



## Oxygen sensitivity of anammox and coupled N-cycle processes in oxygen minimum zones

Kalvelage, Tim; Jensen, Marlene Mark; Contreras, Sergio; Revsbech, Niels Peter; Lam, Phyllis; Günter, Marcel; LaRoche, Julie; Lavik, Gaute; Kuypers, Marcel M. M.

*Published in:*  
P L o S One

*Link to article, DOI:*  
[10.1371/journal.pone.0029299](https://doi.org/10.1371/journal.pone.0029299)

*Publication date:*  
2011

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Kalvelage, T., Jensen, M. M., Contreras, S., Revsbech, N. P., Lam, P., Günter, M., LaRoche, J., Lavik, G., & Kuypers, M. M. M. (2011). Oxygen sensitivity of anammox and coupled N-cycle processes in oxygen minimum zones. *P L o S One*, 6(12), e29299. <https://doi.org/10.1371/journal.pone.0029299>

---

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

# Oxygen Sensitivity of Anammox and Coupled N-Cycle Processes in Oxygen Minimum Zones

Tim Kalvelage<sup>1\*</sup>, Marlene M. Jensen<sup>1a</sup>, Sergio Contreras<sup>1ab</sup>, Niels Peter Revsbech<sup>2</sup>, Phyllis Lam<sup>1</sup>, Marcel Günter<sup>1</sup>, Julie LaRoche<sup>3</sup>, Gaute Lavik<sup>1</sup>, Marcel M. M. Kuypers<sup>1</sup>

**1** Department of Biogeochemistry, Max Planck Institute for Marine Microbiology, Bremen, Germany, **2** Department of Biological Sciences, University of Aarhus, Aarhus C, Denmark, **3** Department of Marine Biogeochemistry, Leibniz Institute of Marine Sciences (IFM-GEOMAR), Kiel, Germany

## Abstract

Nutrient measurements indicate that 30–50% of the total nitrogen (N) loss in the ocean occurs in oxygen minimum zones (OMZs). This pelagic N-removal takes place within only ~0.1% of the ocean volume, hence moderate variations in the extent of OMZs due to global warming may have a large impact on the global N-cycle. We examined the effect of oxygen (O<sub>2</sub>) on anammox, NH<sub>3</sub> oxidation and NO<sub>3</sub><sup>-</sup> reduction in <sup>15</sup>N-labeling experiments with varying O<sub>2</sub> concentrations (0–25 μmol L<sup>-1</sup>) in the Namibian and Peruvian OMZs. Our results show that O<sub>2</sub> is a major controlling factor for anammox activity in OMZ waters. Based on our O<sub>2</sub> assays we estimate the upper limit for anammox to be ~20 μmol L<sup>-1</sup>. In contrast, NH<sub>3</sub> oxidation to NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> reduction to NO<sub>2</sub><sup>-</sup> as the main NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup> sources for anammox were only moderately affected by changing O<sub>2</sub> concentrations. Intriguingly, aerobic NH<sub>3</sub> oxidation was active at non-detectable concentrations of O<sub>2</sub>, while anaerobic NO<sub>3</sub><sup>-</sup> reduction was fully active up to at least 25 μmol L<sup>-1</sup> O<sub>2</sub>. Hence, aerobic and anaerobic N-cycle pathways in OMZs can co-occur over a larger range of O<sub>2</sub> concentrations than previously assumed. The zone where N-loss can occur is primarily controlled by the O<sub>2</sub>-sensitivity of anammox itself, and not by any effects of O<sub>2</sub> on the tightly coupled pathways of aerobic NH<sub>3</sub> oxidation and NO<sub>3</sub><sup>-</sup> reduction. With anammox bacteria in the marine environment being active at O<sub>2</sub> levels ~20 times higher than those known to inhibit their cultured counterparts, the oceanic volume potentially acting as a N-sink increases tenfold. The predicted expansion of OMZs may enlarge this volume even further. Our study provides the first robust estimates of O<sub>2</sub> sensitivities for processes directly and indirectly connected with N-loss. These are essential to assess the effects of ocean de-oxygenation on oceanic N-cycling.

**Citation:** Kalvelage T, Jensen MM, Contreras S, Revsbech NP, Lam P, et al. (2011) Oxygen Sensitivity of Anammox and Coupled N-Cycle Processes in Oxygen Minimum Zones. PLoS ONE 6(12): e29299. doi:10.1371/journal.pone.0029299

**Editor:** Jack Anthony Gilbert, Argonne National Laboratory, United States of America

**Received:** August 31, 2011; **Accepted:** November 23, 2011; **Published:** December 28, 2011

**Copyright:** © 2011 Kalvelage et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** The study was funded by the Max-Planck-Gesellschaft (www.mpg.de), Deutsche Forschungsgemeinschaft (DFG) (www.dfg.de), the DFG-funded Sonderforschungsbereich 754 "Climate-Biogeochemistry Interactions in the Tropical Ocean" (www.sfb754.de), the European Research Council, grant 267233 (www.erc.europa.eu) and the Danish Council for Independent Research: Natural Sciences, grant 10-083140 (http://en.fi.dk/councils-commissions/the-danish-council-for-independent-research). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

\* E-mail: tkalvela@mpi-bremen.de

<sup>a</sup> Current address: Department of Environmental Engineering, Technical University of Denmark, Kongens Lyngby, Denmark

<sup>b</sup> Current address: Large Lakes Observatory, University of Minnesota Duluth, Duluth, Minnesota, United States of America

## Introduction

Oxygen (O<sub>2</sub>) is one of the key regulatory factors of major biogeochemical cycles in the marine environment [1]. The distribution of dissolved O<sub>2</sub> in the world's oceans is regulated by gas exchange between surface waters and the lower atmosphere, advective processes within the ocean, as well as the biological processes of photosynthesis and respiration. Oxygen, entering the ocean interior mainly at high latitudes, is distributed throughout the global ocean via thermohaline circulation. In the ocean's sunlit surface layer, phytoplankton produces O<sub>2</sub> and fixes carbon dioxide (CO<sub>2</sub>) in to biomass. Near the base of the euphotic zone, concentrations of O<sub>2</sub> are generally at their lowest as photosynthesis diminishes or ceases altogether while the respiration of sinking organic matter by heterotrophic micro-organisms consumes O<sub>2</sub> at maximal rates.

Subsurface regions of severely reduced O<sub>2</sub> concentrations (O<sub>2</sub> ≤ 5 μmol L<sup>-1</sup>), the so-called oxygen minimum zones (OMZs), are found along the eastern boundaries of the ocean basins in the

subtropics and tropics (e.g. off California, Namibia, Peru/Chile) and in the Arabian Sea. Typically in these regions, wind-driven circulation results in the upwelling of nutrient-rich deep waters, fueling high primary production in the euphotic zone. The high surface productivity results in high export of organic matter and thus strong respiration in subsurface waters. Combined with the poor ventilation of these water masses [2,3], this leads to permanently O<sub>2</sub>-depleted to anoxic conditions at mid-depths [4–6].

Although OMZs (if defined by O<sub>2</sub> ≤ 5 μmol L<sup>-1</sup>) account for only ~0.1% of the global ocean volume [7], they play a key role in controlling the oceans' nutrient inventory as 30–50% of the oceanic nitrogen (N) loss is estimated to occur therein [7,8]. The recharge of such N-deficient waters from these regions back to adjacent surface waters limits primary production and thus carbon (C) sequestration in large parts of the tropical oceans. N-loss as primarily the formation of gaseous dinitrogen (N<sub>2</sub>) can occur via two pathways: (1) heterotrophic denitrification, the reduction of nitrate (NO<sub>3</sub><sup>-</sup>) to gaseous dinitrogen (N<sub>2</sub>) via a sequence of

intermediates ( $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$ ) and (2) anammox, the anaerobic oxidation of ammonium ( $\text{NH}_4^+$ ) with nitrite ( $\text{NO}_2^-$ ) to  $\text{N}_2$ . In the OMZs of Namibia and Peru/Chile, on which the current study focuses, anammox has been identified as the major N-loss pathway based on <sup>15</sup>N-labeling experiments, whereas heterotrophic denitrification was often not detectable or only measured sporadically [9–11].

In the course of global climate change and increasing anthropogenic pressures on the marine environment, coastal and open ocean OMZs have been expanding and intensifying in the last decades [12,13]. A continuing decline in dissolved O<sub>2</sub> due to reduced O<sub>2</sub> solubility and enhanced stratification [14], as well as coastal and open ocean eutrophication [15,16], is expected. Deoxygenation will have the greatest effect on water masses already deficient in O<sub>2</sub> as these are often at or near the thresholds for anaerobic processes such as anammox or denitrification. Deutsch et al. [17] calculated that a reduction of the mean upper ocean O<sub>2</sub> content by only 1% would mean a doubling of water masses with  $\text{O}_2 \leq 5 \mu\text{mol L}^{-1}$ , thus significantly enlarging the ocean volume potentially affected by N-loss.

However, the sensitivities of anammox and denitrification to changes in dissolved O<sub>2</sub> and their upper O<sub>2</sub> limits in the marine environment are largely unknown. N-loss attributed to denitrification has been reported to occur at up to  $20 \mu\text{mol L}^{-1}$  of O<sub>2</sub> [18]. Nonetheless, direct measurements of denitrification under controlled exposure to low O<sub>2</sub> concentrations in OMZs are lacking. Active anammox bacteria have been found to be abundant at O<sub>2</sub> concentrations up to 9 and  $20 \mu\text{mol L}^{-1}$  in the Namibian and Peruvian upwelling systems, respectively [9,10], and it has been suggested that marine snow aggregates could provide suitable anoxic micro-niches at ambient O<sub>2</sub> concentrations up to  $25 \mu\text{mol L}^{-1}$  [19,20]. Off Peru/Chile the measured anammox rates were often the highest at the base of the oxycline and in the upper OMZ [10,11,21], likely associated with intensified remineralization of organic matter in these water layers. This further indicates that, unlike their cultured counterparts, which are inhibited at O<sub>2</sub> concentrations as low as  $1 \mu\text{mol L}^{-1}$  [22], marine anammox bacteria can tolerate O<sub>2</sub> concentrations higher than the upper O<sub>2</sub> limit ( $5 \mu\text{mol L}^{-1}$ ) often used to restrict anaerobic processes in biogeochemical models [23]. Recently, Jensen et al. [24] investigated the O<sub>2</sub> sensitivity of anammox in the near-anoxic zone of the Black Sea water column and showed that anammox bacteria remained active up to  $\sim 9 \mu\text{mol L}^{-1}$  of O<sub>2</sub>. Still unknown is whether this relatively high O<sub>2</sub> tolerance is widespread amongst anammox bacteria in the major OMZs of the world's oceans.

