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

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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 $\sim$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_2) on anammox, NH_3 oxidation and NO_3^- reduction in ^{15}N-labeling experiments with varying O_2 concentrations (0–25 μmol L^{-1}) in the Namibian and Peruvian OMZs. Our results show that O_2 is a major controlling factor for anammox activity in OMZ waters. Based on our O_2 assays we estimate the upper limit for anammox to be $\sim$20 μmol L^{-1}. In contrast, NH_3 oxidation to NO_2^- and NO_3^- reduction to NO_2^- as the main NH_4^+ and NO_2^- sources for anammox were only moderately affected by changing O_2 concentrations. Intriguingly, aerobic NH_3 oxidation was active at non-detectable concentrations of O_2, while anaerobic NO_3^- reduction was fully active up to at least 25 μmol L^{-1} O_2. Hence, aerobic and anaerobic N-cycle pathways in OMZs can co-occur over a larger range of O_2 concentrations than previously assumed. The zone where N-loss can occur is primarily controlled by the O_2-sensitivity of anammox itself, and not by any effects of O_2 on the tightly coupled pathways of aerobic NH_3 oxidation and NO_3^- reduction. With anammox bacteria in the marine environment being active at O_2 levels $\sim$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_2 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.

Introduction

Oxygen (O_2) is one of the key regulatory factors of major biogeochemical cycles in the marine environment [1]. The distribution of dissolved O_2 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_2 and fixes carbon dioxide (CO_2) in to biomass. Near the base of the euphotic zone, concentrations of O_2 are generally at their lowest as photosynthesis diminishes or ceases altogether while the repiration of sinking organic matter by heterotrophic micro-organisms consumes O_2 at maximal rates.

Subsurface regions of severely reduced O_2 concentrations ($O_2 \leq 5$ μmol L^{-1}), 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_2-depleted to anoxic conditions at mid-depths [4–6].

Although OMZs (if defined by $O_2 \leq 5$ μmol L^{-1}) account for only $\sim$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_2) can occur via two pathways: (1) heterotrophic denitrification, the reduction of nitrate (NO_3^-) to gaseous dinitrogen (N_2) via a sequence of

intermediates ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) and (2) anammox, the anaerobic oxidation of ammonium (NH_4^+) with nitrite (NO_2^-) to 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 ^{15}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_2 due to reduced O_2 solubility and enhanced stratification [14], as well as coastal and open ocean eutrophication [15,16], is expected. De-oxygenation will have the greatest effect on water masses already deficient in O_2 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_2 content by only 1% would mean a doubling of water masses with $O_2 \leq 5$ µmol 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_2 and their upper O_2 limits in the marine environment are largely unknown. N-loss attributed to denitrification has been reported to occur at up to 20 µmol L^{-1} of O_2 [18]. Nonetheless, direct measurements of denitrification under controlled exposure to low O_2 concentrations in OMZs are lacking. Active anammox bacteria have been found to be abundant at O_2 concentrations up to 9 and 20 µmol 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_2 concentrations up to 25 µmol 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_2 concentrations as low as 1 µmol L^{-1} [22], marine anammox bacteria can tolerate O_2 concentrations higher than the upper O_2 limit (5 µmol L^{-1}) often used to restrict anaerobic processes in biogeochemical models [23]. Recently, Jensen et al. [24] investigated the O_2 sensitivity of anammox in the near-anoxic zone of the Black Sea water column and showed that anammox bacteria remained active up to ~9 µmol L^{-1} of O_2. Still unknown is whether this relatively high O_2 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 NO_2^- and NH_4^+, e.g. NH_3 oxidation to NO_2^- and heterotrophic nitrate (NO_3^-) reduction to 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_2 concentrations. Thus far, it remains unclear whether or not processes coupled to anammox can proceed in the same range of O_2 as assumed for anammox (0–20 µmol L^{-1}), or if they show different O_2 sensitivities that might hence restrict N-loss to a narrower O_2 regime. Under anoxic conditions, 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 NO_2^- maxima are often interpreted as active NO_3^- reduction [26,27]. The formation of NO_2^- from 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 NO_3^- to NO_2^- than to N_2 or NH_4^+ [25,28]. Heterotrophic NO_3^- reduction to 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 NO_2^- required for anammox in this region [30]. At the same time, NO_3^- reduction also provides an important source of NH_4^+ released from oxidized organic matter [30,31]. Lipschultz et al. [29] investigated the effect of varying O_2 concentrations on NO_3^- reduction to NO_2^- in the Peruvian OMZ. They observed that NO_3^- reduction rates doubled under anoxic conditions (N_2 atmosphere) compared to in situ conditions (2.5 µmol L^{-1} of O_2), while rates decreased by ~75% at 20 µmol L^{-1} of O_2.

When O_2 is present, NO_2^- can be produced aerobically by NH_3 oxidizing bacteria and archaea in the first step in nitrification. Rates of 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 NH_3 oxidation in this zone [30]. A similarly tight coupling between anammox and NH_3 oxidation was shown earlier for the Black Sea [34]. The occurrence of NH_3 oxidizers is, however, not restricted to the upper OMZ. They have been found active at non-detectable concentrations of O_2 (<1–2 µmol L^{-1}) in the core of OMZs [30,33,35] and are thus obviously well adapted to near-anoxic O_2 conditions. When Lipschultz et al. [29] investigated the O_2 sensitivity of NH_3 oxidation in the Peruvian OMZ, the inferred de-oxygenation of the samples only caused a ~50% decrease in activity relative to ambient O_2 (2.5 µmol L^{-1}), whereas no stimulation was achieved by an increase to ~20 µmol L^{-1} of O_2.

