations, these results provide additional evidence that truly
737 anaerobic NO_2^- oxidation can occur.
738 One argument against our characterization of the NO_2^- oxidation observed at ~1 nM O_2
739 as functionally anoxic is that the K_m of NO_2^- oxidation has been calculated to be as low as 0.5
740 nM (Bristow et al., 2016). However, the data used to calculate this value have the same

741 qualifications discussed previously: (1) the study site is at a location only anoxic during the
742 austral summer, (2) the cruise was conducted during a time when O_2 intrusions would be more
743 likely, and (3) the sampled waters experienced O_2 concentrations as high as 60 μM prior to
744 sampling. Such conditions would favor aerobic NOB and the expression of high affinity NO_2^-
745 oxidation enzymes by these organisms when exposed to low O_2 conditions in incubations. As a
746 result, we argue that the modeled K_m value of 0.5 nM only applies when NOB with higher O_2
747 niches are placed in sub-micromolar O_2 conditions. This value does not apply to NOB observed
748 to prefer ODZ conditions (Sun et al., 2019), which we assume would be favored under our 1 nM
749 treatment.

750 These O_2 manipulation experiments also provided an opportunity to investigate the
751 response of NO_3^- reduction to O_2. The only clear intra-station pattern that emerged from these
752 experiments was that at station PS3, NO_3^- reduction displayed possible inhibition by O_2, as
753 would be expected. Due to the small number of data points in our data set we did not attempt a
754 kinetics fitting for this data. Interestingly, the disparity observed in depth profile experiments
755 between the magnitudes of the NO_3^- reduction and NO_2^- oxidation rates was not observed in the
756 O_2 manipulations across many O_2 concentrations at stations PS1 and PS2. At station PS3 a large
757 disparity in the magnitudes of these processes as well as the highest overall NO_3^- reduction rates
758 were observed, as in the depth profile experiments (Fig. 4, 7). A few of the FK180624 data
759 points also exhibited NO_3^- reduction rates that were elevated far above NO_2^- oxidation (Fig. S3).
760 These results confirm the importance of NO_3^- reduction for the rapid recycling cycle as well as
761 the source of NO_2^- for the SNM.

762

763 **4.4 NO_2^- dismutation**

In the absence of O_2, NO_2^- oxidation would require another oxidant. Many candidate oxidants have been suggested. For example, iodate (IO_3^-), an abundant marine species with global average marine concentrations of ~0.5 μM (Nozaki, 1997; Lam and Kuypers, 2011), has been proposed and shown to stimulate NO_2^- oxidation (Babbin et al., 2017). However, since IO_3^- is usually absent within the ODZ core (Moriyasu et al., 2020), its low concentration makes IO_3^- mediated anaerobic NO_2^- oxidation in that location unlikely (Babbin et al., 2020). NO_2^- oxidation via Mn^{4+} or Fe^{3+} is thermodynamically feasible, but only at low pH (<6) (Luther, 2010; Luther and Popp, 2002). This pH constraint, combined with the fact that concentrations of these ions are on the order of a few nM in OMZs (Kondo and Moffett, 2015; Vedamati et al., 2015), makes these mechanisms unrealistic for the ODZ core. Another proposed mechanism is that the observed NO_2^- oxidation is due to anammox, which if true should result in an observed NO_2^- oxidation to anammox ratio of 0.16 – 0.3 (Kuenen, 2008; Strous et al., 1998; Oshiki et al., 2016). Instead, the observed ratio is sometimes more than 10x this range and NO_2^- oxidation is rarely observed to be less than anammox (Kalvelage et al., 2013; Babbin et al., 2020; Sun et al., 2021a).

Another alternative hypothesis is based on the reversibility of the nitrite oxidoreductase (NXR) enzyme. Since this enzyme has been suggested to both oxidize NO_2^- and reduce NO_3^- (Kemeny et al., 2016; Koch et al., 2015; Wunderlich et al., 2013), NO_3^- reduction by NXR could over time enrich the ^{15}N-NO_3^- pool since lighter $^{14}NO_3^-$ would be favored (Casciotti, 2009). Even in $^{15}NO_2^-$ tracer experiments, in which the NO_2^- pool is highly labeled, this reversibility at the enzyme site could lead to an apparent transfer of ^{15}N from the NO_2^- to the NO_3^- pool if NXR mediated NO_3^- reduction was occurring. This hypothesis is supported by observations of NO_3^- reduction under low O_2 in cultures from the NOB genera *Nitrobacter* (Freitag et al., 1987; Bock et al., 1990), *Nitrospira* (Koch et al., 2015), and in pure cultures of *Nitrococcus mobilis* (Füssel

et al., 2017). In addition, a recent study presented natural abundance isotopic evidence in pure *Nitrococcus mobilis* cultures consistent with this mechanism (Buchwald and Wankel, 2022).

However, NXR reversibility has not been demonstrated for the abundant (Füssel et al., 2011; Mincer et al., 2007) and sometimes dominant (Beman et al., 2013) OMZ NOB genera *Nitrospina*. Furthermore, the sole source of the isotopic evidence for the enzyme reversibility hypothesis, *Nitrococcus mobilis,* has a cytoplasm facing NXR substrate binding domain (Buchwald and Wankel, 2022), a feature found to have an established evolutionary relationship to NAR (the known NO_3^- reductase enzyme family) in other *Nitrobacter* studies (Starkenburg et al., 2008; Kirstein and Bock, 1993). The NXR substrate binding domains in *Nitrospina* are oriented towards the periplasm and are not evolutionarily related to enzymes for NO_3^- reduction (Buchwald and Wankel, 2022; Sun et al., 2019). Due to these structural and phylogenetic differences among NOB NXR, it is possible that the *Nitrospina* NXR may be unable to perform NO_3^- reduction as easily as other NOB genera. For all these reasons, it is not yet clear if the enzyme reversibility hypothesis can explain all NO_2^- oxidation measured under low O_2 conditions and other hypotheses should continue to be explored.

As a result of the above proposals' shortcomings, this paper focused on the remaining, most plausible hypothesis: NO_2^- dismutation. Our tests for dismutation rested on three hypotheses: (1) that NO_3^- additions would inhibit both NO_2^- oxidation and $^{30}N_2$ production by LeChatelier's principle, (2) that increasing $^{15}NO_2^-$ should energetically favor dismutation, especially in treatments with no additional NO_3^-, and (3) that the ratio of non-anammox mediated NO_2^- oxidation to denitrification ($^{30}N_2$ production) should be close to 3:1 if NO_2^- dismutation explains most of the observed NO_2^- oxidation. We observed repeated inhibition of NO_2^- oxidation by NO_3^- but no inhibition of $^{30}N_2$ production due to the fact that denitrification

was consistently low and insignificantly different from zero across all treatments. In treatments with 0 μM added NO_3^-, increasing NO_2^- generally increased NO_2^- oxidation, but not denitrification. In addition, the ratio of anammox corrected NO_2^- oxidation to observed denitrification deviated from dismutation's 3:1 stoichiometry in almost all treatments. However, we did observe simultaneous inhibition of N_2 and NO_3^- production as well as good agreement between the anammox corrected NO_2^- oxidation / denitrification ratio to the NO_2^- dismutation stoichiometry in one treatment - the treatment most similar to in situ conditions (60m, 0.75 μM $^{15}NO_2^-$, 0 μM NO_3^-). As a result, while our results show little evidence for dismutation overall, we recommend additional experiments at tracer levels similar to 0.75 μM $^{15}NO_2^-$ to further test for NO_2^- dismutation.

4.5 Relative balance of anammox and denitrification

4.5.1 Are results consistent with past observations of slow, low, and steady anammox elevated above the predicted maximum of 29% of total N loss?