Although anammox is an autotrophic process, it relies on other N-cycling processes for the required reactive substrates  $\text{NO}_2^-$  and  $\text{NH}_4^+$ , e.g.  $\text{NH}_3$  oxidation to  $\text{NO}_2^-$  and heterotrophic nitrate ( $\text{NO}_3^-$ ) reduction to  $\text{NO}_2^-$ . The co-occurrence of these aerobic and anaerobic processes together with anammox requires them to be adapted to a certain overlapping range of O<sub>2</sub> concentrations. Thus far, it remains unclear whether or not processes coupled to anammox can proceed in the same range of O<sub>2</sub> as assumed for anammox ( $0\text{--}20 \mu\text{mol L}^{-1}$ ), or if they show different O<sub>2</sub> sensitivities that might hence restrict N-loss to a narrower O<sub>2</sub> regime. Under anoxic conditions,  $\text{NO}_3^-$  is the next thermodynamically favored electron acceptor, which can be used by a variety of micro-organisms to oxidize organic matter [25]. In OMZ waters, secondary  $\text{NO}_2^-$  maxima are often interpreted as active  $\text{NO}_3^-$  reduction [26,27]. The formation of  $\text{NO}_2^-$  from  $\text{NO}_3^-$  is the first step in both denitrification and dissimilatory nitrate reduction to ammonium (DNRA), but it can also be considered as a stand-alone process, as more micro-organisms are

known capable of reducing  $\text{NO}_3^-$  to  $\text{NO}_2^-$  than to  $\text{N}_2$  or  $\text{NH}_4^+$  [25,28]. Heterotrophic  $\text{NO}_3^-$  reduction to  $\text{NO}_2^-$  has been measured at high rates in the Peruvian OMZ [29,30], and has been estimated to account for approximately two thirds of the  $\text{NO}_2^-$  required for anammox in this region [30]. At the same time,  $\text{NO}_3^-$  reduction also provides an important source of  $\text{NH}_4^+$  released from oxidized organic matter [30,31]. Lipschultz et al. [29] investigated the effect of varying O<sub>2</sub> concentrations on  $\text{NO}_3^-$  reduction to  $\text{NO}_2^-$  in the Peruvian OMZ. They observed that  $\text{NO}_3^-$  reduction rates doubled under anoxic conditions ( $\text{N}_2$  atmosphere) compared to *in situ* conditions ( $2.5 \mu\text{mol L}^{-1}$  of O<sub>2</sub>), while rates decreased by  $\sim 75\%$  at  $20 \mu\text{mol L}^{-1}$  of O<sub>2</sub>.

When O<sub>2</sub> is present,  $\text{NO}_2^-$  can be produced aerobically by  $\text{NH}_3$  oxidizing bacteria and archaea in the first step in nitrification. Rates of  $\text{NH}_3$  oxidation are generally highest near the upper OMZ boundaries [32,33]. In the Peruvian OMZ, this is also where anammox bacteria are most active [10]. These bacteria are partly fueled by  $\text{NH}_3$  oxidation in this zone [30]. A similarly tight coupling between anammox and  $\text{NH}_3$  oxidation was shown earlier for the Black Sea [34]. The occurrence of  $\text{NH}_3$  oxidizers is, however, not restricted to the upper OMZ. They have been found active at non-detectable concentrations of O<sub>2</sub> ( $< 1\text{--}2 \mu\text{mol L}^{-1}$ ) in the core of OMZs [30,33,35] and are thus obviously well adapted to near-anoxic O<sub>2</sub> conditions. When Lipschultz et al. [29] investigated the O<sub>2</sub> sensitivity of  $\text{NH}_3$  oxidation in the Peruvian OMZ, the inferred de-oxygenation of the samples only caused a  $\sim 50\%$  decrease in activity relative to ambient O<sub>2</sub> ( $2.5 \mu\text{mol L}^{-1}$ ), whereas no stimulation was achieved by an increase to  $\sim 20 \mu\text{mol L}^{-1}$  of O<sub>2</sub>.

With anammox as well as  $\text{NO}_3^-$  reduction being apparently tolerant to relatively high O<sub>2</sub> and  $\text{NH}_3$  oxidation being apparently able to cope with severe O<sub>2</sub> depletion, an expansion of OMZs might indeed drive larger water masses to greater N-deficits. This would potentially exacerbate N-limitation of primary production in large parts of the ocean and thus affect the oceans' capacity to attenuate the rising atmospheric CO<sub>2</sub>. However, at present no study has systematically investigated the O<sub>2</sub> sensitivities of anammox and concurrent N-cycling processes in oceanic OMZs, and thus the future nutrient balance in these regions remains speculative at best.

In this paper, we present results for the Namibian and Peru/Chile upwelling systems, two of the most productive regions in the worlds' oceans associated with massive N-loss, where we explored the effect of O<sub>2</sub> on anammox,  $\text{NH}_3$  oxidation and  $\text{NO}_3^-$  reduction throughout the OMZ.

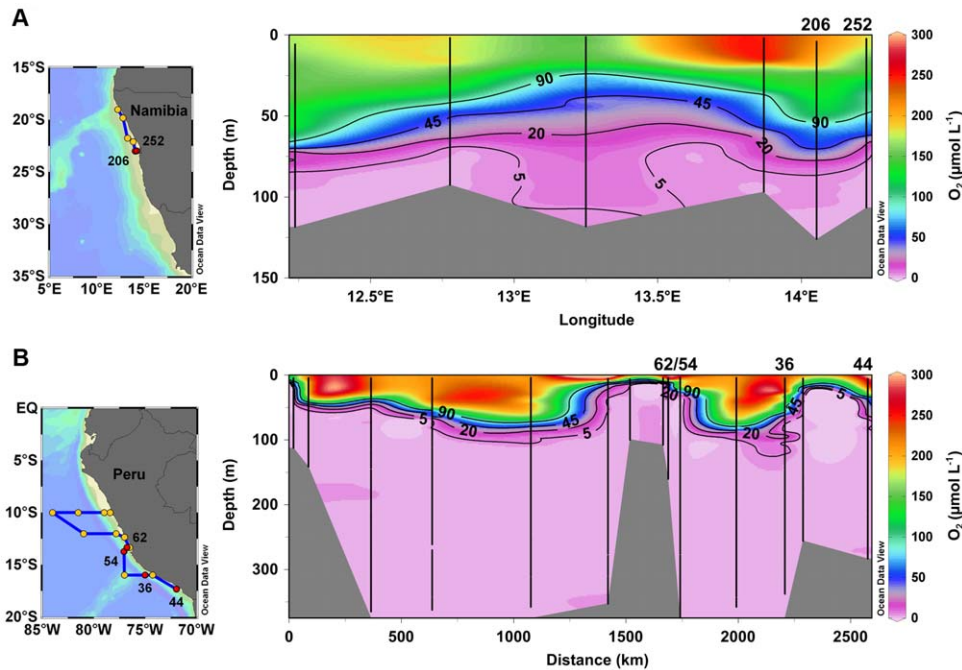
## Materials and Methods

### Ethics Statement

The necessary permissions were obtained from the governments of Namibia and Peru to carry out research in their waters.

### Water sampling and nutrient analyses

Samples were taken on two cruises to the OMZs off Namibia (M76/2) and Peru (M77/3), where upwelling persists year-round, onboard R/V Meteor in May/June 2008 and December/January 2008/2009, respectively (Fig. 1). A pump-CTD system was used to collect water samples just below the oxycline, through the core of the OMZ, down to  $\sim 375$  m depth off the coast of Peru. The pump CTD system was equipped with a conventional amperometric O<sub>2</sub> micro-sensor to obtain vertical profiles of dissolved O<sub>2</sub>. In addition, the recently developed STOX (Switchable Trace amount OXYgen) sensor [6], which allows high-accuracy O<sub>2</sub> measurements in near-anoxic environments (detection limit: 50–



**Figure 1. Locations of the sampled stations and distribution of dissolved O<sub>2</sub>.** Maps show sampling locations on the A) Namibian shelf and in the B) OMZ off Peru during cruises M76-2 and M77-3, respectively. Water samples were collected by pump-CTD (max. sampling depth: ~375 m). The oxygen sensitivities of anammox and coupled N-cycling processes were investigated at sampling stations indicated by numbers (red circles). Vertical distributions of dissolved O<sub>2</sub> are plotted along blue lines.  
doi:10.1371/journal.pone.0029299.g001

100 nmol L<sup>-1</sup> during our deployments), was deployed. At least five measuring cycles after ≥10 min sensor equilibration at a given sampling depth were used to calculate O<sub>2</sub> concentrations. Water samples were taken with a depth resolution of 1–2 m for nutrient analyses. NH<sub>4</sub><sup>+</sup> was measured fluorometrically [36] and NO<sub>2</sub><sup>-</sup> was analyzed spectrophotometrically [37] on board. Water samples for NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3-</sup> were stored frozen until spectrophotometric determination [37] with an autoanalyzer (TRAACS 800, Bran & Lubbe) in a shore-based laboratory. Detection limits for NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3-</sup> were 10, 10, 100 and 100 nmol L<sup>-1</sup>, respectively. N-deficits were calculated from the measured fixed inorganic N- and PO<sub>4</sub><sup>3-</sup> concentrations as N\* (in μmol L<sup>-1</sup>) following Gruber and Sarmiento [8]:  $N^* = [\text{NH}_4^+] + [\text{NO}_2^-] + [\text{NO}_3^-] - 16 \times [\text{PO}_4^{3-}] + 2.9 \mu\text{mol kg}^{-1} \times \text{density in kg L}^{-1}$ .

### <sup>15</sup>N labeling experiments

Incubation experiments were carried out at two shallow shelf stations off Namibia (St. 206 and 252) and four stations off Peru (St. 36, 44, 54 and 63), ranging from coastal to open ocean settings (Fig. 1 and Table 1). Based on O<sub>2</sub> profiles, three to six depths per station were chosen for a standard series of <sup>15</sup>N-labeling experiments. The experimental procedure for <sup>15</sup>N-labeling experiments has been described in detail previously [9,31,38]. Briefly, N-loss by either anammox or heterotrophic denitrification was measured as the production of <sup>15</sup>N-labeled N<sub>2</sub> in <sup>15</sup>NH<sub>4</sub><sup>+</sup> (+<sup>14</sup>NO<sub>2</sub><sup>-</sup>), <sup>15</sup>NO<sub>2</sub><sup>-</sup> (+<sup>14</sup>NH<sub>4</sub><sup>+</sup>) and <sup>15</sup>NO<sub>3</sub><sup>-</sup> (+<sup>14</sup>NO<sub>2</sub><sup>-</sup>) (isotopes: Campro scientific) time-series incubations carried out in 12-ml Exetainers (Labco, UK). At each time interval (about 0, 6, 12, 24 and 48 h) production in one replicate Exetainer was terminated by the addition of saturated mercuric chloride to stop biological activity. The N-isotopic composition of N<sub>2</sub> gas produced in these experiments was determined by GC/

IRMS (Fisons VG Optima). Afterwards, rates of NH<sub>3</sub> oxidation to NO<sub>2</sub><sup>-</sup> and those of NO<sub>3</sub><sup>-</sup> reduction to NO<sub>2</sub><sup>-</sup> were determined in the same samples as net <sup>15</sup>NO<sub>2</sub><sup>-</sup> production in <sup>15</sup>NH<sub>4</sub><sup>+</sup>+<sup>14</sup>NO<sub>2</sub><sup>-</sup> and <sup>15</sup>NO<sub>3</sub><sup>-</sup>+<sup>14</sup>NO<sub>2</sub><sup>-</sup> incubations respectively. The N-isotopic composition of NO<sub>2</sub><sup>-</sup> was determined by GC/IRMS after conversion to either nitrous oxide (N<sub>2</sub>O) by sodium azide [39], or to N<sub>2</sub> by sulfamic acid [40,41]. Rates were calculated from the slope of linear regression of <sup>15</sup>N-production as a function of time. Only significant and linear production of <sup>15</sup>N-species without an initial lag-phase was considered (*t*-tests, *p*<0.05; R<sup>2</sup>>0.8). The net production rates presented here have been corrected for the mole fractions of <sup>15</sup>N in the original substrate pools but not for isotope dilution due to any other concurrent N-consumption or production processes in the course of the incubation.