With anammox as well as NO_3^- reduction being apparently tolerant to relatively high O_2 and NH_3 oxidation being apparently able to cope with severe O_2 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_2. However, at present no study has systematically investigated the O_2 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_2 on anammox, NH_3 oxidation and 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 ~375 m depth off the coast of Peru. The pump CTD system was equipped with a conventional amperometric O_2 micro-sensor to obtain vertical profiles of dissolved O_2. In addition, the recently developed STOX (Switchable Trace amount OXygen) sensor [6], which allows high-accuracy O_2 measurements in near-anoxic environments (detection limit: 50–
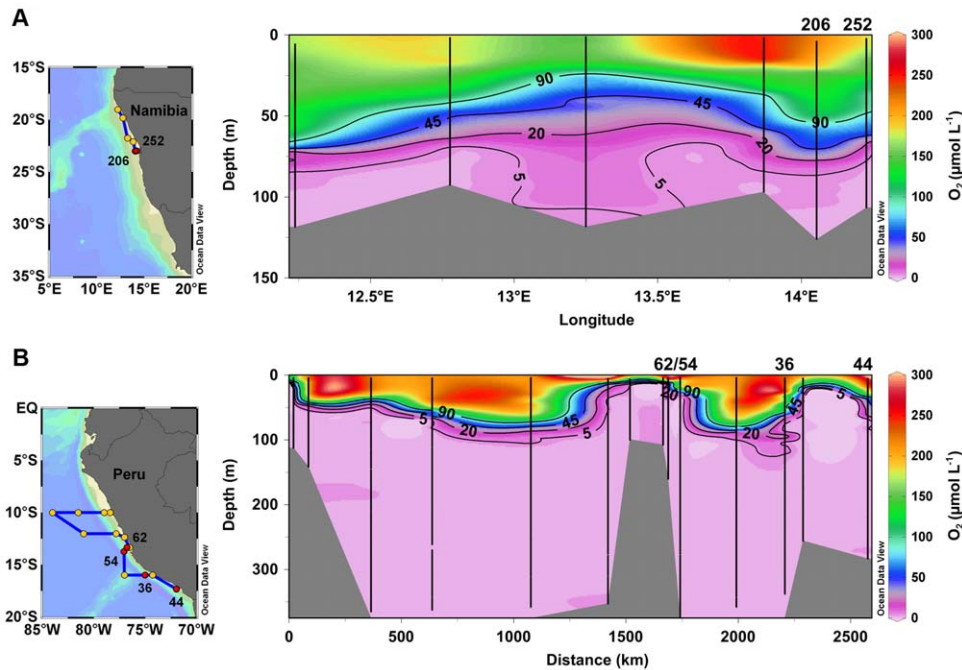
Figure 1. Locations of the sampled stations and distribution of dissolved O$_2$. Maps show sampling locations on the A) Namibian shelf and in the B) OMZ off Peru during cruises M76-2 and M77-3, respectively. Water samples were collected by pump-CTD (max. sampling depth: ~375 m). The oxygen sensitivities of anammox and coupled N-cycling processes were investigated at sampling stations indicated by numbers (red circles). Vertical distributions of dissolved O$_2$ are plotted along blue lines.
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100 nmol L^{-1} 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$_2$ concentrations. Water samples were taken with a depth resolution of 1–2 m for nutrient analyses. NH$_4^+$ was measured fluorometrically [36] and NO$_2^-$ was analyzed spectrophotometrically [37] on board. Water samples for NO$_3^-$ and PO$_4^{3-}$ were stored frozen until spectrophotometric determination [37] with an autoanalyzer (TRAACS 800, Bran & Lubbe) in a shore-based laboratory. Detection limits for NH$_4^+$, NO$_2^-$, NO$_3^-$ and PO$_4^{3-}$ were 10, 10, 100 and 100 nmol L^{-1}, respectively. N-deficits were calculated from the measured fixed inorganic N- and PO$_4^{3-}$ concentrations as N* (in µmol L^{-1}) following Gruber and Sarmiento [8]: N* = [NH$_4^+$]+[NO$_2^-$]+[NO$_3^-$]−16×[PO$_4^{3-}$]+2.9 µmol kg^{-1}× density in kg L^{-1}.

^{15}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$_2$ profiles, three to six depths per station were chosen for a standard series of ^{15}N-labeling experiments. The experimental procedure for ^{15}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 ^{15}N-labeled N$_2$ in ^{15}NH$_4^+$ (+^{14}NO$_2^-$), ^{15}NO$_2^-$ (+^{14}NH$_4^+$) and ^{15}NO$_3^-$ (+^{14}NO$_2^-$) (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$_2$ gas produced in these experiments was determined by GC/

IRMS (Fisons VG Optima). Afterwards, rates of NH$_3$ oxidation to NO$_2^-$ and those of NO$_3^-$ reduction to NO$_2^-$ were determined in the same samples as net ^{15}NO$_2^-$ production in ^{15}NH$_4^+$+^{14}NO$_2^-$ and ^{15}NO$_3^-$+^{14}NO$_2^-$ incubations respectively. The N-isotopic composition of NO$_2^-$ was determined by GC/IRMS after conversion to either nitrous oxide (N$_2$O) by sodium azide [39], or to N$_2$ by sulfamic acid [40,41]. Rates were calculated from the slope of linear regression of ^{15}N-production as a function of time. Only significant and linear production of ^{15}N-species without an initial lag-phase was considered (t-tests, $p < 0.05$; $R^2 > 0.8$). The net production rates presented here have been corrected for the mole fractions of ^{15}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$_2$ concentrations on N-cycle processes, one to two depths per station were sampled for additional O$_2$ 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$_2$ and to lower the N$_2$ background in order to enhance the detection limit of ^{29}N$_2$ and ^{30}N$_2$ [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$_2$ concentration. At St. 206 and 252 (Namibian OMZ) three samples each were

Table 1. Concentrations of O_2, NH_4^+, NO_2^- and N-conversion rates in ^{15}N-labeling experiments in the OMZs off Namibia and Peru.