According to predictions based on the composition of average marine OM (Dalsgaard et al., 2003, 2012) anammox should account for at most 29% of the total N loss flux in OMZ regions. To test this hypothesis under a variety of conditions, regressions of denitrification vs. anammox rates were calculated for all samples from the SR1805, FK180624, TN278, and NBP1305 cruises. In order to compare our new data to a previous study (Babbin et al., 2020), which observed variations in the ratio of anammox and denitrification between samples from the ODZ top or above ($\sigma_\theta < 26.4$, "shallow boundary waters," (Babbin et al., 2020)) and samples from the deoxygenated ODZ core or below ($\sigma_\theta > 26.4$, "ODZ core," (Babbin et al., 2020)), regressions for all data (ODZ core), all data (shallow boundary), 2018 only (ODZ core), 2018

only (shallow boundary), 2012-13 (TN278, and NBP1305) only (ODZ core), and 2012-13 only (shallow boundary) were calculated (Table S6). All regressions deviated from the predicted 29% maximum anammox contour, although the regression from the 2012-13 cruises' ODZ core samples was closest to the 30% anammox contour (Fig. 9A). We observed large differences in the percent anammox contours near 2012-13 and 2018 regressions. ODZ core samples from 2012-13 regressed onto a line between the 40 and 50% anammox contours while ODZ core samples from 2018 regressed onto a line between the 70% and 80% anammox contours. Differences in contouring were smaller for the shallow boundary samples, although the 2018 samples still regressed to a higher contour (just under 80%) than the 2013-13 samples (60%) (Fig. 9A). Our observations that all year and density based regressions fell within contours well above the theoretical prediction (Fig. 9A) and that anammox accounted for as much as 100% of the total N loss at many depths in 2018 samples (Fig. 2, Fig. 9B) is consistent with the many previous studies that observed anammox as the predominant OMZ N loss pathway (Lam et al., 2009; Thamdrup et al., 2006; Kuypers et al., 2005; Hamersley et al., 2007; Jensen et al., 2011).

Our new 2018 results do not contradict the idea (Dalsgaard et al., 2012) that anammox is often measured to be the bulk of total N loss but that large, episodic occurences of denitrification can dwarf the consistent albeit low anammox contribution to total N loss. Under this view, these eruptions in denitrification return the *time integrated* balance of anammox and denitrification to its expected 29 and 71% values. In this scenario, our cruises' sampling, like many but not all others, did not coincide with episodic high rates of denitrification.

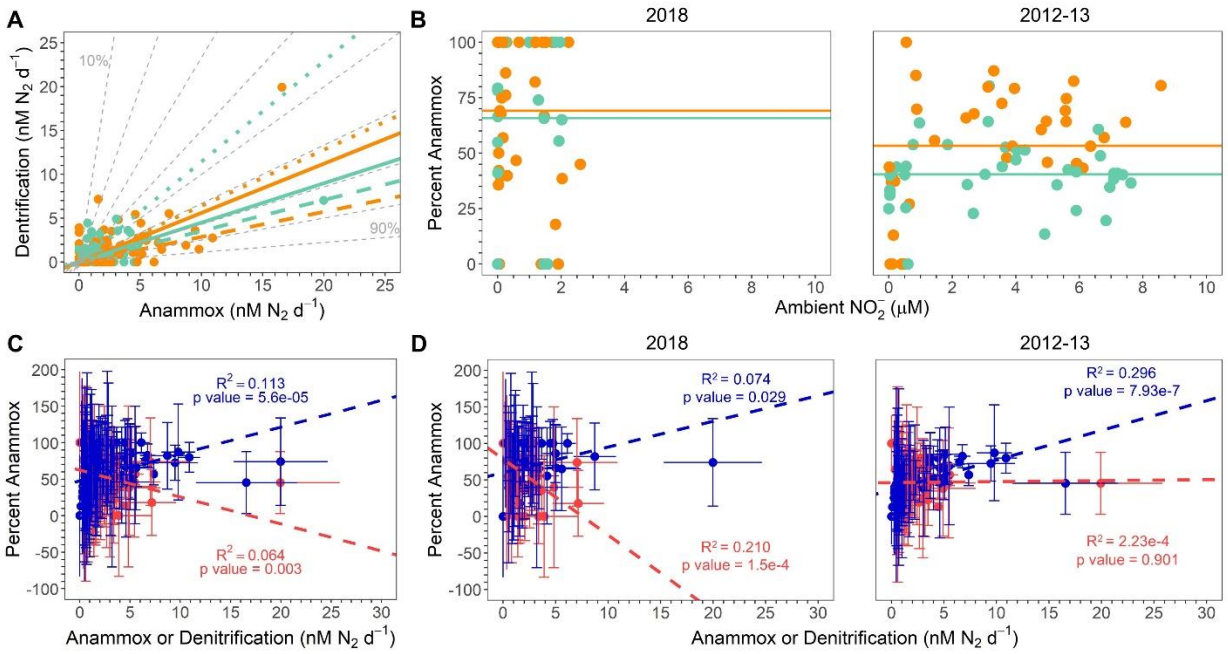


Figure 9: (A) All 2012, 2013, and 2018 denitrification and anammox rates (nM N_2 d^{-1}), color-coded by σ_θ. ODZ core samples and lines are teal (σ_θ > 26.4) while shallow boundary samples and lines are orange (σ_θ < 26.4). Solid, dashed, and dotted lines respectively show regressions for all data, 2018 only, and 2012-13 data only. Dashed grey lines depict contours for percent anammox values. See Supplementary Table S6 for regression statistics. **(B)** Percent anammox vs. ambient NO_2^- for 2018 samples (left) and republished 2012 and 2013 samples (Babbin et al., 2020) (right). Points are colored according to the same scheme as panel A. Lines show the average percent anammox values in shallow boundary waters (orange) and the deoxygenated ODZ core (teal). **(C)** Percent anammox vs. all anammox (blue) and all denitrification (red) rates (nM N_2 d^{-1}). Regression lines shown for % AMX vs. anammox and denitrification rates follow the same color scheme as the data points. Error bars represent the standard error of the regression. **(D)** Percent anammox vs. anammox (blue) and denitrification (red) rates (nM N_2 d^{-1}) for 2018 only (left) and 2012-13 (right). Points and regression lines follow the same color scheme as in panel C. Data shown in the 2012-13 only panel are republished (Babbin et al., 2020).

4.5.2 Do results support a connection between rapid NO_3^- reduction and elevated anammox?

Our 2018 results question the previously proposed view (Babbin et al., 2020) that rapid NO_3^- reduction produces NH_4^+ that in turn elevates anammox in oxycline and upper ODZ waters. While our data (Fig. 2) did find high rates of NO_3^- reduction in shallow boundary

876 waters, the 2018 N loss data do not show elevated shallow boundary (as compared to ODZ core)
877 percent anammox values as would be expected if high NO_3^- reduction were fueling elevated
878 anammox in the oxycline and ODZ top. This difference between our 2018 data and some
879 previous data (Babbin et al., 2020) in support of a connection between rapid NO_3^- reduction and
880 elevated anammox in the oxycline and ODZ top can be seen through a comparison of shallow
881 boundary (σ_θ < 26.4 (Babbin et al., 2020)) and ODZ core (σ_θ >26.4 (Babbin et al., 2020)) percent
882 anammox values in the 2018 SR1805 and FK180624 cruises against the 2012-13 TN278 and
883 NBP1305 cruises (Fig. 9B). 2012-13 samples showed a clear partitioning between the ODZ core
884 and shallow boundary waters in terms of percent anammox values. In 2012-13, as would be
885 expected if high oxycline and ODZ top NO_3^- reduction were supplying NH_4^+ to anammox,
886 shallow boundary samples have a higher average percent anammox value than ODZ core
887 samples (Fig. 9B). In 2018, this partitioning was not present - the difference between the
888 average percent anammox values in ODZ core and shallow boundary samples was much smaller
889 (Fig. 9B). Interestingly, the total number of samples found to be 100% anammox also sharply
890 diverged between 2012-13 and 2018. In the 2012-13 samples, only one shallow boundary
891 sample was found to be 100% anammox. In 2018, many samples from both shallow boundary
892 waters and the ODZ core were 100% anammox (Fig. 9B, Fig. S6).
893 These observed differences in the partitioning of anammox and denitrification between
894 shallow boundary waters and the ODZ core across different years and places do not support the
895 view that NH_4^+ from rapid NO_3^- reduction of oxycline and ODZ top OM always elevates
896 anammox rates. Instead, they suggest that other factors play an important role in setting the
897 balance of anammox and denitrification. Interestingly, NO_2^- concentrations spanned a much
898 narrower range in the two 2018 SR1805 and FK180624 cruises than the 2012-13 TN278 and

NBP1305 cruises (Fig. 9B), a clue that the biogeochemical environment of the OMZ is subject to interannual variability. Observed differences in environmental variables like NO_2^- and percent anammox partitioning between 2012, 2013, and 2018 suggest that the partitioning of total N loss must depend on additional yet to be identified environmental or biological interactions.