### Oxygen sensitivity experiments

In order to determine the effect of varying O<sub>2</sub> concentrations on N-cycle processes, one to two depths per station were sampled for additional O<sub>2</sub> sensitivity experiments. Samples were taken from the upper OMZ, where aerobic and anaerobic N-cycle processes have been shown to co-occur [30], except one sample taken deeper in the core of the Peruvian OMZ (St. 36). Samples were obtained in 250-mL serum bottles and purged with helium (He) for approximately 15 min to remove any initial O<sub>2</sub> and to lower the N<sub>2</sub> background in order to enhance the detection limit of <sup>29</sup>N<sub>2</sub> and <sup>30</sup>N<sub>2</sub> [38]. As a small sample volume was lost during He-purging, the bottles were then refilled with a second He-purged sample from the same depth to avoid headspace. Afterwards, air-saturated water from the same depth was added to the serum bottles in exchange for part of the de-oxygenated water to adjust samples to the desired O<sub>2</sub> concentration. At St. 206 and 252 (Namibian OMZ) three samples each were

**Table 1.** Concentrations of O<sub>2</sub>, NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> and N-conversion rates in <sup>15</sup>N-labeling experiments in the OMZs off Namibia and Peru.

Station (water depth)		Depth (m)	<i>in situ</i> O <sub>2</sub> § ‡	NH <sub>4</sub> <sup>+</sup> §	NO <sub>2</sub> <sup>-</sup> §	NH <sub>3</sub> oxidation†	NO <sub>3</sub> <sup>-</sup> reduction†	Anammox†	
[latitude/longitude]	<sup>15</sup> NH <sub>4</sub> <sup>+</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>					<sup>15</sup> NO <sub>3</sub> <sup>-</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	<sup>15</sup> NH <sub>4</sub> <sup>+</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	<sup>15</sup> NO <sub>2</sub> <sup>-</sup> + <sup>14</sup> NH <sub>4</sub> <sup>+</sup>	
Namibian OMZ	M76-206 (131 m)	90	3.39±0.15	0.01	0.21	29±2*	81±9*	36±1*	13±2*
	[23.01°S/14.05°E]	100	2.14±0.10	0.02	0.60	44±1*	103±19*	107±2*	149±5*
		110	0.60±0.11	2.01	0.90	84±5*	97±23*	144±10*	153±4*
	M76-252 (111 m)	76	1.11±0.25	0.12	0.14	93±9	370±111	42±15	43±8*
	[23.00°S/14.23°E]	95	0.00±0.10	2.24	3.43	110±1	385±21	355±8	399±4*
	105	0.00±0.10	2.51	3.83	92±26	339±77	496±15	462±32*	
Peruvian OMZ	M77-36 (2845 m)	90	1.49±0.11	0.05	0.12	35±3	42±2		2.3±0.4
	[16.00°S/75.00°W]	120	1.17±0.11	0.05	0.04	1.2±0.1	22±2		19±8
		150	0.60±0.10	0.04	0.02	0.5±0.1	7.2±1.0		0.00
		180	0.00±0.05	0.06	2.96	0.0	39±3		19±3
		250	0.01±0.05	0.06	3.36	0.0	48±13		10±3
		337	0.00±0.05	0.04	0.45	0.0	48±7		0.0
	M77-44 (281 m)	75	0.73±0.09	0.14	0.01	19±4	no data	5.1±0.3	
	[17.34°S/71.94°W]	87	0.75±0.10	0.09	0.01	21±2	166±15	18±2	
		125	0.02±0.04	0.07	0.28	0.8±0.1	126±8	14±2	
		150	0.01±0.03	0.06	0.30	0.0	87±17	7.4±1.8	
		200	0.02±0.03	0.07	0.33	0.0	19±5	23±2	
		280	0.01±0.04	0.07	5.50	0.0	145±32	7.8±0.6	
	M77-54 (1893 m)	41	3.64±0.10	0.06	0.28	47±2	72±3	5.8±1.7	
	[13.75°S/77.03°W]	75	0.00±0.05	0.03	0.93	5.0±0.4	71±1	6.3±2.0	
		100	0.00±0.04	0.04	4.01	0.0	71±8	3.0±0.2	
		200	0.00±0.04	0.03	4.87	0.0	0.0	9.4±2.4	
		300	0.00±0.04	0.04	5.75	0.0	0.0	2.6±0.4	
		376	0.00±0.05	0.03	0.46	0.0	77±2	2.2±0.1	
	M77-62 (160 m)	40	9.97±0.10	0.40	0.57	0.2±0.1	108±16		25±3
	[13.35°S/76.75°W]	50	2.56±0.10	0.08	2.30	15±2	83±2		52±2
	70	0.07±0.04	0.05	1.49	4.6±0.1	89±15		78±4	
	100	0.00±0.05	0.04	1.34	2.0±0.2	81±8		39±2	
	130	0.00±0.04	0.05	3.45	1.7±0.2	215±6		44±1	
	160	0.00±0.05	0.05	4.10	0.0	117±8		108±11	

\*No addition of <sup>14</sup>N-species.§In μmol L<sup>-1</sup>.

‡Determined with STOX sensor.

†In nmol N L<sup>-1</sup> d<sup>-1</sup>.

doi:10.1371/journal.pone.0029299.t001

adjusted to ~3.5, 7.5 and 12 μmol L<sup>-1</sup> of O<sub>2</sub>, whereas at St. 36, 44, 54 and 63 (Peruvian OMZ) the experimental setup was extended and five samples each were adjusted to ~1.5, 3, 6, 12, and 24 μmol L<sup>-1</sup> of O<sub>2</sub>. One sample, to which no air-saturated water was added, served as an anoxic control at all stations. After additions of either <sup>15</sup>NH<sub>4</sub><sup>+</sup>+<sup>14</sup>NO<sub>2</sub><sup>-</sup>, <sup>15</sup>NO<sub>2</sub><sup>-</sup> (+<sup>14</sup>NH<sub>4</sub><sup>+</sup>) or <sup>15</sup>NO<sub>3</sub><sup>-</sup>+<sup>14</sup>NO<sub>2</sub><sup>-</sup>, samples were transferred into replicate vials (Exetainers, Labco) for time-series incubations. Except for the incubations with only <sup>15</sup>NO<sub>2</sub><sup>-</sup>, <sup>14</sup>N-species were added to all experiments to exclude substrate limitation, which would otherwise complicate the interpretation of any O<sub>2</sub> effects on the processes of interest. Moreover, keeping the <sup>14</sup>N-pool of the product of a certain reaction well above the expected concentrations produced from the added <sup>15</sup>N-substrate could minimize any further conversion of the newly formed <sup>15</sup>N-products by co-occurring processes. The rate measurements for

the various processes were carried out as described above. To exclude formation of <sup>29</sup>N<sub>2</sub> due to coupled nitrification-denitrification in incubations amended with <sup>15</sup>NH<sub>4</sub><sup>+</sup> we added allylthiourea (ATU; final concentration 84 μmol L<sup>-1</sup>) to an additional sample of the highest O<sub>2</sub> treatment (~11.5 μmol L<sup>-1</sup>) at St. 206 and 252. ATU is a specific inhibitor of aerobic NH<sub>3</sub> oxidation [42–44] and does not affect anammox activity shown at least in sediments [45]. Two sets of incubations were performed in parallel at St. 206 and 252 and one sample per time-point was sacrificed to measure dissolved O<sub>2</sub>. For the remaining stations, O<sub>2</sub> concentrations were determined only for the initial time-point in each <sup>15</sup>N-incubation experiment. We used a custom-built, fast-responding O<sub>2</sub> micro-sensor (Clark-type; MPI Bremen) for most measurements (detection limit: ~0.5 μmol L<sup>-1</sup> of O<sub>2</sub>), except at St. 206 where a STOX sensor was used for selected samples.

## Data analysis

We applied least-squares fitting to each set of samples of the O<sub>2</sub> sensitivity experiments using Excel's solver function [46].

## Results

### Hydrochemistry in the Namibian OMZ

The water column was poorly stratified over the Namibian shelf at St. 206 and 252 during the time of sampling, as indicated by a weak density gradient, along with the vertical profiles of dissolved O<sub>2</sub> and inorganic N-species (Fig. 2A). At both stations O<sub>2</sub> declined gradually with depth, from ~200 μmol L<sup>-1</sup> in the surface waters to less than 10 μmol L<sup>-1</sup> at ~80 m. STOX measurements at the incubation depths revealed O<sub>2</sub> concentrations as low as 0.60±0.11 μmol L<sup>-1</sup> at St. 206. In the central OMZ at St. 252 (Table 1), the sensor was at its detection limit (100 nmol L<sup>-1</sup> of O<sub>2</sub> during M76-2). Ammonium concentrations were typically in the range of 1–3 μmol L<sup>-1</sup> in the oxic zone (<80 m) and decreased to 0.1–0.5 μmol L<sup>-1</sup> at the base of the oxycline (Fig. 2B). Towards the sediment-water interface NH<sub>4</sub><sup>+</sup> concentrations increased up to 4.5 (St. 206) and 2.5 μmol L<sup>-1</sup> (St. 252). Nitrite concentrations were fairly constant in the upper ~100 m (0.1–0.5 μmol L<sup>-1</sup>) and increased to ~2 and ~4 μmol L<sup>-1</sup> in the bottom waters at St. 206 and 252, respectively. The increase in both NO<sub>2</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> in the lower OMZ was accompanied by a sharp decrease in NO<sub>3</sub><sup>-</sup> concentrations, with minimum concentrations of ~12 μmol L<sup>-1</sup> in the lowest sampling depths at both stations.

### Hydrochemistry in the Peruvian OMZ

The stations sampled in the Peruvian OMZ were located on the shelf (St. 62), shelf edge (St. 44) and in the open ocean (St. 36 and 54). Similar to the Namibian shelf stations, the shallowest site (St. 62) was characterized by low density gradients and a gradual decline in O<sub>2</sub> between ~20 and 50 m. In contrast, the water column was highly stratified further offshore. Strong pycnoclines, centered around 65, 30 and 55 m at St. 44, 54 and 36, respectively, and a steep oxycline indicated oxygenated surface waters and OMZ were well separated (Figure 2A). Oxygen decreased from ~250 μmol L<sup>-1</sup> in the surface to less than 10 μmol L<sup>-1</sup> at 66 (St. 44), 35 (St. 54) and 75 m (St. 36). A local O<sub>2</sub> maximum (10 to 25 μmol L<sup>-1</sup>) was found between 90 and 100 m at St. 36, likely due to some lateral advection of more oxygenated water. At all four stations, STOX measurements at the incubation depths revealed traces of O<sub>2</sub> in the central OMZ at best; mostly here O<sub>2</sub> concentrations remained below the detection limit of the STOX sensor (~50 nmol L<sup>-1</sup> of O<sub>2</sub> during M77-3). Ammonium concentrations were low and typically 0.05 to 0.1 μmol L<sup>-1</sup> throughout the OMZ as well as in the surface layer (Fig. 2B). On the shelf, concentrations of NH<sub>4</sub><sup>+</sup> were slightly elevated at the base of the oxycline (up to ~0.4 μmol L<sup>-1</sup> at St. 62). At the open-ocean stations (St. 54 and 36) NH<sub>4</sub><sup>+</sup> maxima of ~2 μmol L<sup>-1</sup> were measured at 20 and 35 m, which coincided with NO<sub>2</sub><sup>-</sup> maxima (up to 1 μmol L<sup>-1</sup>). In general, NO<sub>2</sub><sup>-</sup> concentrations in the surface waters remained below 0.5 μmol L<sup>-1</sup>, whereas NO<sub>2</sub><sup>-</sup> accumulated to over 5 μmol L<sup>-1</sup> in the core of the OMZ at all stations. Nitrate concentrations were as low as ~1 μmol L<sup>-1</sup> on the shelf (St. 62). Further off-shore less pronounced NO<sub>3</sub><sup>-</sup> concentration minima were detected (~12 at St. 44 and ~20 μmol L<sup>-1</sup> at St. 54 and 36).