Station (water depth) [latitude/longitude]	Depth (m)	in situ O_2 § ‡	NH_4^+ §	NO_2^- §	NH$_3$ oxidation† $^{15}NH_4^+ + ^{14}NO_2^-$	NO$_3^-$ reduction† $^{15}NO_3^- + ^{14}NO_2^-)$	Anammox† $^{15}NH_4^+ + ^{14}NO_2^-$	$^{15}NO_2^- + ^{14}NH_4^+$
Namibian OMZ **M76-206 (131 m)** [23.01°S/14.05°E]	90	3.39±0.15	0.01	0.21	29±2*	81±9*	36±1*	13±2*
	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) [23.00°S/14.23°E]	76	1.11±0.25	0.12	0.14	93±9	370±111	42±15	43±8*
	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)** [16.00°S/75.00°W]	90	1.49±0.11	0.05	0.12	35±3	42±2		2.3±0.4
	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) [17.34°S/71.94°W]	75	0.73±0.09	0.14	0.01	19±4	no data	5.1±0.3	
	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) [13.75°S/77.03°W]	41	3.64±0.10	0.06	0.28	47±2	72±3	5.8±1.7	
	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) [13.35°S/76.75°W]	40	9.97±0.10	0.40	0.57	0.2±0.1	108±16		25±3
	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 ^{14}N-species.
§In µmol L^{-1}.
‡Determined with STOX sensor.
†In nmol N L^{-1} d^{-1}.
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adjusted to ~3.5, 7.5 and 12 µmol L^{-1} of O_2, 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^{-1} of O_2. One sample, to which no air-saturated water was added, served as an anoxic control at all stations. After additions of either $^{15}NH_4^+ + ^{14}NO_2^-$, $^{15}NO_2^-$ $(+^{14}NH_4^+)$ or $^{15}NO_3^- + ^{14}NO_2^-$, samples were transferred into replicate vials (Exetainers, Labco) for time-series incubations. Except for the incubations with only $^{15}NO_2^-$, ^{14}N-species were added to all experiments to exclude substrate limitation, which would otherwise complicate the interpretation of any O_2 effects on the processes of interest. Moreover, keeping the ^{14}N-pool of the product of a certain reaction well above the expected concentrations produced from the added ^{15}N-substrate could minimize any further conversion of the newly formed ^{15}N-products by co-occurring processes. The rate measurements for the various processes were carried out as described above. To exclude formation of $^{29}N_2$ due to coupled nitrification-denitrification in incubations amended with $^{15}NH_4^+$ we added allylthiourea (ATU; final concentration 84 µmol L^{-1}) to an additional sample of the highest O_2 treatment (~11.5 µmol L^{-1}) at St. 206 and 252. ATU is a specific inhibitor of aerobic NH$_3$ 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_2. For the remaining stations, O_2 concentrations were determined only for the initial time-point in each ^{15}N-incubation experiment. We used a custom-built, fast-responding O_2 micro-sensor (Clark-type; MPI Bremen) for most measurements (detection limit: ~0.5 µmol L^{-1} of O_2), 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$_2$ 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$_2$ and inorganic N-species (Fig. 2A). At both stations O$_2$ declined gradually with depth, from ~200 μmol L^{-1} in the surface waters to less than 10 μmol L^{-1} at ~80 m. STOX measurements at the incubation depths revealed O$_2$ concentrations as low as 0.60 ± 0.11 μmol L^{-1} at St. 206. In the central OMZ at St. 252 (Table 1), the sensor was at its detection limit (100 nmol L^{-1} of O$_2$ during M76-2). Ammonium concentrations were typically in the range of 1–3 μmol L^{-1} in the oxic zone (<80 m) and decreased to 0.1–0.5 μmol L^{-1} at the base of the oxycline (Fig. 2B). Towards