4.5.3 Correlations of percent anammox values to anammox and denitrification rates - comparison to previous literature

In order to re-examine the result (Babbin et al., 2020) that enhanced fractions of anammox are correlated to greater anammox rates and not lower dentrification (Fig. 9D right), we created percent anammox vs. anammox and denitrification regressions with the 2018 SR1805 and FK180624 data. In 2018, unlike in 2012-13 (Babbin et al., 2020), we observed significant relationships between percent anammox values and both the anammox and denitrification rates (Fig. 9D left). Regressions for the 2012-13 data showed that increases in % anammox values are correlated only to increases in anammox values, not decreases in denitrification (Babbin et al., 2020) (Fig. 9D right). The 2018 regressions, on the other hand, indicate that increases in % anammox are correlated with both increasing anammox and decreasing denitrification rates. The influence of this difference in the 2018 samples can be seen in regressions of % anammox against anammox and denitrification from all three cruises where a similar pattern to the 2018 data is observed (Fig. 9C). As above, this indicates a clear difference in the partitioning of anammox and denitrification between the 2018 SR1805 and FK180624 ETNP cruises and the 2012-13 TN278 and NBP1305 cruises to the ETNP and ETSP. Despite the significance of the relationships, the low R^2 values indicate that these relationships do not explain most of the

variation in the anammox to denitrification ratio. As above, the causal mechanisms behind this variability remains to be elucidated.

4.5.4 Caveats about measurements of anammox and denitrification rates

One bias of our sampling scheme for N loss rates is that we do not capture particle adhering denitrifiers. Most denitrifiers that encode the last two steps of denitrification are found on large particles (Ganesh et al., 2013, 2015; Fuchsman et al., 2017). As a result, measurements of complete denitrification from $^{15}NO_2^-$ to $^{30}N_2$ that do not capture large particle communities will underestimate the rate. Unfortunately, due to the hydrodynamics of the CTD rosette it is unlikely that large particles will be trapped inside the Niskin bottle. In addition, the nipple of each Niskin is above the bottom of the bottle. As a result, the large particles that are successfully sampled by the CTD sink to the bottom of the Niskin and are not transferred into the experiment (Suter et al., 2017).

Another important caveat to some of the above conclusions in section 4.5 is that the detection limits for anammox and denitrification rates are not identical. It is easier to detect anammox for a variety of reasons. For example, anammox from a $^{15}NH_4^+$ tracer is more easily detected due to low background NH_4^+ across most of the OMZ. Anammox from the $^{15}NO_2^-$ tracer is more detectible due to its reliance on incorporation of only a single ^{15}N atom into the $^{29}N_2$ product. Denitrification, on the other hand, is more difficult to detect because of higher background NO_2^- concentrations and because definitive denitrification requires the rarer combination of two $^{15}NO_2^-$ molecules (Babbin et al., 2017).

We suspect that our sampling bias against particle based denitrification and denitrification's higher detection limit may have played a role in our observations of

944 denitrification rates in the 2012, 2013, and 2018 cruises where, for example, significant
945 denitrification rates were only detected at four of the thirty depths sampled during SR1805
946 (Supplementary Table S3). As a result, while the comparisons made above are helpful to
947 examine differences in N biogeochemistry across years and stations, the true biogeochemical
948 role of denitrification is likely greater than our tracer experiments suggest.
949 An additional important consideration is the possibility that anammox was stimulated by
950 our tracer additions, which substantially enriched the NO_2^- and especially the NH_4^+
951 concentrations above their in situ values (see Table S7 for enrichment factors for these two
952 nutrients' concentrations). As mentioned above, the differential control of anammox and
953 denitrification by substrate concentration may affect the observed ratio of the two rates in tracer
954 incubations. Tracer additions above ambient nutrient levels are necessary to detect a mass
955 spectrometric signal but often can result in rates above true in situ levels. Data on the kinetic
956 responses of anammox and denitrification are scarce, yet another area where further research
957 would be very useful.
958
959 **4.6 Possibility of N loss via AOA and other N cycling processes**
960 A recent paper (Kraft et al., 2022) reported that dense cultures of the ammonium
961 oxidizing archaea (AOA) *Nitrosopumilus maritimus* can support the O_2 dependent process of
962 NH_4^+ oxidation in deoxygenated waters via NO disproportionation to O_2 and N_2. This
963 mechanism would be a third N loss process that, if occuring in OMZs, would be measured as
964 anammox or denitrification. In order to investigate the possible significance of this N loss
965 pathway in ODZ waters, we calculated the maximum possible N loss from NH_4^+ oxidation – the
966 N loss that would result if all of the $^{15}N\text{-}NO_2^-$ produced in our NH_4^+ oxidation experiments was

converted into N_2 via the proposed NO disportionation reaction. These maximum NH_4^+ oxidation derived N loss rates were a small fraction of the total N loss rates at most depths (Supplementary Table S5). As a result, even these unrealistically high estimates of N_2 production from AOA do not suggest that AOA are significant agents for fixed N loss. The depths where this was not the case are all either oxic or upper oxycline depths where NH_4^+ oxidation rates peak and do not require NO disproportionation to supply O_2, or depths where equally low NH_4^+ oxidation, anammox, and denitrification rates would allow a higher percentage of the total N loss to be due to NH_4^+ oxidation. As a result, our calculation argues that N loss derived from NH_4^+ oxidation is not a significant N loss flux in ODZs. Thus, we argue that our conclusions regarding the relative balance of anammox and denitrification, as well as the relationship of these two N loss processes to other parts of the N cycle, do not need to be revised to account for N loss via NO disproportionation in AOA.

We note that an additional N recycling pathway, dissimilatory nitrate/nitrite reduction to ammonium (DNRA) can occur under low O_2 conditions similar to those preferred by anammox and denitrification. While some OMZ studies have found rates and *nrfA* abundances comparable to anammox, denitrification, and NH_4^+ oxidation rates and marker gene abundances (Lam et al., 2009; Jensen et al., 2011), DNRA is best described as an extremely variable process. Other past OMZ studies have often found negligible rates (De Brabandere et al., 2014; Kalvelage et al., 2013; Füssel et al., 2011) and little genetic evidence for DNRA (Kalvelage et al., 2013; Fuchsman et al., 2017). Due to this variability we chose to focus this study on what are arguably the most consistently relevant rates for OMZ N biogeochemistry.

5 Conclusions

Nitrogen is an essential component of life and as a result, its availability can function as a cap on biological productivity in many marine ecosystems. Since all the ocean is linked through an intricate web of currents that span the globe, the N biogeochemistry of small regions can affect the biogeochemistry of the rest of the ocean. Although OMZs account for just 0.1 - 1% of the ocean's total volume (Lam and Kuypers, 2011; Codispoti and Richards, 1976; Naqvi, 1987; Bange et al., 2000; Codispoti et al., 2005) they account for 30-50% of all total marine N loss (DeVries et al., 2013). As a result, developing an understanding of N cycling within OMZs is critical for comprehending the total marine N budget. Here we presented measurements from the ETNP OMZ of five microbial N cycling metabolisms, all of which have NO_2^- as a product, reactant, or intermediate. Understanding the magnitudes of these rates is key to determining the OMZ inventory of N species as well as an important piece of understanding the marine N budget.