### N-cycling in the Namibian and Peruvian OMZs

**Distribution of anammox activity.** Over the Namibian shelf a strong increase in the N-deficit was observed below the oxycline. Minimum values for N\* (down to -19 μmol L<sup>-1</sup>) were

found in the central OMZ, suggesting N-loss therein. We measured <sup>15</sup>N<sup>14</sup>N formation in all of our <sup>15</sup>NH<sub>4</sub><sup>+</sup> (+<sup>14</sup>NO<sub>2</sub><sup>-</sup>) and <sup>15</sup>NO<sub>2</sub><sup>-</sup>-incubations at the three depths sampled per station (Table 1). Corrected for the labeling percentage (i.e. the mole fraction of <sup>15</sup>N in the respective N-substrate pool), rates were comparable in <sup>15</sup>NH<sub>4</sub><sup>+</sup> and <sup>15</sup>NO<sub>2</sub><sup>-</sup> experiments. As no increase in <sup>15</sup>N<sup>15</sup>N was detectable in either <sup>15</sup>NO<sub>2</sub><sup>-</sup> or <sup>15</sup>NO<sub>3</sub><sup>-</sup> incubations, the formation of <sup>15</sup>N-labeled N<sub>2</sub> was attributed to anammox activity and not denitrification. At both stations, anammox rates and N-loss inferred from N\* increased with depth (Fig. 2C). Rates ranged from 13 to 43 nmol N L<sup>-1</sup> d<sup>-1</sup> at the base of the oxycline to 144 to 496 nmol N L<sup>-1</sup> d<sup>-1</sup> in the central OMZ and were generally higher at St. 252.

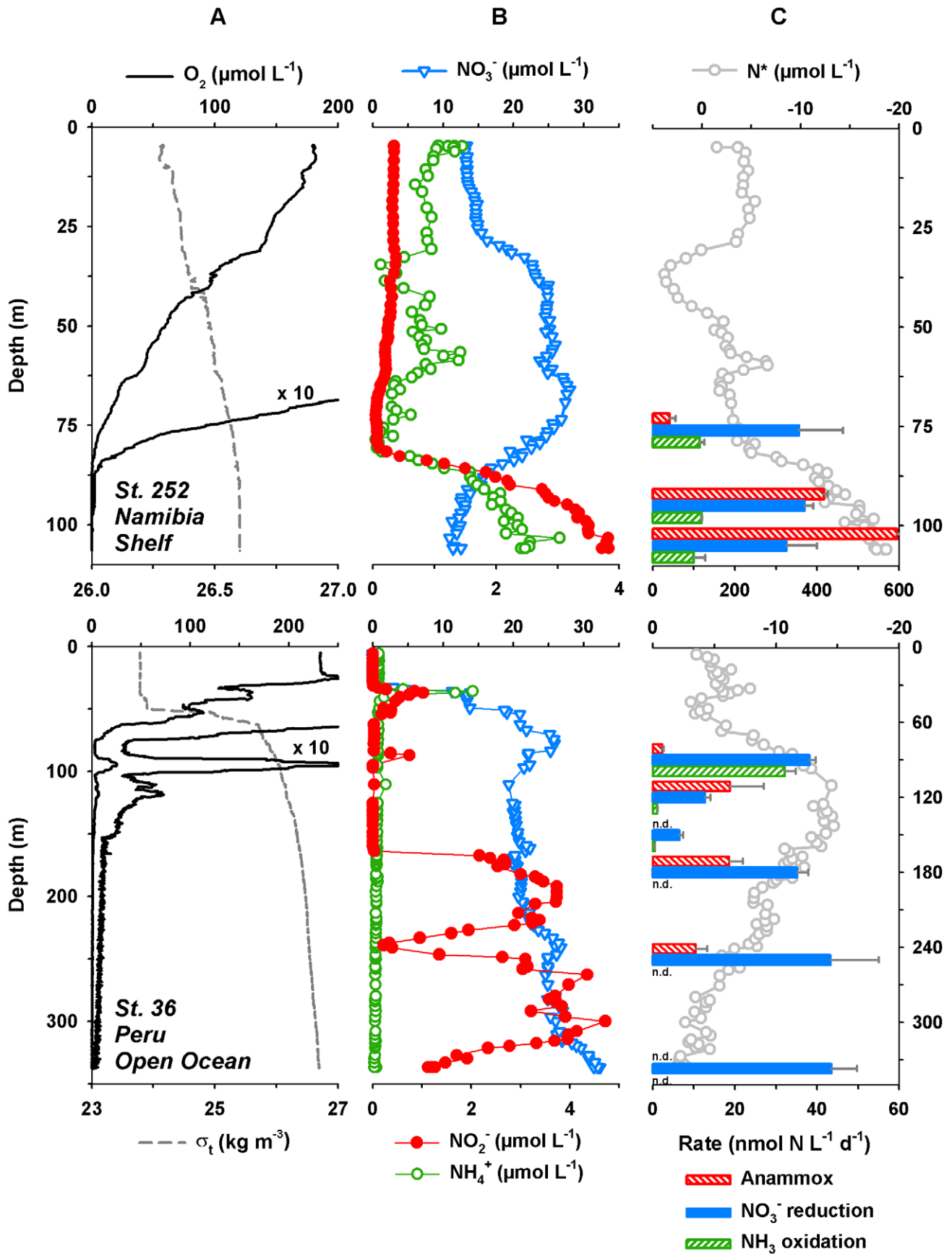
In the OMZ off Peru, the N-deficit was strongest over the shelf (N\* = -33 μmol L<sup>-1</sup>; St. 62) and less pronounced towards the open ocean (N\* = 10 μmol L<sup>-1</sup>; St. 54), indicating the highest N-loss likely occurred near the coast. Six depths per station were sampled and <sup>15</sup>N<sup>14</sup>N formation in <sup>15</sup>NH<sub>4</sub><sup>+</sup>+<sup>14</sup>NO<sub>2</sub><sup>-</sup> and <sup>15</sup>NO<sub>2</sub><sup>-</sup>+<sup>14</sup>NH<sub>4</sub><sup>+</sup> was measured in 22 out of 24 incubation depths (Table 1). No formation of <sup>15</sup>N-labeled N<sub>2</sub> was detectable at 150 and 337 m at St. 36. As for the Namibian OMZ, whenever N<sub>2</sub> formation occurred all of the <sup>15</sup>N-labeled N<sub>2</sub> produced was recovered as <sup>29</sup>N<sub>2</sub> and there was no detectable increase in <sup>15</sup>N<sup>15</sup>N over time detected in either <sup>15</sup>NO<sub>2</sub><sup>-</sup> or <sup>15</sup>NO<sub>3</sub><sup>-</sup> incubations. Thus, anammox was the only detectable active N<sub>2</sub>-producing pathway, while there was no clear evidence for denitrification activity at the time of our sampling. In general, high anammox activity corresponded with more negative N\*, i.e. a more pronounced N-deficit (Fig. 2C). Over the Peruvian shelf, anammox rates (25 to 108 nmol N L<sup>-1</sup> d<sup>-1</sup>; St. 62) were comparable to those measured over the Namibian shelf (St. 206). Further offshore in the Peruvian OMZ, rates dropped to as low as one tenth of those measured near the coast (2.2 to 9.4 nmol N L<sup>-1</sup> d<sup>-1</sup>; St. 54).

**Distribution of nitrate reduction to nitrite activity.** Nitrate reduction was measured as <sup>15</sup>NO<sub>2</sub><sup>-</sup> production in all <sup>15</sup>NO<sub>3</sub><sup>-</sup>+<sup>14</sup>NO<sub>2</sub><sup>-</sup> incubations carried out in the OMZ overlying the Namibian shelf. Nitrate reduction occurred uniformly over the three sampled depths, at rates around 100 and 360 nmol N L<sup>-1</sup> d<sup>-1</sup> at St. 206 and 252, respectively (Table 1).

Off Peru, NO<sub>3</sub><sup>-</sup> reduction could be detected in 21 out of 23 <sup>15</sup>NO<sub>3</sub><sup>-</sup>+<sup>14</sup>NO<sub>2</sub><sup>-</sup> incubation experiments. The vertical distribution of NO<sub>3</sub><sup>-</sup> reducing activity was slightly variable and high NO<sub>3</sub><sup>-</sup> reduction rates did not always coincide with a noticeable accumulation of NO<sub>2</sub><sup>-</sup>. Similar to anammox activity, maximum rates of NO<sub>3</sub><sup>-</sup> reduction were generally detected over the shelf (up to 215 nmol N L<sup>-1</sup> d<sup>-1</sup>) and decreased towards the open ocean (up to 48 nmol N L<sup>-1</sup> d<sup>-1</sup>).

**Distribution of ammonia oxidation activity.** Ammonia oxidation, measured as <sup>15</sup>NO<sub>2</sub><sup>-</sup> production in <sup>15</sup>NH<sub>4</sub><sup>+</sup>+<sup>14</sup>NO<sub>2</sub><sup>-</sup> incubation experiments, was detected at all incubation depths (Table 1). At St. 206 <sup>15</sup>N-labeling experiments were carried out under anoxic conditions, whereas samples were incubated at *in situ* O<sub>2</sub> (<1 μmol L<sup>-1</sup>) at St. 252. Rates increased with depth at St. 206 (from 29 to 84 nmol N L<sup>-1</sup> d<sup>-1</sup>) but remained rather constant at St. 252 (~100 nmol N L<sup>-1</sup> d<sup>-1</sup>).

Off Peru, NH<sub>3</sub> oxidation to NO<sub>2</sub><sup>-</sup> was determined in <sup>15</sup>NH<sub>4</sub><sup>+</sup>+<sup>14</sup>NO<sub>2</sub><sup>-</sup> incubations under anoxic conditions (St. 44 and 54) or at *in situ* O<sub>2</sub> levels (St. 36 and 62). Maximum NH<sub>3</sub> oxidation rates ranged between 15 and 47 nmol N L<sup>-1</sup> d<sup>-1</sup>. There was no obvious trend in nitrifying activity between coastal and open-ocean stations. Ammonia oxidation was generally confined to the upper OMZ, where O<sub>2</sub> was still measurable.



**Figure 2. Physicochemical zonation and N-conversion rates at selected stations.** Stations are plotted for cruises M76-2 and M77-3 to the OMZs off A) Namibia and B) Peru, respectively. Water depths were 111 m at St. 252 and 2845 m at St. 36. N\* was calculated from the fixed inorganic N- and PO<sub>4</sub><sup>3-</sup> concentrations (data not shown). Anammox rates were determined in <sup>15</sup>NH<sub>4</sub><sup>+</sup> (St. 206) and <sup>15</sup>NO<sub>2</sub><sup>-</sup>+<sup>14</sup>NH<sub>4</sub><sup>+</sup> incubations (St. 36). All rates are net rates corrected for the percentage of <sup>15</sup>N in the pool of the respective N-species. Error bars for rates are standard errors calculated from linear regression.

doi:10.1371/journal.pone.0029299.g002

However, despite an apparent lack of O<sub>2</sub> *in situ* (i.e. O<sub>2</sub> concentrations were below detection) shipboard experiments revealed NH<sub>3</sub> oxidation activity also at St. 54 at 75 m as well as in the central OMZ at St. 62 (1.7 to 5.0 nmol N L<sup>-1</sup> d<sup>-1</sup>).