the sediment-water interface NH$_4^+$ concentrations increased up to 4.5 (St. 206) and 2.5 μmol L^{-1} (St. 252). Nitrite concentrations were fairly constant in the upper ~100 m (0.1–0.5 μmol L^{-1}) and increased to ~2 and ~4 μmol L^{-1} in the bottom waters at St. 206 and 252, respectively. The increase in both NO$_2^-$ and NH$_4^+$ in the lower OMZ was accompanied by a sharp decrease in NO$_3^-$ concentrations, with minimum concentrations of ~12 μmol L^{-1} 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$_2$ 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^{-1} in the surface to less than 10 μmol L^{-1} at 66 (St. 44), 35 (St. 54) and 75 m (St. 36). A local O$_2$ maximum (10 to 25 μmol L^{-1}) 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$_2$ in the central OMZ at best; mostly here O$_2$ concentrations remained below the detection limit of the STOX sensor (~50 nmol L^{-1} of O$_2$ during M77-3). Ammonium concentrations were low and typically 0.05 to 0.1 μmol L^{-1} throughout the OMZ as well as in the surface layer (Fig. 2B). On the shelf, concentrations of NH$_4^+$ were slightly elevated at the base of the oxycline (up to ~0.4 μmol L^{-1} at St. 62). At the open-ocean stations (St. 54 and 36) NH$_4^+$ maxima of ~2 μmol L^{-1} were measured at 20 and 35 m, which coincided with NO$_2^-$ maxima (up to 1 μmol L^{-1}). In general, NO$_2^-$ concentrations in the surface waters remained below 0.5 μmol L^{-1}, whereas NO$_2^-$ accumulated to over 5 μmol L^{-1} in the core of the OMZ at all stations. Nitrate concentrations were as low as ~1 μmol L^{-1} on the shelf (St. 62). Further off-shore less pronounced NO$_3^-$ concentration minima were detected (~12 at St. 44 and ~20 μmol L^{-1} 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^{-1}) were found in the central OMZ, suggesting N-loss therein. We measured ^{15}N^{14}N formation in all of our ^{15}NH$_4^+$ (+^{14}NO$_2^-$) and ^{15}NO$_2^-$-incubations at the three depths sampled per station (Table 1). Corrected for the labeling percentage (i.e. the mole fraction of ^{15}N in the respective N-substrate pool), rates were comparable in ^{15}NH$_4^+$ and ^{15}NO$_2^-$ experiments. As no increase in ^{15}N^{15}N was detectable in either ^{15}NO$_2^-$ or ^{15}NO$_3^-$ incubations, the formation of ^{15}N-labeled N$_2$ 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^{-1} d^{-1} at the base of the oxycline to 144 to 496 nmol N L^{-1} d^{-1} in the central OMZ and were generally higher at St. 252.
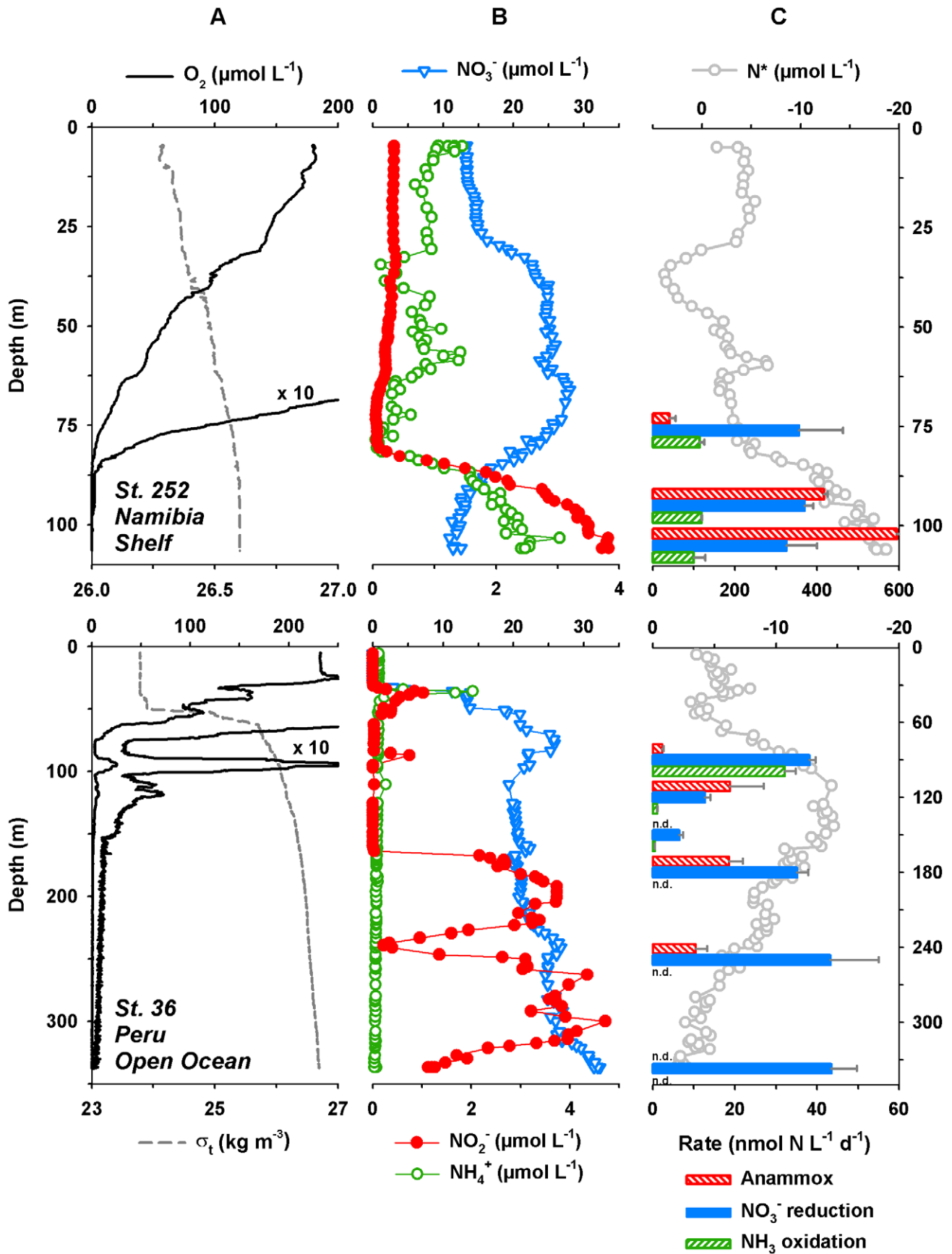
In the OMZ off Peru, the N-deficit was strongest over the shelf (N* = −33 μmol L^{-1}; St. 62) and less pronounced towards the open ocean (N* = 10 μmol L^{-1}; St. 54), indicating the highest N-loss likely occurred near the coast. Six depths per station were sampled and ^{15}N^{14}N formation in ^{15}NH$_4^+$+^{14}NO$_2^-$ and ^{15}NO$_2^-$+^{14}NH$_4^+$ was measured in 22 out of 24 incubation depths (Table 1). No formation of ^{15}N-labeled N$_2$ was detectable at 150 and 337 m at St. 36. As for the Namibian OMZ, whenever N$_2$ formation occurred all of the ^{15}N-labeled N$_2$ produced was recovered as ^{29}N$_2$ and there was no detectable increase in ^{15}N^{15}N over time detected in either ^{15}NO$_2^-$ or ^{15}NO$_3^-$ incubations. Thus, anammox was the only detectable active N$_2$-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^{-1} d^{-1}; 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^{-1} d^{-1}; St. 54).