Our results add to the growing evidence that the N recycling process of NO_3^- reduction is the largest OMZ N flux followed by the recycling process of NO_2^- oxidation back to NO_3^-. These two processes peaked in the oxycline or ODZ top and were usually much greater than the two N loss processes of anammox and denitrification, a departure from the established view that understanding N loss processes alone is the key to understanding OMZ biogeochemistry. We also add further evidence to the body of literature that supports the occurrence of anaerobic NO_2^- oxidation in OMZ regions, most strikingly through a series of O_2 manipulation experiments that show NO_2^- oxidation at putative O_2 concentrations as low as 1 nM. We conducted experiments on waters from two deoxygenated depths to evaluate if NO_2^- dismutation provides the oxidative power for observed anaerobic NO_2^- oxidation and found no evidence of NO_2^- dismutation except in one treatment – the closest to in situ NO_2^- conditions. Further exploration of the dismutation

hypothesis might therefore usefully focus on conditions near in situ NO_2^- concentrations. Across our experiments, the percent of N loss due to anammox was consistently above the theoretical prediction of at most 29% anammox. Our observations that NO_3^- reduction and NO_2^- oxidation greatly surpass N loss, especially in shallow boundary waters, further reinforce the view that NO_2^- in the SNM is sourced from NO_3^- reduction.

Together, these observations provide additional data that supports several new views of OMZ biogeochemistry. We hope that our work inspires additional isotopic experiments, culturing efforts, or genomic studies, especially those that seek to further test the occurrence of NO_2^- oxidation under functionally anoxic conditions and to examine alternative oxidants for this process. In addition, we emphasize the importance of integrating our experimental results into future OMZ N and C biogeochemical models, especially our results showing the predominance of NO_3^- reduction and NO_2^- oxidation over N loss. The development of an accurate model of OMZ N cycling is essential towards forecasting future changes in marine productivity and ecology as OMZs respond to climate change and other anthropogenic environmental changes.

Author contributions

XS, CF and BBW designed, and CF performed, measured, and calculated the NO_3^- reduction and NH_4^+ oxidation rates. BBW and JCT designed, BBW and JCT performed, and JCT measured and calculated the anammox and denitrification depth profile experiments. BBW and XS designed, JCT, BBW, and XS performed, XS and KD measured, and KD, EW, and JCT calculated the NO_2^- oxidation depth profiles. TT and ARB designed, TT performed, DEM and JCT measured, and EW and JCT calculated the anammox and denitrification profiles from the FK180624 cruise. TT and ARB designed, and TT performed, measured, and calculated the NO_2^-

oxidation O_2 variation experiments. ARB and TT designed, TT performed, EW, XS, and JCT measured, and EW and JCT calculated the dismutation experiments. SO provided critical help in running the mass spectrometer to measure all samples except the oxygen variation experiments. BBW performed the correlation and RDA analyses. JCT drafted the paper with inputs from all authors.

Competing Interests

The authors declare that they have no conflicts of interest.

Data Availability

All data discussed in this manuscript is archived at 10.5281/zenodo.7920778 .

Acknowledgments

We would like to acknowledge the crew and scientists of the R/V *Sally Ride* and the R/V *Falkor* for logistical and scientific support during our 2018 cruises. We thank Amal Jayakumar for providing *amoA* and *nirS* gene abundances for the RDA and PCA analyses. We thank Emilio Robledo-Garcia for assistance using the LUMOS sensor for the NO_2^- oxidation O_2 gradient experiments. We thank Matthias Spieler for supporting NO_3^- reduction rate measurements in Basel. We thank Patrick Boduch for his aid in reviewing graphics before submission. We also acknowledge the Schmidt Ocean Institute which provided R/V *Falkor* ship time to ARB. We thank the Simons Foundation and National Science Foundation for supporting ARB, TT, and DEM on Simons Foundation grant 622065 and NSF grants OCE-2138890 and OCE-2142998 as well as support for BBW, CF, JCT, and XS through NSF grant OCE-1657663.

References

Anderson, J. J., Okubo, A., Robbins, A. S., and Richards, F. A.: A model for nitrate distributions in oceanic oxygen minimum zones, Deep Sea Res. Part A. Oceanogr. Res. Pap., 29, 1113–1140, https://doi.org/10.1016/0198-0149(82)90031-0, 1982.

ASTM International: Standard Guide for Spiking into Aqueous Samples, West Conshohocken, PA, 2006.

Babbin, A. R., Keil, R. G., Devol, A. H., and Ward, B. B.: Organic matter stoichiometry, flux, and oxygen control nitrogen loss in the ocean, Science (80-.)., 344, 406–408, https://doi.org/10.1126/science.1248364, 2014.

Babbin, A. R., Peters, B. D., Mordy, C. W., Widner, B., Casciotti, K. L., and Ward, B. B.: Multiple metabolisms constrain the anaerobic nitrite budget in the Eastern Tropical South Pacific, Global Biogeochem. Cycles, 31, 258–271, https://doi.org/10.1002/2016GB005407, 2017.

Babbin, A. R., Buchwald, C., Morel, F. M. M., Wankel, S. D., and Ward, B. B.: Nitrite oxidation exceeds reduction and fixed nitrogen loss in anoxic Pacific waters, Mar. Chem., 224, 103814, https://doi.org/10.1016/J.MARCHEM.2020.103814, 2020.

Bange, H. W., Rixen, T., Johansen, A. M., Siefert, R. L., Ramesh, R., Ittekkot, V., Hoffmann, M. R., and Andreae, M. O.: A revised nitrogen budget of the Arabian Sea, Global Biogeochem. Cycles, 14, 1283–1297, https://doi.org/10.1029/1999GB001228, 2000.

Beman, J. M., Leilei Shih, J., and Popp, B. N.: Nitrite oxidation in the upper water column and oxygen minimum zone of the eastern tropical North Pacific Ocean, ISME J. 2013 711, 7, 2192–2205, https://doi.org/10.1038/ismej.2013.96, 2013.

Berg, J. S., Ahmerkamp, S., Pjevac, P., Hausmann, B., Milucka, J., and Kuypers, M. M. M.: How low can they go? Aerobic respiration by microorganisms under apparent anoxia, FEMS Microbiol. Rev., 46, 1–14, https://doi.org/10.1093/FEMSRE/FUAC006, 2022.

Bianchi, D., Babbin, A. R., and Galbraith, E. D.: Enhancement of anammox by the excretion of diel vertical migrators, Proc. Natl. Acad. Sci., 111, 15653–15658, https://doi.org/10.1073/PNAS.1410790111, 2014.

Bock, E., Koops, H. P., Möller, U. C., and Rudert, M.: A new facultatively nitrite oxidizing bacterium, Nitrobacter vulgaris sp. nov., Arch. Microbiol. 1990 1532, 153, 105–110, https://doi.org/10.1007/BF00247805, 1990.

De Brabandere, L., Canfield, D. E., Dalsgaard, T., Friederich, G. E., Revsbech, N. P., Ulloa, O., and Thamdrup, B.: Vertical partitioning of nitrogen-loss processes across the oxic-anoxic interface of an oceanic oxygen minimum zone, Environ. Microbiol., 16, 3041–3054, https://doi.org/10.1111/1462-2920.12255, 2014.

Braman, R. S. and Hendrix, S. A.: Nanogram Nitrite and Nitrate Determination in Environmental and Biological Materials by Vanadium(III) Reduction with Chemiluminescence Detection, Anal. Chem., 61, 2715–2718, 1989.