### Oxygen sensitivity of anammox and coupled N-cycle processes

**Oxygen sensitivity of anammox.** Anammox activity, as indicated by <sup>15</sup>N<sup>14</sup>N production from <sup>15</sup>NH<sub>4</sub><sup>+</sup> and <sup>15</sup>NO<sub>2</sub><sup>-</sup>, was measurable in all O<sub>2</sub> manipulation experiments without lag phase at the Namibian shelf stations (Table 2). Oxygen concentration and N<sub>2</sub> formation showed a significant negative correlation for the incubations with <sup>15</sup>NH<sub>4</sub><sup>+</sup> as well as <sup>15</sup>NO<sub>2</sub><sup>-</sup> at St. 206 and the one with <sup>15</sup>NH<sub>4</sub><sup>+</sup> at St. 252 (Pearson  $r = -0.95$  to  $-0.99$ ,  $P < 0.05$ ). Similar responses to increased O<sub>2</sub> were observed for the incubations amended with <sup>15</sup>NH<sub>4</sub><sup>+</sup> and <sup>15</sup>NO<sub>2</sub><sup>-</sup> at both stations. Activity decreased with increasing O<sub>2</sub> and was, on average, ~85%, ~70% and ~50% of the anoxic control at ~3.7, ~8.1 and ~11.3 μmol L<sup>-1</sup> of oxygen, respectively (Fig. 3A). Over the course of the incubation (0–48 h) O<sub>2</sub> concentrations in the <sup>15</sup>N-labeling experiments did not vary significantly ( $\pm 0.44$  μmol L<sup>-1</sup> on average). No substantial difference in <sup>15</sup>N<sup>14</sup>N production was observed between <sup>15</sup>NH<sub>4</sub><sup>+</sup>-labeled incubations with and without ATU. This indicates that anammox rather than coupled nitrification-denitrification was the process responsible for the production of <sup>15</sup>N-labeled N<sub>2</sub> at 11–12 μmol L<sup>-1</sup> of dissolved O<sub>2</sub>.

In the OMZ off Peru, <sup>15</sup>N<sup>14</sup>N production rates in <sup>15</sup>NH<sub>4</sub><sup>+</sup> and <sup>15</sup>NO<sub>2</sub><sup>-</sup> incubations decreased with increasing O<sub>2</sub> concentrations in all O<sub>2</sub> manipulation experiments. However, substantial differences in the O<sub>2</sub> sensitivity of anammox were found between stations. Over the Peruvian shelf, adjusted O<sub>2</sub> levels and N<sub>2</sub> production were linearly and negatively correlated up to 14.4 μmol L<sup>-1</sup> O<sub>2</sub> at St. 44 (Pearson  $r = -0.99$ ,  $P < 0.05$ ) and 10.9 μmol L<sup>-1</sup> at St. 62 (Pearson  $r = -0.96$ ,  $P < 0.05$ ). No rates were detectable beyond ~20 μmol L<sup>-1</sup> of O<sub>2</sub>. At the open-ocean stations in the Peruvian OMZ, anammox activity appeared to be more sensitive to the added O<sub>2</sub> (Fig. 3A). At St. 36, ~30% activity of the anoxic control experiment remained detectable when O<sub>2</sub> was increased from the *in situ* ~1.2 μmol L<sup>-1</sup> (measured by STOX) to 5.5 μmol L<sup>-1</sup> of O<sub>2</sub> in the 120 m sample. In comparison, anammox was fully inhibited at 2.8 μmol L<sup>-1</sup> of O<sub>2</sub> already in the 180 m sample, where O<sub>2</sub> was not detectable by the STOX sensor *in situ*. A similarly strong O<sub>2</sub> response was seen at St. 54, where rates dropped to zero at 4.0 μmol L<sup>-1</sup> of O<sub>2</sub> in the 75 m incubation experiment.

**Oxygen sensitivity of nitrate reduction to nitrite.** Nitrate reduction rates in the O<sub>2</sub> sensitivity assay carried out for the Namibian OMZ waters, decreased with increasing O<sub>2</sub> concentrations (Table 2). The incubation experiments at St. 206 revealed a stronger negative response to elevated O<sub>2</sub> levels than those performed at St. 252. Activity at St. 206 was reduced to ~30% of the anoxic control in the highest O<sub>2</sub> treatment (7.3 μmol L<sup>-1</sup>), whereas a doubling of the O<sub>2</sub> concentration (14.7 μmol L<sup>-1</sup>) led to a decrease in NO<sub>3</sub><sup>-</sup> reduction rates to ~60% of the control experiment at St. 252 (Fig. 3B).

In the Peruvian OMZ, production of <sup>15</sup>NO<sub>2</sub><sup>-</sup> from <sup>15</sup>NO<sub>3</sub><sup>-</sup> was never fully inhibited by O<sub>2</sub>, not even in the highest O<sub>2</sub>

treatments (~25 μmol L<sup>-1</sup> of O<sub>2</sub>). Nevertheless, NO<sub>3</sub><sup>-</sup> reduction rates showed marked differences in their sensitivity towards elevated O<sub>2</sub> levels between and within our experimental stations. For example at St. 36, NO<sub>3</sub><sup>-</sup> reduction activity in the upper OMZ sample (120 m) at St. 36 did not vary significantly among the various O<sub>2</sub> treatments (1.4 to 27.1 μmol L<sup>-1</sup> of O<sub>2</sub>), while activity decreased to ~10% of the control experiment in samples taken deeper (180 m) in the OMZ when adjusted to 25.5 μmol L<sup>-1</sup> of O<sub>2</sub> (Figure 3B).

**Oxygen sensitivity of ammonia oxidation.** Rates of NH<sub>3</sub> oxidation to NO<sub>2</sub><sup>-</sup> showed no significant difference over the range of the applied O<sub>2</sub> concentrations (~1–12 μmol L<sup>-1</sup>) in the Namibian OMZ samples (Table 2). Activity varied by a maximum of ~15% among the different O<sub>2</sub> treatments but without any systematic trends (Fig. 3C).

Similar to the observations for the Namibian shelf, <sup>15</sup>NO<sub>2</sub><sup>-</sup> production in the <sup>15</sup>NH<sub>4</sub><sup>+</sup> experiments conducted for the Peruvian shelf (St. 44) and at open-ocean (St. 54) stations showed no marked differences among the different O<sub>2</sub> treatments (~1–25 μmol L<sup>-1</sup>). Only the control experiment (0.8 μmol L<sup>-1</sup> O<sub>2</sub>) at St. 54 suggested a slightly lower NH<sub>3</sub> oxidation rate (~35%) compared to the higher O<sub>2</sub> treatments (Fig. 3C).

## Discussion

### Oxygen sensitivity of anammox in OMZ waters

In the investigated samples from both the Namibian and Peruvian OMZ, the only N<sub>2</sub>-forming pathway detected by <sup>15</sup>N-labeling experiments was anammox. This confirms the results from earlier studies, which detected N-loss due to anammox but not denitrification in these regions [9–11]. The highest anammox rates (on the order of 500 nmol N L<sup>-1</sup> d<sup>-1</sup>) were measured in the Namibian shelf waters. Off Peru, rates declined from ~50 nmol N L<sup>-1</sup> d<sup>-1</sup> over the shelf to <10 nmol N L<sup>-1</sup> d<sup>-1</sup> at the open ocean sites. This may be explained by differences in surface productivity between the two upwelling systems [47] as well as between Peruvian coastal and open-ocean waters, since organic matter transport ultimately fuels all processes delivering NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup> for the anammox reaction [30,31]. Anammox often showed the highest rates in the upper OMZ, as seen in previous studies [10,11,21] probably in response to the high NH<sub>4</sub><sup>+</sup> release from the enhanced remineralization of particulate organic matter at the base of the oxycline, below which all three activities decreased with depth. There were exceptions, however, particularly at depths close to the seafloor on the shelf, where exceptionally high rates were likely supported by NH<sub>4</sub><sup>+</sup> diffusing out of the sediment [9,48,49] (S. Sommer, pers. comm.).

In the O<sub>2</sub> tolerance assays, N-loss due to anammox was in fact detectable at O<sub>2</sub> levels significantly higher (up to ~15 μmol L<sup>-1</sup>) than that generally used to define OMZs (<5 μmol L<sup>-1</sup> of O<sub>2</sub>). Anammox activity in samples taken at the shallow sites appeared the least affected by increasing O<sub>2</sub>. The rates therein remained measurable even at adjusted O<sub>2</sub> concentrations of 10 to 15 μmol L<sup>-1</sup>. These are almost twice as high as the anammox O<sub>2</sub>-tolerance level previously determined in the Black Sea suboxic zone [24]. In comparison, anammox activity appeared increas-



**Table 2.** Rates of NH<sub>3</sub> oxidation, NO<sub>3</sub><sup>-</sup> reduction and anammox measured at varying concentrations of dissolved O<sub>2</sub>.

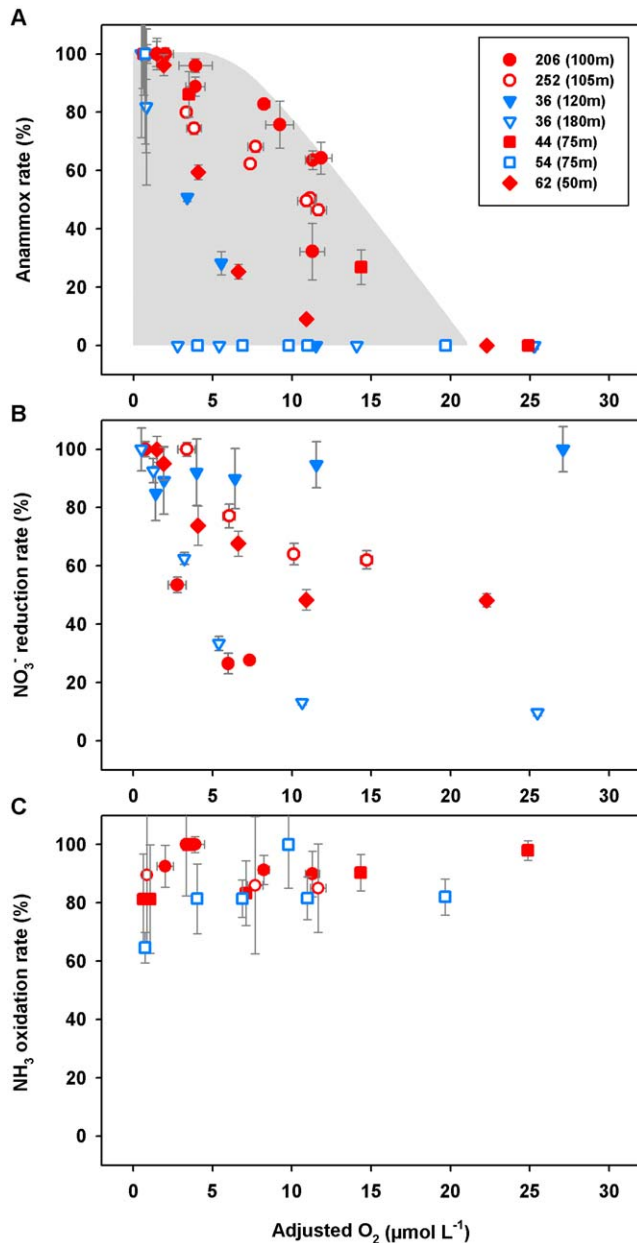
Substrate additions:		NH <sub>3</sub> oxidation		NO <sub>3</sub> <sup>-</sup> reduction		Anammox				
		<sup>15</sup> NH <sub>4</sub> <sup>+</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>		<sup>15</sup> NO <sub>3</sub> <sup>-</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>		<sup>15</sup> NH <sub>4</sub> <sup>+</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>		<sup>15</sup> NO <sub>2</sub> <sup>-</sup> + <sup>14</sup> NH <sub>4</sub> <sup>+</sup>		
		O <sub>2</sub> § ‡	Rate†	O <sub>2</sub> § ‡	Rate†	O <sub>2</sub> § ‡	Rate†	O <sub>2</sub> § ‡	Rate†	
Namibian OMZ	M76-206	2.0	70±5	0.8	65±2	2.0	122±3	0.8	119±10 *	
	(100 m)	3.9	76±2	2.8	35±2	3.9	108±4	3.9	114±3 *	
		8.2	69±4	6.0	17±2	8.2	101±2	9.2	90±10 *	
		11.3	68±6	7.3	18±1	11.3	77±4	11.3	38±12 *	
	+ATU					11.8	78±7			
	M76-252	0.9	92±26	3.4	192±4	0.9	361±12	1.5	430±18 *	
	(105 m)	3.3	103±18	6.0	148±8	3.3	289±7	3.8	320±9 *	
		7.7	89±24	10.1	123±7	7.7	246±7	7.4	267±5 *	
		11.7	88±16	14.7	119±6	11.7	167±7	11.1	217±8 *	
	+ATU					10.9	179±7			
	Peruvian OMZ	M77-36			1.4	22.3±2.5			0.6	10.1±1.2
		(120 m)			1.9	23.5±3.1			0.8	8.2±2.7
				4.0	24.2±3.0			3.4	5.1±0.1	
				6.4	23.7±2.7			5.5	2.8±0.4	
				11.5	24.9±2.1			11.5	0	
			27.1	26.3±2.0			25.3	0		
M77-36				0.5	38.7±2.9			0.5	15.8±4.5	
(180 m)				1.3	35.9±1.6			0.8	12.9±2.5	
				3.2	24.2±0.8			2.8	0	
				5.4	13.0±0.9			5.4	0	
				10.6	5.1±0.3			14.1	0	
			25.5	3.8±0.4			25.3	0		
M77-44		0.6	12.0±2.3			0.6	4.1±0.6			
(75 m)		1.1	12.0±2.7			1.1	no data			
		3.5	14.7±0.2			3.5	3.5±0.3			
		7.1	12.3±1.6			7.1	no data			
		14.4	13.3±0.9			14.4	1.1±0.2			
		24.9	14.5±0.5			24.9	0			
M77-54		0.8	5.6±0.4			0.8	6.3±2.0			
(75 m)		4.0	6.3±0.9			4.0	0			
	6.9	6.3±0.5			6.9	0				
	9.8	7.8±1.2			9.8	0				
	11.0	6.3±0.6			11.0	0				
	19.7	6.4±0.5			19.7	0				
M77-62			1.5	105±5			1.5	33±1.8		
(50 m)			1.9	100±6			1.9	31±1.2		
			4.1	77±7			4.1	19±0.8		
			6.6	71±4			6.6	8.2±0.8		
			10.9	51±4			10.9	2.9±0.5		
		22.3	51±2			22.3	0			