Distribution of nitrate reduction to nitrite activity. Nitrate reduction was measured as ^{15}NO$_2^-$ production in all ^{15}NO$_3^-$+^{14}NO$_2^-$ 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^{-1} d^{-1} at St. 206 and 252, respectively (Table 1).

Off Peru, NO$_3^-$ reduction could be detected in 21 out of 23 ^{15}NO$_3^-$+^{14}NO$_2^-$ incubation experiments. The vertical distribution of NO$_3^-$ reducing activity was slightly variable and high NO$_3^-$ reduction rates did not always coincide with a noticeable accumulation of NO$_2^-$. Similar to anammox activity, maximum rates of NO$_3^-$ reduction were generally detected over the shelf (up to 215 nmol N L^{-1} d^{-1}) and decreased towards the open ocean (up to 48 nmol N L^{-1} d^{-1}).

Distribution of ammonia oxidation activity. Ammonia oxidation, measured as ^{15}NO$_2^-$ production in ^{15}NH$_4^+$+(^{14}NO$_2^-$) incubation experiments, was detected at all incubation depths (Table 1). At St. 206 ^{15}N-labeling experiments were carried out under anoxic conditions, whereas samples were incubated at *in situ* O$_2$ (<1 μmol L^{-1}) at St. 252. Rates increased with depth at St. 206 (from 29 to 84 nmol N L^{-1} d^{-1}) but remained rather constant at St. 252 (~100 nmol N L^{-1} d^{-1}).

Off Peru, NH$_3$ oxidation to NO$_2^-$ was determined in ^{15}NH$_4^+$+^{14}NO$_2^-$ incubations under anoxic conditions (St. 44 and 54) or at *in situ* O$_2$ levels (St. 36 and 62). Maximum NH$_3$ oxidation rates ranged between 15 and 47 nmol N L^{-1} d^{-1}. 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$_2$ was still measurable.

However, despite an apparent lack of O₂ *in situ* (i.e. O₂ concentrations were below detection) shipboard experiments revealed NH₃ 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^{-1} d^{-1}).

Oxygen sensitivity of anammox and coupled N-cycle processes

Oxygen sensitivity of anammox. Anammox activity, as indicated by $^{15}N^{14}N$ production from $^{15}NH_4^+$ and $^{15}NO_2^-$, was measurable in all O₂ manipulation experiments without lag phase at the Namibian shelf stations (Table 2). Oxygen concentration and N₂ formation showed a significant negative correlation for the incubations with $^{15}NH_4^+$ as well as $^{15}NO_2^-$ at St. 206 and the one with $^{15}NH_4^+$ at St. 252 (Pearson $r = -0.95$ to -0.99, P<0.05). Similar responses to increased O₂ were observed for the incubations amended with $^{15}NH_4^+$ and $^{15}NO_2^-$ at both stations. Activity decreased with increasing O₂ and was, on average, ~85%, ~70% and ~50% of the anoxic control at ~3.7, ~8.1 and ~11.3 μmol L^{-1} of oxygen, respectively (Fig. 3A). Over the course of the incubation (0–48 h) O₂ concentrations in the ^{15}N-labeling experiments did not vary significantly (±0.44 μmol L^{-1} on average). No substantial difference in $^{15}N^{14}N$ production was observed between $^{15}NH_4^+$-labeled incubations with and without ATU. This indicates that anammox rather than coupled nitrification-denitrification was the process responsible for the production of ^{15}N-labeled N₂ at 11–12 μmol L^{-1} of dissolved O₂.

In the OMZ off Peru, $^{15}N^{14}N$ production rates in $^{15}NH_4^+$ and $^{15}NO_2^-$ incubations decreased with increasing O₂ concentrations in all O₂ manipulation experiments. However, substantial differences in the O₂ sensitivity of anammox were found between stations. Over the Peruvian shelf, adjusted O₂ levels and N₂ production were linearly and negatively correlated up to 14.4 μmol L^{-1} O₂ at St. 44 (Pearson $r = -0.99$, P<0.05) and 10.9 μmol L^{-1} at St. 62 (Pearson $r = -0.96$, P<0.05). No rates were detectable beyond ~20 μmol L^{-1} of O₂. At the open-ocean stations in the Peruvian OMZ, anammox activity appeared to be more sensitive to the added O₂ (Fig. 3A). At St. 36, ~30% activity of the anoxic control experiment remained detectable when O₂ was increased from the in situ ~1.2 μmol L^{-1} (measured by STOX) to 5.5 μmol L^{-1} of O₂ in the 120 m sample. In comparison, anammox was fully inhibited at 2.8 μmol L^{-1} of O₂ already in the 180 m sample, where O₂ was not detectable by the STOX sensor *in situ*. A similarly strong O₂ response was seen at St. 54, where rates dropped to zero at 4.0 μmol L^{-1} of O₂ in the 75 m incubation experiment.

Oxygen sensitivity of nitrate reduction to nitrite. Nitrate reduction rates in the O₂ sensitivity assay carried out for the Namibian OMZ waters, decreased with increasing O₂ concentrations (Table 2). The incubation experiments at St. 206 revealed a stronger negative response to elevated O₂ levels than those performed at St. 252. Activity at St. 206 was reduced to ~30% of the anoxic control in the highest O₂ treatment (7.3 μmol L^{-1}), whereas a doubling of the O₂ concentration (14.7 μmol L^{-1}) led to a decrease in NO_3^- reduction rates to ~60% of the control experiment at St. 252 (Fig. 3B).