Brandhorst, W.: Nitrification and Denitrification in the Eastern Tropical North Pacific, J. du Cons. Cons. Perm. Int. pour l'exploration la mer, 25, 3–20, 1959.

Bristow, L. A., Dalsgaard, T., Tiano, L., Mills, D. B., Bertagnolli, A. D., Wright, J. J., Hallam, S. J., Ulloa, O., Canfield, D. E., Revsbech, N. P., and Thamdrup, B.: Ammonium and nitrite oxidation at nanomolar oxygen concentrations in oxygen minimum zone waters, Proc. Natl. Acad. Sci. U. S. A., 113, 10601–10606, https://doi.org/10.1073/PNAS.1600359113, 2016.

Bristow, L. A., Callbeck, C. M., Larsen, M., Altabet, M. A., Dekaezemacker, J., Forth, M.,

Gauns, M., Glud, R. N., Kuypers, M. M. M., Lavik, G., Milucka, J., Naqvi, S. W. A., Pratihary, A., Revsbech, N. P., Thamdrup, B., Treusch, A. H., and Canfield, D. E.: N2 production rates limited by nitrite availability in the Bay of Bengal oxygen minimum zone, Nat. Geosci., 10, 24–29, https://doi.org/10.1038/ngeo2847, 2017.

Buchwald, C. and Wankel, S. D.: Enzyme-catalyzed isotope equilibrium: A hypothesis to explain apparent N cycling phenomena in low oxygen environments, Mar. Chem., 244, 104140, https://doi.org/10.1016/J.MARCHEM.2022.104140, 2022.

Buchwald, C., Santoro, A. E., Stanley, R. H. R., and Casciotti, K. L.: Nitrogen cycling in the secondary nitrite maximum of the eastern tropical North Pacific off Costa Rica, Global Biogeochem. Cycles, 29, 2061–2081, https://doi.org/10.1002/2015GB005187, 2015.

Bulow, S. E., Rich, J. J., Naik, H. S., Pratihary, A. K., and Ward, B. B.: Denitrification exceeds anammox as a nitrogen loss pathway in the Arabian Sea oxygen minimum zone, Deep Sea Res. Part I Oceanogr. Res. Pap., 57, 384–393, https://doi.org/10.1016/J.DSR.2009.10.014, 2010.

Busecke, J. J. M., Resplandy, L., Ditkovsky, S. J., and John, J. G.: Diverging Fates of the Pacific Ocean Oxygen Minimum Zone and Its Core in a Warming World, AGU Adv., 3, e2021AV000470, https://doi.org/10.1029/2021AV000470, 2022.

Casciotti, K. L.: Inverse kinetic isotope fractionation during bacterial nitrite oxidation, Geochim. Cosmochim. Acta, 73, 2061–2076, https://doi.org/10.1016/J.GCA.2008.12.022, 2009.

Casciotti, K. L., Buchwald, C., and McIlvin, M.: Implications of nitrate and nitrite isotopic measurements for the mechanisms of nitrogen cycling in the Peru oxygen deficient zone, Deep Sea Res. Part I Oceanogr. Res. Pap., 80, 78–93, https://doi.org/10.1016/J.DSR.2013.05.017, 2013.

Codispoti, L. A. and Packard, T. T.: Denitrification rates in the eastern tropical South Pacific, J.

Mar. Res., 38, 453–477, 1980.

Codispoti, L. A. and Richards, F. A.: An analysis of the horizontal regime of denitrification in the eastern tropical North Pacific, Limnol. Oceanogr., 21, 379–388, https://doi.org/10.4319/LO.1976.21.3.0379, 1976.

Codispoti, L. A., Brandes, J. A., Christensen, J. P., Devol, A. H., Naqvi, S. W. A., Paerl, H. W., and Yoshinari, T.: The oceanic fixed nitrogen and nitrous oxide budgets: Moving targets as we enter the anthropocene?, Sci. Mar., 65, 85–105, https://doi.org/10.3989/SCIMAR.2001.65S285, 2001.

Codispoti, L. A., Yoshinari, T., and Devol, A. H.: Suboxic respiration in the oceanic water column, in: Respiration in Aquatic Ecosystems, edited by: del Giorgio, P. A. and Williams, P. J. L., Oxford University Press, 225–247, 2005.

Cram, J. A., Fuchsman, C. A., Duffy, M. E., Pretty, J. L., Lekanoff, R. M., Neibauer, J. A., Leung, S. W., Huebert, K. B., Weber, T. S., Bianchi, D., Evans, N., Devol, A. H., Keil, R. G., and McDonnell, A. M. P.: Slow Particle Remineralization, Rather Than Suppressed Disaggregation, Drives Efficient Flux Transfer Through the Eastern Tropical North Pacific Oxygen Deficient Zone, Global Biogeochem. Cycles, 36, e2021GB007080, https://doi.org/10.1029/2021GB007080, 2022.

Dalsgaard, T., Canfield, D. E., Peterson, J., Thamdrup, B., and Acuna-Gonzales, J.: N production by anamox in the anoxic water column of Golfo Dulce, Costa Rica., Nature, 422, 606–608, https://doi.org/10.1038/nature01526, 2003.

Dalsgaard, T., Thamdrup, B., Farías, L., and Revsbech, N. P.: Anammox and denitrification in the oxygen minimum zone of the eastern South Pacific, Limnol. Oceanogr., 57, 1331–1346, https://doi.org/10.4319/lo.2012.57.5.1331, 2012.

DeVries, T., Deutsch, C., Rafter, P. A., and Primeau, F.: Marine denitrification rates determined from a global 3-D inverse model, Biogeosciences, 10, 2481–2496, https://doi.org/10.5194/BG-10-2481-2013, 2013.

Freitag, A., Rudert, M., and Bock, E.: Growth of Nitrobacter by dissimilatoric nitrate reduction, FEMS Microbiol. Lett., 48, 105–109, https://doi.org/10.1111/J.1574-6968.1987.TB02524.X, 1987.

Frey, C., Bange, H. W., Achterberg, E. P., Jayakumar, A., Löscher, C. R., Arévalo-Martínez, D. L., León-Palmero, E., Sun, M., Sun, X., Xie, R. C., Oleynik, S., and Ward, B. B.: Regulation of nitrous oxide production in low-oxygen waters off the coast of Peru, Biogeosciences, 17, 2263–2287, https://doi.org/10.5194/BG-17-2263-2020, 2020.

Frey, C., Sun, X., Szemberski, L., Casciotti, K. L., Garcia-Robledo, E., Jayakumar, A., Kelly, C. L., and Lehmann, M. F.: Nitrous oxide production kinetics from ammonia oxidation in the Eastern Tropical North Pacific, Limnol. Oceanogr., Under review, 2022.

Fuchsman, C. A., Devol, A. H., Saunders, J. K., McKay, C., and Rocap, G.: Niche partitioning of the N cycling microbial community of an offshore oxygen deficient zone, Front. Microbiol., 8, 2384, https://doi.org/10.3389/FMICB.2017.02384/BIBTEX, 2017.

Fuchsman, C. A., Palevsky, H. I., Widner, B., Duffy, M., Carlson, M. C. G., Neibauer, J. A., Mulholland, M. R., Keil, R. G., Devol, A. H., and Rocap, G.: Cyanobacteria and cyanophage contributions to carbon and nitrogen cycling in an oligotrophic oxygen-deficient zone, ISME J. 2019 1311, 13, 2714–2726, https://doi.org/10.1038/s41396-019-0452-6, 2019.

Füssel, J., Lam, P., Lavik, G., Jensen, M. M., Holtappels, M., Günter, M., and Kuypers, M. M. M.: Nitrite oxidation in the Namibian oxygen minimum zone, ISME J., 6, 1200–1209, https://doi.org/10.1038/ismej.2011.178, 2011.