\*No addition of <sup>14</sup>N-species.§In μmol L<sup>-1</sup>.‡Adjusted concentrations of O<sub>2</sub>, determined by μ-sensor measurements.†In nmol N L<sup>-1</sup> d<sup>-1</sup>.

doi:10.1371/journal.pone.0029299.t002

ingly sensitive to O<sub>2</sub> towards the open ocean and deeper in the OMZ, where rates were not detectable above 2.8 to 5.5 μmol L<sup>-1</sup> of O<sub>2</sub> (St. 36 and 54). Based on the observed negative linear

correlation between the measured rates and adjusted O<sub>2</sub> levels, the upper O<sub>2</sub> limit for anammox to proceed in the OMZs is estimated to be ~20 μmol L<sup>-1</sup> (Table 3 & Fig. 3).



**Figure 3. N-cycle processes in Namibian and Peruvian OMZ waters with respect to dissolved oxygen.** A) Anammox measured as <sup>15</sup>N<sup>14</sup>N production in <sup>15</sup>NO<sub>2</sub><sup>-</sup> (+<sup>14</sup>NH<sub>4</sub><sup>+</sup>) and <sup>15</sup>NH<sub>4</sub><sup>+</sup>+<sup>14</sup>NO<sub>2</sub><sup>-</sup> incubations. B) NO<sub>3</sub><sup>-</sup> reduction measured as <sup>15</sup>NO<sub>2</sub><sup>-</sup> production in <sup>15</sup>NO<sub>3</sub><sup>-</sup>+<sup>14</sup>NO<sub>2</sub><sup>-</sup> incubations. C) NH<sub>3</sub> oxidation measured as <sup>15</sup>NO<sub>2</sub><sup>-</sup> production in <sup>15</sup>NH<sub>4</sub><sup>+</sup>+<sup>14</sup>NO<sub>2</sub><sup>-</sup> incubations. N-conversion rates are given as percentages of the highest rate observed (=100%) for the different O<sub>2</sub> treatments at each incubation depth. Adjusted O<sub>2</sub> concentrations were verified by micro-sensor measurements. Parentheses in figure legend indicate the corresponding sampling depths at each station shown. Station numbers with double digits and triple digits represent the Peruvian and Namibian stations, respectively. Shelf and open ocean stations are represented by red and blue symbols, respectively. The O<sub>2</sub> sensitivity assays indicate an upper O<sub>2</sub> limit for N-loss due to anammox of ~20 μmol L<sup>-1</sup> (grey shading). doi:10.1371/journal.pone.0029299.g003

The apparently higher O<sub>2</sub> tolerance at the shelf stations may be explained by an adaptation of anammox bacteria to fluctuations in dissolved O<sub>2</sub> due to the presence of a less stable oxycline at the

upper boundary of the OMZ. Vertical mixing is usually enhanced in coastal upwelling regions. This was indicated by a weak density gradients and a gradual O<sub>2</sub> decline over the Namibian shelf, where the level of dissolved O<sub>2</sub> are known to be variable [50]. In the open-ocean off Peru, ventilation of the OMZ from above is hindered due to strong stratification [51]. The dissolved O<sub>2</sub> content is perhaps most stable within the core of the OMZ, where the highest O<sub>2</sub> sensitivity of anammox was measured in our current study (180 m at St. 36). With O<sub>2</sub> concentrations consistently below 1–2 μmol L<sup>-1</sup>, anammox bacteria thriving therein are unlikely to have adapted to higher O<sub>2</sub> levels compared to their counterparts in more dynamic environments.

Alternatively, marine snow particles have been speculated to provide “anoxic” micro-environments in which O<sub>2</sub> is sufficiently depleted to favor N-loss at ambient O<sub>2</sub> levels <25 μmol L<sup>-1</sup> [9,20], while some anammox bacteria have been shown to be potentially particle-associated in the Namibian OMZ [20]. Hence, higher abundance of particles in coastal waters than further offshore or in the core of the OMZ might also explain the apparently higher O<sub>2</sub> tolerance by anammox bacteria near the coast.

### Oxygen sensitivity of nitrate reduction in OMZ waters

The reduction of NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup>, was detected at high rates at the shallow shelf stations both off Namibia and Peru (~100 to 360 nmol L<sup>-1</sup> d<sup>-1</sup>) and decreased with increasing distance from the coast in the Peruvian OMZ (~10 to 50 nmol L<sup>-1</sup> d<sup>-1</sup> at St. 36). The rates measured off Peru are consistent with earlier results from <sup>15</sup>N-labeling experiments in the same region [29,30] and a similar rate distribution was recently reported for the Arabian Sea OMZ [52,53].

Reduction of NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup> showed a high degree of variability in O<sub>2</sub> sensitivity amongst stations. No effect of increasing O<sub>2</sub> on NO<sub>3</sub><sup>-</sup> reduction was observed in the 120 m incubations at St. 36. At the remaining stations, the correlation between activity and adjusted O<sub>2</sub> concentrations was non-linear and could be best described by an exponential function, as determined by least-squares fitting (Table 3 & Fig. 3b). Our results from two shelf stations in the Namibian (St. 252) and Peruvian (St. 62) OMZs further confirmed earlier observations by Lipschultz et al. [29] that NO<sub>3</sub><sup>-</sup> reduction was only moderately affected by increasing O<sub>2</sub>. About 50% of NO<sub>3</sub><sup>-</sup> reduction activity remained when O<sub>2</sub> was adjusted to ~14 to 17 μmol L<sup>-1</sup> in our above-mentioned samples (Table 3). More pronounced sensitivity to O<sub>2</sub> was detected at St. 206 on the Namibian shelf and at 180 m at St. 36 off Peru, where rates were reduced by ~50% relative to the control already at ~4 μmol L<sup>-1</sup> of O<sub>2</sub>.

The observation, that in general NO<sub>3</sub><sup>-</sup> reduction activity was only moderately affected by increasing concentrations of O<sub>2</sub> may at first seem at odds with the fact that NO<sub>3</sub><sup>-</sup> respiration is generally considered an anaerobic process. However, it has been reported from experiments with cultures and environmental samples that complete or partial denitrification can take place under aerobic conditions [54–56]. Moreover, the different enzymes involved in the step-wise reduction on NO<sub>3</sub><sup>-</sup> to N<sub>2</sub> during denitrification, differ in their O<sub>2</sub> sensitivity. In various bacterial strains the NO<sub>2</sub><sup>-</sup> and nitrous oxide (N<sub>2</sub>O) reductase appear to be most sensitive with respect to O<sub>2</sub>, whereas the NO<sub>3</sub><sup>-</sup> reductase is the most O<sub>2</sub>-tolerant enzyme [57–59]. This O<sub>2</sub> tolerance could explain the observation that even the highest O<sub>2</sub> additions did not lead to a full inhibition of NO<sub>3</sub><sup>-</sup> reduction in the samples taken from the Namibian and Peruvian OMZ waters. However, the detected variability in terms of O<sub>2</sub> sensitivity among the different incubation experiments and the lack of any response

**Table 3.** Overview of the response of NH<sub>3</sub> oxidation, NO<sub>3</sub><sup>-</sup> reduction and anammox to changes in dissolved O<sub>2</sub>.

Process	Region	Station	Sampled depth (m)	Substrate addition	Upper OMZ boundary (m) †	<i>in situ</i> O <sub>2</sub> ‡	O <sub>2</sub> at 50% rate reduction §
<b>NH<sub>3</sub> oxidation</b>	Namibian OMZ	206	100	<sup>15</sup> NH <sub>4</sub> <sup>+</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	77	2.1	no trend observed
	Namibian OMZ	252	105	<sup>15</sup> NH <sub>4</sub> <sup>+</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	64	0.0	no trend observed
	Peruvian OMZ	44	75	<sup>15</sup> NH <sub>4</sub> <sup>+</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	52	0.7	no trend observed
	Peruvian OMZ	54	75	<sup>15</sup> NH <sub>4</sub> <sup>+</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	26	0.0	no trend observed
<b>NO<sub>3</sub><sup>-</sup> reduction</b>	Peruvian OMZ	36	120	<sup>15</sup> NO <sub>3</sub> <sup>-</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	51	1.2	no trend observed
	Namibian OMZ	252	105	<sup>15</sup> NO <sub>3</sub> <sup>-</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	64	0.0	17.3
	Peruvian OMZ	62	50	<sup>15</sup> NO <sub>3</sub> <sup>-</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	26	2.6	14.7
	Peruvian OMZ	36	180	<sup>15</sup> NO <sub>3</sub> <sup>-</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	51	0.0	4.1
	Namibian OMZ	206	100	<sup>15</sup> NO <sub>3</sub> <sup>-</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	77	2.1	3.6
<b>Anammox</b>	Namibian OMZ	206	100	<sup>15</sup> NH <sub>4</sub> <sup>+</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	77	2.1	16.0
	Namibian OMZ	252	105	<sup>15</sup> NH <sub>4</sub> <sup>+</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	64	0.0	11.0
	Namibian OMZ	206	100	<sup>15</sup> NO <sub>2</sub> <sup>-</sup>	77	0.0	10.9
	Namibian OMZ	252	105	<sup>15</sup> NO <sub>2</sub> <sup>-</sup>	64	2.1	10.6
	Peruvian OMZ	44	75	<sup>15</sup> NH <sub>4</sub> <sup>+</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	52	0.7	10.1
	Black Sea*	1	100	<sup>15</sup> NH <sub>4</sub> <sup>+</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	~75	<1	8.6
	Black Sea*	1	100	<sup>15</sup> NO <sub>2</sub> <sup>-</sup>	~75	<1	7.1
	Peruvian OMZ	62	50	<sup>15</sup> NO <sub>2</sub> <sup>-</sup> + <sup>14</sup> NH <sub>4</sub> <sup>+</sup>	26	2.6	5.8
	Peruvian OMZ	36	120	<sup>15</sup> NO <sub>2</sub> <sup>-</sup> + <sup>14</sup> NH <sub>4</sub> <sup>+</sup>	51	1.2	4.7
	Peruvian OMZ	54	75	<sup>15</sup> NH <sub>4</sub> <sup>+</sup> + <sup>14</sup> NO <sub>2</sub> <sup>-</sup>	26	0.0	2.4
Peruvian OMZ	36	180	<sup>15</sup> NO <sub>2</sub> <sup>-</sup> + <sup>14</sup> NH <sub>4</sub> <sup>+</sup>	51	0.0	1.9	

†Here defined as water depth where O<sub>2</sub> drops below 25 μmol L<sup>-1</sup>.