In the Peruvian OMZ, production of $^{15}NO_2^-$ from $^{15}NO_3^-$ was never fully inhibited by O₂, not even in the highest O₂

treatments (~25 μmol L^{-1} of O₂). Nevertheless, NO_3^- reduction rates showed marked differences in their sensitivity towards elevated O₂ levels between and within our experimental stations. For example at St. 36, NO_3^- reduction activity in the upper OMZ sample (120 m) at St. 36 did not vary significantly among the various O₂ treatments (1.4 to 27.1 μmol L^{-1} of O₂), 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^{-1} of O₂ (Figure 3B).

Oxygen sensitivity of ammonia oxidation. Rates of NH₃ oxidation to NO_2^- showed no significant difference over the range of the applied O₂ concentrations (~1–12 μmol L^{-1}) in the Namibian OMZ samples (Table 2). Activity varied by a maximum of ~15% among the different O₂ treatments but without any systematic trends (Fig. 3C).

Similar to the observations for the Namibian shelf, $^{15}NO_2^-$ production in the $^{15}NH_4^+$ experiments conducted for the Peruvian shelf (St. 44) and at open-ocean (St. 54) stations showed no marked differences among the different O₂ treatments (~1–25 μmol L^{-1}). Only the control experiment (0.8 μmol L^{-1} O₂) at St. 54 suggested a slightly lower NH₃ oxidation rate (−35%) compared to the higher O₂ treatments (Fig. 3C).

Discussion

Oxygen sensitivity of anammox in OMZ waters

In the investigated samples from both the Namibian and Peruvian OMZ, the only N₂-forming pathway detected by ^{15}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^{-1} d^{-1}) were measured in the Namibian shelf waters. Off Peru, rates declined from ~50 nmol N L^{-1} d^{-1} over the shelf to <10 nmol N L^{-1} d^{-1} 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_4^+ and NO_2^- 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_4^+ 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_4^+ diffusing out of the sediment [9,48,49] (S. Sommer, pers. comm.).

In the O₂ tolerance assays, N-loss due to anammox was in fact detectable at O₂ levels significantly higher (up to ~15 μmol L^{-1}) than that generally used to define OMZs (<5 μmol L^{-1} of O₂). Anammox activity in samples taken at the shallow sites appeared the least affected by increasing O₂. The rates therein remained measurable even at adjusted O₂ concentrations of 10 to 15 μmol L^{-1}. These are almost twice as high as the anammox O₂-tolerance level previously determined in the Black Sea suboxic zone [24]. In comparison, anammox activity appeared increas-

Table 2. Rates of NH$_3$ oxidation, NO$_3^-$ reduction and anammox measured at varying concentrations of dissolved O$_2$.

Substrate additions:		NH$_3$ oxidation ^{15}NH$_4^+$+^{14}NO$_2^-$ O$_2$ §‡	Rate†	NO$_3^-$ reduction ^{15}NO$_3^-$+^{14}NO$_2^-$ O$_2$ §‡	Rate†	Anammox ^{15}NH$_4^+$+^{14}NO$_2^-$ O$_2$ §‡	Rate†	^{15}NO$_2^-$+^{14}NH$_4^+$ O$_2$ §‡	Rate†
Namibian	M76-206	2.0	70±5	0.8	65±2	2.0	122±3	0.8	119±10 *
OMZ	(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	M77-36			1.4	22.3±2.5			0.6	10.1±1.2
OMZ	(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 ^{14}N-species.
§In µmol L^{-1}.
‡Adjusted concentrations of O$_2$, determined by µ-sensor measurements.
†In nmol N L^{-1} d^{-1}.
doi:10.1371/journal.pone.0029299.t002