Füssel, J., Lücker, S., Yilmaz, P., Nowka, B., van Kessel, M. A. H. J., Bourceau, P., Hach, P. F., Littmann, S., Berg, J., Spieck, E., Daims, H., Kuypers, M. M. M., and Lam, P.: Adaptability as the key to success for the ubiquitous marine nitrite oxidizer Nitrococcus, Sci. Adv., 3, https://doi.org/10.1126/SCIADV.1700807/SUPPL_FILE/1700807_TABLES1_TO_S10.ZIP, 2017.

Ganesh, S., Parris, D. J., Delong, E. F., and Stewart, F. J.: Metagenomic analysis of size-fractionated picoplankton in a marine oxygen minimum zone, ISME J. 2014 81, 8, 187–211, https://doi.org/10.1038/ismej.2013.144, 2013.

Ganesh, S., Bristow, L. A., Larsen, M., Sarode, N., Thamdrup, B., and Stewart, F. J.: Size-fraction partitioning of community gene transcription and nitrogen metabolism in a marine oxygen minimum zone, ISME J. 2015 912, 9, 2682–2696, https://doi.org/10.1038/ismej.2015.44, 2015.

Garcia-Robledo, E., Borisov, S., Klimant, I., and Revsbech, N. P.: Determination of respiration rates in water with sub-micromolar oxygen concentrations, Front. Mar. Sci., 3, 244, https://doi.org/10.3389/FMARS.2016.00244/BIBTEX, 2016.

Garcia-Robledo, E., Padilla, C. C., Aldunate, M., Stewart, F. J., Ulloa, O., Paulmier, A., Gregori, G., and Revsbech, N. P.: Cryptic oxygen cycling in anoxic marine zones, Proc. Natl. Acad. Sci. U. S. A., 114, 8319–8324, https://doi.org/10.1073/PNAS.1619844114, 2017.

Garcia-Robledo, E., Paulmier, A., Borisov, S. M., and Revsbech, N. P.: Sampling in low oxygen aquatic environments: The deviation from anoxic conditions, Limnol. Oceanogr. Methods, 19, 733–740, https://doi.org/10.1002/LOM3.10457, 2021.

Granger, J., & Sigman, D. M.: Removal of nitrite with sulfamic acid for nitrate N and O isotope analysis with the denitrifier method, Rapid Commun. Mass Spectrom, 23, 3753-3762., Rapid

Commun. Mass Spectrom., 23, 3753–3762, https://doi.org/10.1002/rcm, 2009.

Granger, J. and Wankel, S. D.: Isotopic overprinting of nitrification on denitrification as a ubiquitous and unifying feature of environmental nitrogen cycling, Proc. Natl. Acad. Sci. U. S. A., 113, E6391–E6400, https://doi.org/10.1073/PNAS.1601383113/SUPPL_FILE/PNAS.201601383SI.PDF, 2016.

Hamersley, M. R., Lavik, G., Woebken, D., Rattray, J. E., Lam, P., Hopmans, E. C., Sinninghe Damsté, J. S., Krüger, S., Graco, M., Gutiérrez, D., and Kuypers, M. M. M.: Anaerobic ammonium oxidation in the Peruvian oxygen minimum zone, Limnol. Oceanogr., 52, 923–933, https://doi.org/10.4319/LO.2007.52.3.0923, 2007.

Holmes, R. M., Aminot, A., Kérouel, R., Hooker, B. A., and Peterson, B. J.: A simple and precise method for measuring ammonium in marine and freshwater ecosystems, Can. J. Fish. Aquat. Sci., 56, 1801–1808, https://doi.org/10.1139/f99-128, 1999.

Horak, R. E. A., Ruef, W., Ward, B. B., and Devol, A. H.: Expansion of denitrification and anoxia in the eastern tropical North Pacific from 1972 to 2012, Geophys. Res. Lett., 43, 5252–5260, https://doi.org/10.1002/2016GL068871, 2016.

Ito, T., Minobe, S., Long, M. C., and Deutsch, C.: Upper ocean O2 trends: 1958–2015, Geophys. Res. Lett., 44, 4214–4223, https://doi.org/10.1002/2017GL073613, 2017.

Jayakumar, A., Wajih, S., Naqvi, A., and Ward, B. B.: Distribution and Relative Quantification of Key Genes Involved in Fixed Nitrogen Loss From the Arabian Sea Oxygen Minimum Zone, Indian Ocean Biogeochem. Process. Ecol. Var. Geophys. Monogr. Ser., 185, https://doi.org/10.1029/2008GM000730, 2009.

Jensen, M. M., Lam, P., Revsbech, N. P., Nagel, B., Gaye, B., Jetten, M. S. M., and Kuypers, M. M. M.: Intensive nitrogen loss over the Omani Shelf due to anammox coupled with dissimilatory

nitrite reduction to ammonium, ISME J. 2011 510, 5, 1660–1670, https://doi.org/10.1038/ismej.2011.44, 2011.

Kalvelage, T., Lavik, G., Lam, P., Contreras, S., Arteaga, L., Löscher, C. R., Oschlies, A., Paulmier, A., Stramma, L., and M Kuypers, M. M.: Nitrogen cycling driven by organic matter export in the South Pacific oxygen minimum zone, 55812, https://doi.org/10.1038/NGEO1739, 2013.

Keeling, R. F., Körtzinger, A., and Gruber, N.: Ocean deoxygenation in a warming world, Ann. Rev. Mar. Sci., 2, 199–229, https://doi.org/10.1146/annurev.marine.010908.163855, 2010.

Kemeny, P. C., Weigand, M. A., Zhang, R., Carter, B. R., Karsh, K. L., Fawcett, S. E., and Sigman, D. M.: Enzyme-level interconversion of nitrate and nitrite in the fall mixed layer of the Antarctic Ocean, Global Biogeochem. Cycles, 30, 1069–1085, https://doi.org/10.1002/2015GB005350, 2016.

Kirstein, K. and Bock, E.: Close genetic relationship between Nitrobacter hamburgensis nitrite oxidoreductase and Escherichia coli nitrate reductases, Arch. Microbiol. 1993 1606, 160, 447–453, https://doi.org/10.1007/BF00245305, 1993.

Koch, H., Lücker, S., Albertsen, M., Kitzinger, K., Herbold, C., Spieck, E., Nielsen, P. H., Wagner, M., and Daims, H.: Expanded metabolic versatility of ubiquitous nitrite-oxidizing bacteria from the genus Nitrospira, Proc. Natl. Acad. Sci. U. S. A., 112, 11371–11376, https://doi.org/10.1073/PNAS.1506533112, 2015.

Kondo, Y. and Moffett, J. W.: Iron redox cycling and subsurface offshore transport in the eastern tropical South Pacific oxygen minimum zone, Mar. Chem., 168, 95–103, https://doi.org/10.1016/J.MARCHEM.2014.11.007, 2015.

Kraft, B., Jehmlich, N., Larsen, M., Bristow, L. A., Könneke, M., Thamdrup, B., and Canfield,

D. E.: Oxygen and nitrogen production by an ammonia-oxidizing archaeon, Science (80-.)., 375, 97–100, https://doi.org/10.1126/SCIENCE.ABE6733/SUPPL_FILE/SCIENCE.ABE6733_MDAR_REPRODUCIBILITY_CHECKLIST.PDF, 2022.

Kuenen, J. G.: Anammox bacteria from discovery, 6, https://doi.org/10.1038/nrmicro1857, 2008.

Kuypers, M. M. M., Lavik, G., Woebken, D., Schmid, M., Fuchs, B. M., Amann, R., Jørgensen, B. B., and Jetten, M. S. M.: Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation, Proc. Natl. Acad. Sci. U. S. A., 102, 6478–6483, https://doi.org/10.1073/PNAS.0502088102, 2005.

Lam, P. and Kuypers, M. M. M.: Microbial Nitrogen Cycling Processes in Oxygen Minimum Zones, Ann. Rev. Mar. Sci., 3, 317–345, https://doi.org/10.1146/annurev-marine-120709-142814, 2011.