‡In μmol L<sup>-1</sup>. Calculated from regression functions obtained by least-squares fitting of the data given in Table 2.

\*Jensen et al. 2008.

doi:10.1371/journal.pone.0029299.t003

at 120 m at St. 36 remains puzzling. One possible explanation might be the high phylogenetic diversity and thus variable physiology of the NO<sub>3</sub><sup>-</sup> reducers inhabiting the OMZ waters [30,60].

### Oxygen sensitivity of ammonia oxidation in OMZ waters

Ammonia oxidizing activity seemed widespread throughout the OMZ overlying the Namibian shelf, as indicated by high NO<sub>2</sub><sup>-</sup> production rates. Off Peru, nitrifying activity peaked at the base of the oxycline, where the highest NH<sub>4</sub><sup>+</sup> release due to remineralization of sinking organic matter can be expected. Though O<sub>2</sub> was not always detectable *in situ*, NH<sub>3</sub> oxidation rates could be detected at these upper OMZ depths, consistent with previous studies [30,33,35].

In the O<sub>2</sub> sensitivity assays, NH<sub>3</sub> oxidation at most decreased slightly in the anoxic control (St. 54) when compared to the higher O<sub>2</sub> treatments. No stimulation at higher O<sub>2</sub> levels (20 to 25 μmol L<sup>-1</sup> of O<sub>2</sub>) was achieved. A similar observation was made by Lipschultz et al. [29], though they detected a 50% reduction of activity in their assumedly anoxic control. Our results suggest a relatively high O<sub>2</sub> affinity of aerobic NH<sub>3</sub> oxidizers in both OMZs investigated. It has been shown that cultured bacterial NH<sub>3</sub> oxidizers, including marine nitrifiers, are, in principle, able to cope with very low O<sub>2</sub> concentrations down to at least ~2 μmol L<sup>-1</sup> [61–63]. The only cultured marine aerobic ammonia oxidizing archaea investigated so far appears to have a limited capacity to survive under near anoxic conditions [64]. However, a higher O<sub>2</sub> affinity of archaeal NH<sub>3</sub> oxidizers in the environment is indicated by results from the Peruvian OMZ,

which suggest that both bacterial and archaeal NH<sub>3</sub> oxidizers are active at undetectable *in situ* O<sub>2</sub> levels (<1.5–2 μmol L<sup>-1</sup>) [30].

Based on our findings, the minimum O<sub>2</sub> concentration for NH<sub>3</sub> oxidizer to be active in OMZ waters is most likely in the nanomolar range. An adaptation of aerobic micro-organisms to extremely low O<sub>2</sub> has been shown in a recent study by Stolper et al. [65]. They demonstrated aerobic growth in a culture experiment at an O<sub>2</sub> concentration ≤3 nmol L<sup>-1</sup>. Alternatively, when O<sub>2</sub> is scarce, NH<sub>3</sub> oxidizer may also grow anaerobically via the oxidation of NH<sub>3</sub> with gaseous nitrogen dioxide (NO<sub>2</sub>) or tetraoxide (N<sub>2</sub>O<sub>4</sub>) [66]. However, as these compounds are rare in the marine environment, it is unlikely that this is of major ecological significance.

### Implications for N-loss in the future ocean and our understanding of N-cycling in modern OMZs

In summary, the current study shows that O<sub>2</sub> is a major controlling factor for anammox activity in OMZ waters. Based on our O<sub>2</sub> assays we estimate the upper limit for anammox to be ~20 μmol L<sup>-1</sup> O<sub>2</sub>, which is significantly higher than previously shown for the Black Sea (Table 3 & Fig. 3). In contrast, NH<sub>3</sub> oxidation and NO<sub>3</sub><sup>-</sup> reduction as the main NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup> sources for anammox were little or only moderately affected by changing concentrations of dissolved O<sub>2</sub>. Intriguingly, aerobic NH<sub>3</sub> oxidation was active at non-detectable O<sub>2</sub> concentrations, while NO<sub>3</sub><sup>-</sup> reduction to NO<sub>2</sub><sup>-</sup>, which is generally considered to be an anaerobic process, was fully active up to at least 25 μmol L<sup>-1</sup> O<sub>2</sub>. Hence, aerobic and anaerobic N-cycle pathways in OMZs can co-occur over a larger range of O<sub>2</sub> concentrations

than previously assumed. The zone where N-loss can occur is primarily controlled by the O<sub>2</sub>-sensitivity of anammox and not by the O<sub>2</sub>-sensitivity of the tightly coupled aerobic NH<sub>3</sub> oxidation and anaerobic NO<sub>3</sub><sup>-</sup> reduction.

Additionally, our results indicate that N-loss and other N-cycling processes within such O<sub>2</sub> regimes would be controlled by other environmental factors such as substrate availability. For instance, the (near) anoxic conditions in the core of the OMZ do not confer the highest NO<sub>3</sub><sup>-</sup> reduction and anammox rates despite the ideal O<sub>2</sub> regime. Surface water productivity and therewith export of particulate organic matter into the OMZ might play an important role in controlling anammox activity. Sinking organic matter is the ultimate source of the required reactive substrates NO<sub>2</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> for anammox and it may also provide suitable anoxic micro-environments for anammox bacteria in zones of higher ambient O<sub>2</sub> [9,20].

The fact that anammox in the marine environment can proceed at O<sub>2</sub> levels ~20 times higher than those known to inhibit enrichment cultures of anammox bacteria (~1 μmol L<sup>-1</sup>) [22] enlarges the global oceanic volume potentially affected by N-loss from the previously estimated 0.1% tenfold to ~1% (O<sub>2</sub> ≤ 20 μmol L<sup>-1</sup>) [67]. In addition, recent reports show that OMZs have been expanding and intensifying worldwide, particularly in the tropical Atlantic and Pacific [13]. Such expansions of the OMZs would mean an even greater increase in ocean volume potentially subject to active N-loss processes in the coming years. In other words, progressively more fixed inorganic N may be removed from the oceans, and larger areas in the subtropics and tropics might experience enhanced N-limitation due to the recharge of N-deficient waters back to the surface in the future. In the long run, negative feedbacks might also ensue from increasing N-loss and ocean warming. Less productive surface waters would export less organic matter to subsurface waters and lead to reduced O<sub>2</sub> consumption rates. The stronger stratification due to the warming of the upper ocean might also hamper

upwelling of nutrient-rich water to the surface, therewith reducing export production and the respiration of O<sub>2</sub> in OMZs.

The relative significance of these positive and negative feedback mechanisms, or how they may counteract each other and eventually influence global oceanic nutrient budgets, would require further investigations complemented with realistic global biogeochemical modeling. To date, the models used to develop future scenarios of the global ocean nutrient balance have rarely taken into account coupling N-cycling processes, and certainly not their respective O<sub>2</sub> sensitivities.

In light of the above presented results, the simple switching from aerobic to anaerobic respiration at ~5 μmol L<sup>-1</sup> of O<sub>2</sub> often implemented in models [23] appears not realistic. The current study provides the first robust estimates of O<sub>2</sub> sensitivities for processes directly and indirectly connected with N-loss. These factors are necessary for biogeochemical models to collectively and accurately assess the effects of ocean de-oxygenation on N-cycling in OMZs and neighboring water masses, and hence global oceanic N-balance.

## Acknowledgments

We sincerely thank the cruise leaders Kay Emeis (M76/2) and Martin Frank (M77/3) as well as the crews of the cruises onboard R/V Meteor for their support at sea. We are grateful for the technical and analytical assistance of Gabriele Klockgether, Daniela Franzke, Inka Boosmann, Violeta Leon, Aurelien Paulmier, Moritz Holtappels, Andreas Ellrott, Volker Meyer, Philipp Hach and Michael Jünemann. We thank Gail Arnold and Rachel Foster for reading the early version of the manuscript and offering valuable comments to improve the article.

## Author Contributions

Conceived and designed the experiments: MMJ JL GL MMMK. Performed the experiments: TK MMJ SC GL. Analyzed the data: TK MMJ MG PL. Contributed reagents/materials/analysis tools: NPR. Wrote the paper: TK PL GL MMMK.