ingly sensitive to O$_2$ towards the open ocean and deeper in the OMZ, where rates were not detectable above 2.8 to 5.5 µmol L^{-1} of O$_2$ (St. 36 and 54). Based on the observed negative linear correlation between the measured rates and adjusted O$_2$ levels, the upper O$_2$ limit for anammox to proceed in the OMZs is estimated to be ~20 µmol L^{-1} (Table 3 & Fig. 3).
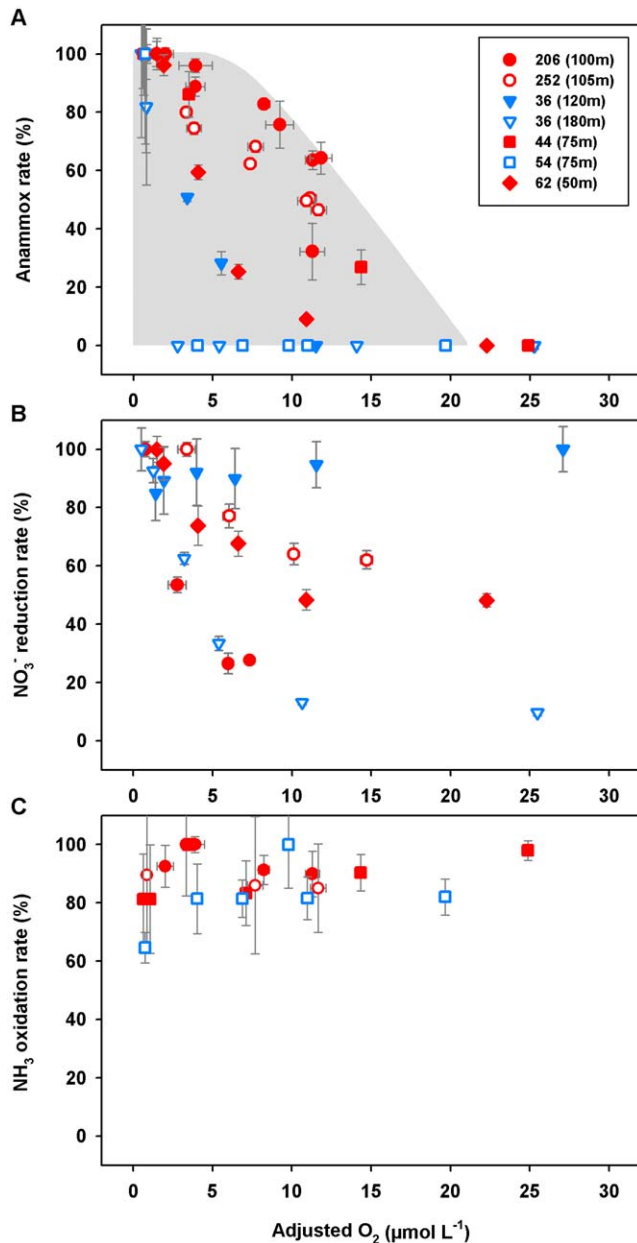
Figure 3. N-cycle processes in Namibian and Peruvian OMZ waters with respect to dissolved oxygen. A) Anammox measured as $^{15}N^{14}N$ production in $^{15}NO_2^-$ ($+^{14}NH_4^+$) and $^{15}NH_4^+ + ^{14}NO_2^-$ incubations. B) NO_3^- reduction measured as $^{15}NO_2^-$ production in $^{15}NO_3^- + ^{14}NO_2^-$ incubations. C) NH$_3$ oxidation measured as $^{15}NO_2^-$ production in $^{15}NH_4^+ + ^{14}NO_2^-$ incubations. N-conversion rates are given as percentages of the highest rate observed (= 100%) for the different O$_2$ treatments at each incubation depth. Adjusted O$_2$ 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$_2$ sensitivity assays indicate an upper O$_2$ limit for N-loss due to anammox of ~20 µmol L^{-1} (grey shading).
doi:10.1371/journal.pone.0029299.g003

The apparently higher O$_2$ tolerance at the shelf stations may be explained by an adaptation of anammox bacteria to fluctuations in dissolved O$_2$ 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$_2$ decline over the Namibian shelf, where the level of dissolved O$_2$ 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$_2$ content is perhaps most stable within the core of the OMZ, where the highest O$_2$ sensitivity of anammox was measured in our current study (180 m at St. 36). With O$_2$ concentrations consistently below 1–2 µmol L^{-1}, anammox bacteria thriving therein are unlikely to have adapted to higher O$_2$ levels compared to their counterparts in more dynamic environments.

Alternatively, marine snow particles have been speculated to provide "anoxic" micro-environments in which O$_2$ is sufficiently depleted to favor N-loss at ambient O$_2$ levels <25 µmol L^{-1} [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$_2$ tolerance by anammox bacteria near the coast.

Oxygen sensitivity of nitrate reduction in OMZ waters

The reduction of NO_3^- to NO_2^-, was detected at high rates at the shallow shelf stations both off Namibia and Peru (~100 to 360 nmol L^{-1} d^{-1}) and decreased with increasing distance from the coast in the Peruvian OMZ (~10 to 50 nmol L^{-1} d^{-1} at St. 36). The rates measured off Peru are consistent with earlier results from ^{15}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_3^- to NO_2^- showed a high degree of variability in O$_2$ sensitivity amongst stations. No effect of increasing O$_2$ on NO_3^- reduction was observed in the 120 m incubations at St. 36. At the remaining stations, the correlation between activity and adjusted O$_2$ 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_3^- reduction was only moderately affected by increasing O$_2$. About 50% of NO_3^- reduction activity remained when O$_2$ was adjusted to ~14 to 17 µmol L^{-1} in our above-mentioned samples (Table 3). More pronounced sensitivity to O$_2$ 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^{-1} of O$_2$.

The observation, that in general NO_3^- reduction activity was only moderately affected by increasing concentrations of O$_2$ may at first seem at odds with the fact that NO_3^- 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_3^- to N_2 during denitrification, differ in their O$_2$ sensitivity. In various bacterial strains the NO_2^- and nitrous oxide (N_2O) reductase appear to be most sensitive with respect to O$_2$, whereas the NO_3^- reductase is the most O$_2$-tolerant enzyme [57–59]. This O$_2$ tolerance could explain the observation that even the highest O$_2$ additions did not lead to a full inhibition of NO_3^- reduction in the samples taken from the Namibian and Peruvian OMZ waters. However, the detected variability in terms of O$_2$ sensitivity among the different incubation experiments and the lack of any response

Table 3. Overview of the response of NH$_3$ oxidation, NO$_3^-$ reduction and anammox to changes in dissolved O$_2$.