Lam, P., Lavik, G., Jensen, M. M., Van Vossenberg, J. De, Schmid, M., Woebken, D., Gutiérrez, D., Amann, R., Jetten, M. S. M., and Kuypers, M. M. M.: Revising the nitrogen cycle in the Peruvian oxygen minimum zone, Proc. Natl. Acad. Sci. U. S. A., 106, 4752–4757, https://doi.org/10.1073/PNAS.0812444106, 2009.

Lam, P., Jensen, M. M., Kock, A., Lettmann, K. A., Plancherel, Y., Lavik, G., Bange, H. W., and Kuypers, M. M. M.: Origin and fate of the secondary nitrite maximum in the Arabian Sea, Biogeosciences, 8, 1565–1577, https://doi.org/10.5194/BG-8-1565-2011, 2011.

Van de Leemput, I. A., Veraart, A. J., Dakos, V., De Klein, J. J. M., Strous, M., and Scheffer, M.: Predicting microbial nitrogen pathways from basic principles, Environ. Microbiol., 13, 1477–1487, https://doi.org/10.1111/J.1462-2920.2011.02450.X, 2011.

Lehner, P., Larndorfer, C., Garcia-Robledo, E., Larsen, M., Borisov, S. M., Revsbech, N. P.,

Glud, R. N., Canfield, D. E., and Klimant, I.: LUMOS - A Sensitive and Reliable Optode System for Measuring Dissolved Oxygen in the Nanomolar Range, PLoS One, 10, e0128125, https://doi.org/10.1371/JOURNAL.PONE.0128125, 2015.

Lipschultz, F., Wofsy, S. C., Ward, B. B., Codispoti, L. A., Friedrich, G., and Elkins, J. W.: Bacterial transformations of inorganic nitrogen in the oxygen-deficient waters of the Eastern Tropical South Pacific Ocean, Deep Sea Res. Part A. Oceanogr. Res. Pap., 37, 1513–1541, https://doi.org/10.1016/0198-0149(90)90060-9, 1990.

Lomas, M. W. and Lipschultz, F.: Forming the primary nitrite maximum: Nitrifiers or phytoplankton?, Limnol. Oceanogr., 51, 2453–2467, https://doi.org/10.4319/LO.2006.51.5.2453, 2006.

Luther, G. W.: The role of one- and two-electron transfer reactions in forming thermodynamically unstable intermediates as barriers in multi-electron redox reactions, Aquat. Geochemistry, 16, 395–420, https://doi.org/10.1007/S10498-009-9082-3/FIGURES/14, 2010.

Luther, G. W. and Popp, J. I.: Kinetics of the Abiotic Reduction of Polymeric Manganese Dioxide by Nitrite: An Anaerobic Nitrification Reaction, Aquat. Geochemistry 2002 81, 8, 15–36, https://doi.org/10.1023/A:1020325604920, 2002.

Margolskee, A., Frenzel, H., Emerson, S., and Deutsch, C.: Ventilation Pathways for the North Pacific Oxygen Deficient Zone, Global Biogeochem. Cycles, 33, 875–890, https://doi.org/10.1029/2018GB006149, 2019.

Martin, J. H., Knauer, G. A., Karl, D. M., and Broenkow, W. W.: VERTEX: carbon cycling in the northeast Pacific, Deep Sea Res. Part A. Oceanogr. Res. Pap., 34, 267–285, https://doi.org/10.1016/0198-0149(87)90086-0, 1987.

McIlvin, M. R. and Altabet, M. A.: Chemical conversion of nitrate and nitrite to nitrous oxide for

1289 nitrogen and oxygen isotopic analysis in freshwater and seawater, Anal. Chem., 77, 5589–5595,
1290 https://doi.org/10.1021/ac050528s, 2005.
1291 Mincer, T. J., Church, M. J., Taylor, L. T., Preston, C., Karl, D. M., and DeLong, E. F.:
1292 Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the
1293 North Pacific Subtropical Gyre, Environ. Microbiol., 9, 1162–1175,
1294 https://doi.org/10.1111/J.1462-2920.2007.01239.X, 2007.
1295 Moriyasu, R., Evans, N., Bolster, K. M., Hardisty, D. S., and Moffett, J. W.: The Distribution
1296 and Redox Speciation of Iodine in the Eastern Tropical North Pacific Ocean, Global
1297 Biogeochem. Cycles, 34, e2019GB006302, https://doi.org/10.1029/2019GB006302, 2020.
1298 Naqvi, S. W. A.: Some aspects of the oxygen-deficient conditions and denitrification in the
1299 Arabian Sea, J. Mar. Res., 45, 1049–1072, 1987.
1300 Nozaki, Y.: A fresh look at element distribution in the North Pacific Ocean, Eos, Trans. Am.
1301 Geophys. Union, 78, 221–221, https://doi.org/10.1029/97EO00148, 1997.
1302 Oshiki, M., Satoh, H., and Okabe, S.: Ecology and physiology of anaerobic ammonium oxidizing
1303 bacteria, Environ. Microbiol., 18, 2784–2796, https://doi.org/10.1111/1462-
1304 2920.13134/SUPPINFO, 2016.
1305 Padilla, C. C., Bristow, L. A., Sarode, N., Garcia-Robledo, E., Gómez Ramírez, E., Benson, C.
1306 R., Bourbonnais, A., Altabet, M. A., Girguis, P. R., Thamdrup, B., and Stewart, F. J.: NC10
1307 bacteria in marine oxygen minimum zones, ISME J. 2016 108, 10, 2067–2071,
1308 https://doi.org/10.1038/ismej.2015.262, 2016.
1309 Peng, X., Fuchsman, C. A., Jayakumar, A., Oleynik, S., Martens-Habbena, W., Devol, A. H., and
1310 Ward, B. B.: Ammonia and nitrite oxidation in the Eastern Tropical North Pacific, Global
1311 Biogeochem. Cycles, 29, 2034–2049, https://doi.org/10.1002/2015GB005278, 2015.

Peng, X., Fuchsman, C. A., Jayakumar, A., Warner, M. J., Devol, A. H., and Ward, B. B.: Revisiting nitrification in the Eastern Tropical South Pacific: A focus on controls, J. Geophys. Res. Ocean., 121, 1667–1684, https://doi.org/10.1002/2015JC011455, 2016.

Penn, J., Weber, T., and Deutsch, C.: Microbial functional diversity alters the structure and sensitivity of oxygen deficient zones, Geophys. Res. Lett., 43, 9773–9780, https://doi.org/10.1002/2016GL070438, 2016.

Peters, B. D., Babbin, A. R., Lettmann, K. A., Mordy, C. W., Ulloa, O., Ward, B. B., and Casciotti, K. L.: Vertical modeling of the nitrogen cycle in the eastern tropical South Pacific oxygen deficient zone using high-resolution concentration and isotope measurements, Global Biogeochem. Cycles, 30, 1661–1681, https://doi.org/10.1002/2016GB005415, 2016.

R: A language and environment for statistical computing:

Starkenburg, S. R., Larimer, F. W., Stein, L. Y., Klotz, M. G., Chain, P. S. G., Sayavedra-Soto, L. A., Poret-Peterson, A. T., Gentry, M. E., Arp, D. J., Ward, B., and Bottomley, P. J.: Complete genome sequence of Nitrobacter hamburgensis X14 and comparative genomic analysis of species within the genus Nitrobacter, Appl. Environ. Microbiol., 74, 2852–2863, https://doi.org/10.1128/AEM.02311-07/SUPPL_FILE/SUPPLEMENTAL_TABLE_2.PDF, 2008.

Stramma, L., Johnson, G. C., Sprintall, J., and Mohrholz, V.: Expanding oxygen-minimum zones in the tropical oceans, Science (80-.)., 320, 655–658, https://doi.org/10.1126/SCIENCE.1153847/ASSET/09F7EF41-9228-40BD-8E17-06C4E9218CC5/ASSETS/GRAPHIC/320_655_F2.JPEG, 2008.