## References

- Falkowski PG (2008) Earth's Biogeochemical Cycles. *Science* 320: 1034–1039.
- Wyrski K (1962) Circulation and Water Masses in the Eastern Equatorial Pacific Ocean. *Deep-Sea Research*. pp 11–13.
- Karstensen J, Stramma L, Visbeck M (2008) Oxygen minimum zones in the eastern tropical Atlantic and Pacific oceans. *Progress in Oceanography* 77: 331–350.
- Kamykowski D, Zentara S (1990) Hypoxia in the world ocean as recorded in the historical data set. *Deep-Sea Research* 37(12): 1861–1874.
- Helly JJ, Levin LA (2004) Global distribution of naturally occurring marine hypoxia on continental margins. *Deep-Sea Research* 51: 1159–1168.
- Revsbech NP, Larsen LH, Gundersen J, Dalsgaard T, Ulloa O, et al. (2009) Determination of ultra-low oxygen concentrations in oxygen minimum zones by the STOX sensor. *Limnology and Oceanography: Methods* 7: 371–381.
- Codispoti LA, Brandes JA, Christensen JP, Devol AH, Naqvi SW, et al. (2001) The oceanic fixed nitrogen and nitrous oxide budgets: Moving targets as we enter the anthropocene? *Scientia Marina* 65: 85–105.
- Gruber N, Sarmiento JL (1997) Global Patterns of Marine Nitrogen Fixation and Denitrification. *Global Biogeochemical Cycles* 11: 235–266.
- Kuypers MM, Lavik G, Woebken D, Schmid M, Fuchs BM, et al. (2005) Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation. *Proc Natl Acad Sci USA* 102(18): 6478–6483.
- Hamersley MR, Lavik G, Woebken D, Rattray JE, Lam P, et al. (2007) Anaerobic ammonium oxidation in the Peruvian oxygen minimum zone. *Limnology and Oceanography* 52(3): 923–933.
- Thamdrup B, Dalsgaard T, Jensen MM, Ulloa O, Farias L, et al. (2006) Anaerobic ammonium oxidation in the oxygen-deficient waters off northern Chile. *Limnology and Oceanography* 51(5): 2145–2156.
- Naqvi SW, Jayakumar DA, Narvekar PV, Naik H, Sarma VV, et al. (2000) Increased marine production of N<sub>2</sub>O due to intensifying anoxia on the Indian continental shelf. *Nature* 408(6810): 346–349.
- Stramma L, Johnson GC, Sprintall J, Mohrholz V (2008) Expanding Oxygen-Minimum Zones in the Tropical Oceans. *Science* 320(5876): 655–658.
- Keeling RF, Körtzinger A, Gruber N (2009) Ocean Deoxygenation in a Warming World. *Annual Review of Marine Science*. pp 463–493.
- Diaz RJ, Rosenberg R (2008) Spreading Dead Zones and Consequences for Marine Ecosystems. *Science* 321(5891): 926–929.
- Duce RA, LaRoche J, Altieri K, Arrigo KR, Baker AR, et al. (2008) Impacts of Atmospheric Anthropogenic Nitrogen on the Open Ocean. *Science* 320(5878): 893–897.
- Deutsch C, Brix H, Ito T, Frenzel H, Thompson L (2011) Climate-Forced Variability of Ocean Hypoxia. *Science* 33: 336–339.
- Smethie Jr, WM (1987) Nutrient regeneration and denitrification in low oxygen fjords. *Deep-Sea Research* 34(5–6): 983–1006.
- Ploug H (2001) Small-scale oxygen fluxes and remineralization in sinking aggregates. *Limnology and Oceanography* 46(7): 1624–1631.
- Woebken D, Fuchs BA, Kuypers MA, Amann R (2007) Potential interactions of particle-associated anammox bacteria with bacterial and archaeal partners in the Namibian upwelling system. *Applied and Environmental Microbiology* 73(14): 4648–4657.
- Galán A, Molina V, Thamdrup B, Woebken D, Lavik G, et al. (2009) Anammox bacteria and the anaerobic oxidation of ammonium in the oxygen minimum zone off northern Chile. *Deep-Sea Research Part II* 56(16): 1021–1031.
- Strous M, Van Gerven E, Kuenen JG, Jetten M (1997) Effects of Aerobic and Microaerobic Conditions on Anaerobic Ammonium-Oxidizing (Anammox) Sludge. *Applied and Environmental Microbiology* 63(6): 2446–2448.
- Paulmier A, Krist I, Oschlies A (2009) Stoichiometries of remineralisation and denitrification in global biogeochemical ocean models. *Biogeosciences* 6(1): 2539–2566.
- Jensen MM, Kuypers MM, Lavik G, Thamdrup B (2008) Rates and regulation of anaerobic ammonium oxidation and denitrification in the Black Sea. *Limnology and Oceanography* 53(1): 23–36.
- Zumft WG (1997) Cell Biology and Molecular Basis of Denitrification. *Microbiology and Molecular Biology Reviews* 61(4): 533–616.
- Cline JD, Richards FA (1972) Oxygen Deficient Conditions and Nitrate Reduction in the Eastern Tropical North Pacific Ocean. *Limnology and Oceanography* 17(6): 885–900.
- Codispoti LA, Packard TT (1980) Denitrification Rates in the Eastern Tropical South-Pacific. *Journal of Marine Research* 38(3): 453–477.

28. Gonzales PJ, Correia C, Moura I, Brondino CD, Moura JJ (2006) Bacterial nitrate reductases: Molecular and biological aspects of nitrate reduction. *Journal of Inorganic Biochemistry* 100: 1015–1023.
29. Lipschultz F, Wofsy SC, Ward BB, Codispoti LA, Friedrich G, et al. (1990) Bacterial transformations of inorganic nitrogen in the oxygen-deficient waters of the Eastern Tropical South Pacific Ocean. *Deep-Sea Research* 37(10): 1513–1541.
30. Lam P, Lavik G, Jensen MM, van De Vossenbergh J, Schmid M, et al. (2009) Revising the nitrogen cycle in the Peruvian oxygen minimum zone. *Proc Natl Acad Sci USA* 106(12): 4752–4757.
31. Dalsgaard T, Canfield DE, Petersen J, Thamdrup B, Acuna-Gonzalez J (2003) N<sub>2</sub> production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. *Nature* 422(6932): 606–608.
32. Ward BB, Zafriou OC (1988) Nitrification and nitric oxide in the oxygen minimum of the eastern tropical North Pacific. *Deep-Sea Research* 35(7): 1127–1142.
33. Ward BB, Glover HE, Lipschultz F (1989) Chemoautotrophic Activity and Nitrification in the Oxygen Minimum Zone off Peru. *Deep-Sea Research* 36(7): 1031–1051.
34. Lam P, Jensen MM, Lavik G, McGinnis DF, Muller B, et al. (2007) Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea. *Proc Natl Acad Sci USA* 104(17): 7104–7109.
35. Molina V, Farias L (2009) Aerobic ammonium oxidation in the oxycline and oxygen minimum zone of the eastern tropical South Pacific off northern Chile (~20°S). *Deep-Sea Research Part II* 56(16): 1032–1041.
36. Holmes RM, Aminot A, Keroul R, Hooker BA, Peterson BJ (1999) A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* 56: 1801–1808.
37. Grasshoff K, Ehrhardt K, Kremling K, Anderson LG (1999) Determination of nutrients. In: *Methods of seawater analysis*. Wiley, pp 159–226.
38. Holtappels M, Lavik G, Jensen MM, Kuypers MM (2011) N-labeling experiments to dissect the contributions of heterotrophic denitrification and anammox to nitrogen removal in the OMZ waters of the ocean. *Methods in Enzymology* 486: 223–251.
39. McIlvin MR, Altabet MA (2005) Chemical Conversion of Nitrate and Nitrite to Nitrous Oxide for Nitrogen and Oxygen Isotopic Analysis in Freshwater and Seawater. *Analytical Chemistry* 77(17): 5589–5595.
40. Granger J, Sigman DM, Prokopenko MG, Lehmann MF, Tortell PD (2006) A method for nitrite removal in nitrate N and O isotope analyses. *Limnology and Oceanography: Methods* 4: 205–212.
41. Füssel J, Lam P, Lavik G, Jensen MM, Holtappels M, et al. (2011) Nitrite oxidation in the Namibian oxygen minimum zone. *ISME Journal*. In press.
42. Hall GH (1984) Measurement of Nitrification Rates in Lake Sediments: Comparison of the Nitrification Inhibitors Nitrapyrin and Allylthiourea. *Microbial Ecology* 10(1): 25–36.
43. Hall GH, Jeffries C (1984) The Contribution of Nitrification in the Water Column and Profundal Sediments to the Total Oxygen Deficit of the Hypolimnion of a Mesotrophic Lake (Grasmere, English Lake District). *Microbial Ecology* 10(1): 37–46.
44. Bedard C, Knowles R (1989) Physiology, Biochemistry, and Specific Inhibitors of CH<sub>4</sub>, NH<sub>4</sub><sup>+</sup>, and CO Oxidation by Methanotrophs and Nitrifiers. *Microbiological Reviews* 53(1): 68–84.
45. Jensen MM, Thamdrup B, Dalsgaard T (2007) Effects of Specific Inhibitors on Anammox and Denitrification in Marine Sediments. *Applied and Environmental Microbiology* 73(10): 3151–3158.
46. Kemmer G, Keller S (2010) Nonlinear least-squares data fitting in Excel spreadsheets. *Nature Protocols* 5(2): 267–281.
47. Carr M (2002) Estimation of potential productivity in Eastern Boundary Currents using remote sensing. *Deep-Sea Research* 49: 59–80.
48. Lavik G, Stührmann T, Bruchert V, Van Der Plas A, Mohrholz V, et al. (2008) Detoxification of sulphidic African shelf waters by blooming chemolithotrophs. *Nature* 457: 581–584.
49. Bohlen L, Dale AW, Sommer S, Mosch T, Hensen C, et al. (2011) Benthic nitrogen cycling traversing the Peruvian oxygen minimum zone. *Geochimica et Cosmochimica Acta* 75(20): 6094–6111.
50. Chapman P, Shannon LV (1985) The Benguela ecosystem. Part II. Chemistry and related processes. *Mar Biol Ann Rev* 23: 183–251.
51. Fuenzalida R, Schneider W, Garces-Vargas J, Bravo L, Lange C (2009) Vertical and horizontal extension of the oxygen minimum zone in the eastern South Pacific Ocean. *Deep-Sea Research II* 56: 992–1003.
52. Nicholls JC, Davies CA, Trimmer M (2007) High-resolution profiles and nitrogen isotope tracing reveal a dominant source of nitrous oxide and multiple pathways of nitrogen gas formation in the central Arabian Sea. *Limnology and Oceanography* 52(1): 156–168.
53. Lam P, Jensen MM, Kock A, Lettmann KA, Plancherel Y, et al. (2011) Origin and fate of the secondary nitrite maximum in the Arabian Sea. *Biogeosciences* 8: 1565–1577.
54. Robertson LA, Kuenen JG (1984) Aerobic denitrification: a controversy revived. *Archives of Microbiology* 139(4): 351–354.
55. Robertson LA, Dalsgaard T, Revsbech N, Kuenen JG (1995) Confirmation of ‘aerobic denitrification’ in batch cultures, using gas chromatography and <sup>15</sup>N mass spectrometry. *FEMS Microbiology Ecology* 18(2): 113–119.
56. Gao H, Schreiber F, Collins G, Jensen MM, Kostka JE, et al. (2009) Aerobic denitrification in permeable Wadden Sea sediments. *ISME Journal* 4(3): 417–426.
57. Körner H, Zumft WG (1989) Expression of Denitrification Enzymes in Response to the Dissolved Oxygen Level and Respiratory Substrate in Continuous Culture of *Pseudomonas stutzeri*. *Applied and Environmental Microbiology* 55(7): 1670–1676.
58. Coyne MS, Tiedje JM (1990) Induction of denitrifying enzymes in oxygen-limited *Achromobacter cycloclastes* continuous culture. *FEMS Microbiol Ecology* 73: 263–270.
59. McKenney DJ, Drury CF, Findlay WI, Mutus B, McDonnell T, et al. (1994) Kinetics of denitrification by *Pseudomonas fluorescens*: Oxygen Effects. *Soil Biology & Biochemistry* 26(7): 901–908.
60. Hartsock A, Shapleigh JP (2011) Physiological Roles for Two Periplasmic Nitrate Reductases in *Rhodobacter Sphaeroides* 2.4.3 (ATCC 17025). *Journal of Bacteriology* 193(23): 6483–6489.
61. Gundersen K (1966) The Growth and Respiration of *Nitrosocystis oceanus* at Different Partial Pressures of Oxygen. *J gen Microbiol* 42: 387–396.
62. Carlucci AF, McNally PM (1969) Nitrification by marine bacteria in low concentrations of substrate and oxygen. *Limnology and Oceanography* 14(5): 736–739.
63. Goreau TJ, Kaplan WA, Wofsy SC, McElroy MB, Valois FW, et al. (1980) Production of NO<sub>2</sub><sup>-</sup> and N<sub>2</sub>O by Nitrifying Bacteria at Reduced Concentrations of Oxygen. *Applied and Environmental Microbiology* 40(3): 526–532.
64. Martens-Habbena W, Berube PM, Urakawa H, de La Torre JR, Stahl DA (2009) Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature* 461(7266): 976–979.
65. Stolper DA, Revsbech NP, Canfield DE (2010) Aerobic growth at nanomolar oxygen concentrations. *Proc Natl Acad Sci USA* 107(44): 18755–18760.
66. Schmidt I, Bock E (1997) Anaerobic ammonia oxidation with nitrogen dioxide by *Nitrosomonas eutropha*. *Arch Microbiol* 167: 106–111.
67. Lam P, Kuypers MM (2011) Microbial Nitrogen Cycling Processes in Oxygen Minimum Zones. *Annual Review of Marine Science*. pp 317–347.