Process	Region	Station	Sampled depth (m)	Substrate addition	Upper OMZ boundary (m) †	in situ O$_2$ §	O$_2$ at 50% rate reduction §
NH$_3$ oxidation	Namibian OMZ	206	100	$^{15}NH_4^+ + ^{14}NO_2^-$	77	2.1	no trend observed
	Namibian OMZ	252	105	$^{15}NH_4^+ + ^{14}NO_2^-$	64	0.0	no trend observed
	Peruvian OMZ	44	75	$^{15}NH_4^+ + ^{14}NO_2^-$	52	0.7	no trend observed
	Peruvian OMZ	54	75	$^{15}NH_4^+ + ^{14}NO_2^-$	26	0.0	no trend observed
NO$_3^-$ reduction	Peruvian OMZ	36	120	$^{15}NO_3^- + ^{14}NO_2^-$	51	1.2	no trend observed
	Namibian OMZ	252	105	$^{15}NO_3^- + ^{14}NO_2^-$	64	0.0	17.3
	Peruvian OMZ	62	50	$^{15}NO_3^- + ^{14}NO_2^-$	26	2.6	14.7
	Peruvian OMZ	36	180	$^{15}NO_3^- + ^{14}NO_2^-$	51	0.0	4.1
	Namibian OMZ	206	100	$^{15}NO_3^- + ^{14}NO_2^-$	77	2.1	3.6
Anammox	Namibian OMZ	206	100	$^{15}NH_4^+ + ^{14}NO_2^-$	77	2.1	16.0
	Namibian OMZ	252	105	$^{15}NH_4^+ + ^{14}NO_2^-$	64	0.0	11.0
	Namibian OMZ	206	100	$^{15}NO_2^-$	77	0.0	10.9
	Namibian OMZ	252	105	$^{15}NO_2^-$	64	2.1	10.6
	Peruvian OMZ	44	75	$^{15}NH_4^+ + ^{14}NO_2^-$	52	0.7	10.1
	Black Sea*	1	100	$^{15}NH_4^+ + ^{14}NO_2^-$	~75	<1	8.6
	Black Sea*	1	100	$^{15}NO_2^-$	~75	<1	7.1
	Peruvian OMZ	62	50	$^{15}NO_2^- + ^{14}NH_4^+$	26	2.6	5.8
	Peruvian OMZ	36	120	$^{15}NO_2^- + ^{14}NH_4^+$	51	1.2	4.7
	Peruvian OMZ	54	75	$^{15}NH_4^+ + ^{14}NO_2^-$	26	0.0	2.4
	Peruvian OMZ	36	180	$^{15}NO_2^- + ^{14}NH_4^+$	51	0.0	1.9

† Here defined as water depth where O$_2$ drops below 25 µmol L^{-1}.
§ In µmol L^{-1}. 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$_3^-$ 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$_2^-$ production rates. Off Peru, nitrifying activity peaked at the base of the oxycline, where the highest NH$_4^+$ release due to remineralization of sinking organic matter can be expected. Though O$_2$ was not always detectable in situ, NH$_3$ oxidation rates could be detected at these upper OMZ depths, consistent with previous studies [30,33,35].

In the O$_2$ sensitivity assays, NH$_3$ oxidation at most decreased slightly in the anoxic control (St. 54) when compared to the higher O$_2$ treatments. No stimulation at higher O$_2$ levels (20 to 25 µmol L^{-1} of O$_2$) 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$_2$ affinity of aerobic NH$_3$ oxidizers in both OMZs investigated. It has been shown that cultured bacterial NH$_3$ oxidizers, including marine nitrifiers, are, in principle, able to cope with very low O$_2$ concentrations down to at least ~2 µmol L^{-1} [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$_2$ affinity of archaeal NH$_3$ oxidizers in the environment is indicated by results from the Peruvian OMZ, which suggest that both bacterial and archaeal NH$_3$ oxidizers are active at undetectable in situ O$_2$ levels (<1.5–2 µmol L^{-1}) [30].

Based on our findings, the minimum O$_2$ concentration for NH$_3$ oxidizer to be active in OMZ waters is most likely in the nanomolar range. An adaptation of aerobic micro-organisms to extremely low O$_2$ has been shown in a recent study by Stolper et al. [65]. They demonstrated aerobic growth in a culture experiment at an O$_2$ concentration ≤3 nmol L^{-1}. Alternatively, when O$_2$ is scarce, NH$_3$ oxidizer may also grow anaerobically via the oxidation of NH$_3$ with gaseous nitrogen dioxide (NO$_2$) or tetraoxide (N$_2$O$_4$) [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$_2$ is a major controlling factor for anammox activity in OMZ waters. Based on our O$_2$ assays we estimate the upper limit for anammox to be ~20 µmol L^{-1} O$_2$, which is significantly higher than previously shown for the Black Sea (Table 3 & Fig. 3). In contrast, NH$_3$ oxidation and NO$_3^-$ reduction as the main NH$_4^+$ and NO$_2^-$ sources for anammox were little or only moderately affected by changing concentrations of dissolved O$_2$. Intriguingly, aerobic NH$_3$ oxidation was active at non-detectable O$_2$ concentrations, while NO$_3^-$ reduction to NO$_2^-$, which is generally considered to be an anaerobic process, was fully active up to at least 25 µmol L^{-1} O$_2$. Hence, aerobic and anaerobic N-cycle pathways in OMZs can co-occur over a larger range of O$_2$ concentrations

than previously assumed. The zone where N-loss can occur is primarily controlled by the O$_2$-senstivity of anammox and not by the O$_2$-senstivity of the tightly coupled aerobic NH$_3$ oxidation and anaerobic NO$_3^-$ reduction.

Additionally, our results indicate that N-loss and other N-cycling processes within such O$_2$ 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$_3^-$ reduction and anammox rates despite the ideal O$_2$ 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$_2^-$ and NH$_4^+$ for anammox and it may also provide suitable anoxic micro-environments for anammox bacteria in zones of higher ambient O$_2$ [9,20].

The fact that anammox in the marine environment can proceed at O$_2$ levels ~20 times higher than those known to inhibit enrichment cultures of anammox bacteria (~1 µmol L^{-1}) [22] enlarges the global oceanic volume potentially affected by N-loss from the previously estimated 0.1% tenfold to ~1% (O$_2 \leq$ 20 µmol L^{-1}) [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$_2$ 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$_2$ 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$_2$ sensitivities.

In light of the above presented results, the simple switching from aerobic to anaerobic respiration at ~5 µmol L^{-1} of O$_2$ often implemented in models [23] appears not realistic. The current study provides the first robust estimates of O$_2$ 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.

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.

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