Strickland, J. D. H. and Parsons, T. R.: A Practical Handbook of Seawater Analysis, Fisheries Research Board of Canada, Ottawa, 1972.

Strohm, T. O., Griffin, B., Zumft, W. G., and Schink, B.: Growth yields in bacterial denitrification and nitrate ammonification, Appl. Environ. Microbiol., 73, 1420–1424, https://doi.org/10.1128/AEM.02508-06/ASSET/88AA1997-7BB3-42B6-A761-DCB47D972CA6/ASSETS/GRAPHIC/ZAM0050775730001.JPEG, 2007.

Strous, M., Heijnen, J. J., Kuenen, J. G., and Jetten, M. S. M.: The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms, Appl. Microbiol. Biotechnol. 1998 505, 50, 589–596, https://doi.org/10.1007/S002530051340, 1998.

Sun, X. and Ward, B. B.: Novel metagenome-assembled genomes involved in the nitrogen cycle from a Pacific oxygen minimum zone, ISME Commun. 2021 11, 1, 1–5, https://doi.org/10.1038/s43705-021-00030-2, 2021.

Sun, X., Ji, Q., Jayakumar, A., and Ward, B. B.: Dependence of nitrite oxidation on nitrite and oxygen in low-oxygen seawater, Geophys. Res. Lett., 44, 7883–7891, https://doi.org/10.1002/2017GL074355, 2017.

Sun, X., Kop, L. F. M., Lau, M. C. Y., Frank, J., Jayakumar, A., Lücker, S., and Ward, B. B.: Uncultured Nitrospina-like species are major nitrite oxidizing bacteria in oxygen minimum zones, ISME J., https://doi.org/10.1038/s41396-019-0443-7, 2019.

Sun, X., Frey, C., Garcia-Robledo, E., Jayakumar, A., and Ward, B. B.: Microbial niche differentiation explains nitrite oxidation in marine oxygen minimum zones, ISME J. 2021 155, 15, 1317–1329, https://doi.org/10.1038/s41396-020-00852-3, 2021.

Sun, X., Frey, C., and Ward, B. B.: Nitrite Oxidation Across the Full Oxygen Spectrum in the Ocean, Global Biogeochem. Cycles, 37, e2022GB007548, https://doi.org/10.1029/2022GB007548, 2023.

Suter, E. A., Scranton, M. I., Chow, S., Stinton, D., Medina Faull, L., and Taylor, G. T.: Niskin bottle sample collection aliases microbial community composition and biogeochemical interpretation, Limnol. Oceanogr., 62, 606–617, https://doi.org/10.1002/LNO.10447, 2017.

Tang, W., Tracey, J. C., Carroll, J., Wallace, E., Lee, J. A., Nathan, L., Sun, X., Jayakumar, A., and Ward, B. B.: Nitrous oxide production in the Chesapeake Bay, Limnol. Oceanogr., 67, 2101–2116, https://doi.org/10.1002/LNO.12191, 2022.

Taylor, B. W., Keep, C. F., Hall, R. O., Koch, B. J., Tronstad, L. M., Flecker, A. S., and Ulseth, A. J.: Improving the fluorometric ammonium method: matrix effects, background fluorescence, and standard additions, Am. Benthol. Soc, 26, 167–177, https://doi.org/10.1899/0887-3593, 2007.

Thamdrup, B. and Dalsgaard, T.: The fate of ammonium in anoxic manganese oxide-rich marine sediment, Geochim. Cosmochim. Acta, 64, 4157–4164, https://doi.org/10.1016/S0016-7037(00)00496-8, 2000.

Thamdrup, B. and Dalsgaard, T.: Production of N2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments, Appl. Environ. Microbiol., 68, 1312–1318, https://doi.org/10.1128/AEM.68.3.1312-1318.2002/ASSET/F1579424-64C0-464C-AF9C-5BEB3458C869/ASSETS/GRAPHIC/AM0321630005.JPEG, 2002.

Thamdrup, B., Dalsgaard, T., Jensen, M. M., Ulloa, O., Farías, L., and Escribano, R.: Anaerobic ammonium oxidation in the oxygen-deficient waters off northern Chile, Limnol. Oceanogr., 51, 2145–2156, https://doi.org/10.4319/LO.2006.51.5.2145, 2006.

Tiano, L., Garcia-Robledo, E., Dalsgaard, T., Devol, A. H., Ward, B. B., Ulloa, O., Canfield, D. E., and Peter Revsbech, N.: Oxygen distribution and aerobic respiration in the north and south eastern tropical Pacific oxygen minimum zones, Deep Sea Res. Part I Oceanogr. Res. Pap., 94,

173–183, https://doi.org/10.1016/J.DSR.2014.10.001, 2014.

Tsementzi, D., Wu, J., Deutsch, S., Nath, S., Rodriguez-R, L. M., Burns, A. S., Ranjan, P., Sarode, N., Malmstrom, R. R., Padilla, C. C., Stone, B. K., Bristow, L. A., Larsen, M., Glass, J. B., Thamdrup, B., Woyke, T., Konstantinidis, K. T., and Stewart, F. J.: SAR11 bacteria linked to ocean anoxia and nitrogen loss, Nature, 536, 179–183, https://doi.org/10.1038/nature19068, 2016.

Ulloa, O., Canfield, D. E., DeLong, E. F., Letelier, R. M., and Stewart, F. J.: Microbial oceanography of anoxic oxygen minimum zones, Proc. Natl. Acad. Sci. U. S. A., 109, 15996–16003, https://doi.org/10.1073/PNAS.1205009109, 2012.

Vedamati, J., Chan, C., and Moffett, J. W.: Distribution of dissolved manganese in the Peruvian Upwelling and Oxygen Minimum Zone, Geochim. Cosmochim. Acta, 156, 222–240, https://doi.org/10.1016/J.GCA.2014.10.026, 2015.

Wanninkhof, R.: Relationship between wind speed and gas exchange over the ocean, J. Geophys. Res. Ocean., 97, 7373–7382, https://doi.org/10.1029/92JC00188, 1992.

Ward, B. B., Glover, H. E., and Lipschultz, F.: Chemoautotrophic activity and nitrification in the oxygen minimum zone off Peru, Deep Sea Res. Part A. Oceanogr. Res. Pap., 36, 1031–1051, https://doi.org/10.1016/0198-0149(89)90076-9, 1989.

Ward, B. B., Tuit, C. B., Jayakumar, A., Rich, J. J., Moffett, J., and Naqvi, S. W. A.: Organic carbon, and not copper, controls denitrification in oxygen minimum zones of the ocean, Deep Sea Res. Part I Oceanogr. Res. Pap., 55, 1672–1683, https://doi.org/10.1016/J.DSR.2008.07.005, 2008.

Ward, B. B., Devol, A. H., Rich, J. J., Chang, B. X., Bulow, S. E., Naik, H., Pratihary, A., and Jayakumar, A.: Denitrification as the dominant nitrogen loss process in the Arabian Sea, Nat.

2009 4617260, 461, 78–81, https://doi.org/10.1038/nature08276, 2009.

Weigand, M. A., Foriel, J., Barnett, B., Oleynik, S., and Sigman, D. M.: Updates to instrumentation and protocols for isotopic analysis of nitrate by the denitrifier method, Rapid Commun. Mass Spectrom., 30, 1365–1383, https://doi.org/10.1002/rcm.7570, 2016.

Wunderlich, A., Meckenstock, R. U., and Einsiedl, F.: A mixture of nitrite-oxidizing and denitrifying microorganisms affects the δ18O of dissolved nitrate during anaerobic microbial denitrification depending on the δ18O of ambient water, Geochim. Cosmochim. Acta, 119, 31–45, https://doi.org/10.1016/J.GCA.2013.05.028, 2013.