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

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 Kalvelage1*, Marlene M. Jensen1a, Sergio Contreras1a, Niels Peter Revsbech2, Phyllis Lam1, Marcel Günter1, Julie LaRoche3, Gaute Lavik1, Marcel M. M. Kuypers1

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 (O2) on anammox, NH4 oxidation and NO3 reduction in 15N-labeling experiments with varying O2 concentrations (0–25 μmol L−1) in the Namibian and Peruvian OMZs. Our results show that O2 is a major controlling factor for anammox activity in OMZ waters. Based on our O2 assays we estimate the upper limit for anammox to be ~20 μmol L−1. In contrast, NH4 oxidation to NO2 and NO3 reduction to NO2 as the main NH4 and NO3 sources for anammox were only moderately affected by changing O2 concentrations. Intriguingly, aerobic NH4 oxidation was active at non-detectable concentrations of O2, while anaerobic NO3− reduction was fully active up to at least 25 μmol L−1 O2. Hence, aerobic and anaerobic N-cycle pathways in OMZs can co-occur over a larger range of O2 concentrations than previously assumed. The zone where N-loss can occur is primarily controlled by the O2-sensitivity of anammox itself, and not by any effects of O2 on the tightly coupled pathways of aerobic NH4 oxidation and NO3− reduction. With anammox bacteria in the marine environment being active at O2 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 O2 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 (O2) is one of the key regulatory factors of major biogeochemical cycles in the marine environment [1]. The distribution of dissolved O2 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 O2 and fixes carbon dioxide (CO2) in to biomass. Near the base of the euphotic zone, concentrations of O2 are generally at their lowest as photosynthesis diminishes or ceases altogether while the respiration of sinking organic matter by heterotrophic micro-organisms consumes O2 at maximal rates.

Subsurface regions of severely reduced O2 concentrations (O2≤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 O2-depleted to anoxic conditions at mid-depths [4–6].

Although OMZs (if defined by O2≤5 μmol L−1) 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 (N2) can occur via two pathways: (1) heterotrophic denitrification, the reduction of nitrate (NO3−) to gaseous dinitrogen (N2) 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 \(^15\text{N}\)-labeling experiments, whereas heterotrophic denitrification was often not detectable or only measured sporadically \cite{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 \cite{12,13}. A continuing decline in dissolved \(\text{O}_2\) due to reduced \(\text{O}_2\) solubility and enhanced stratification \cite{14}, as well as coastal and open ocean eutrophication \cite{15,16}, is expected. Deoxygenation will have the greatest effect on water masses already deficient in \(\text{O}_2\) as these are often at or near the thresholds for anaerobic processes such as anammox or denitrification. Deutsch et al. \cite{17} calculated that a reduction of the mean upper ocean \(\text{O}_2\) 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 \(\text{O}_2\) and their upper \(\text{O}_2\) 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 \(\text{O}_2\) \cite{18}. Nonetheless, direct measurements of denitrification under controlled exposure to low \(\text{O}_2\) concentrations in OMZs are lacking. Active anammox bacteria have been found to be abundant at \(\text{O}_2\) concentrations up to 9 and 20 \(\mu\text{mol L}^{-1}\) in the Namibian and Peruvian upwelling systems, respectively \cite{9,10}, and it has been suggested that marine snow aggregates could provide suitable anoxic micro-niches at ambient \(\text{O}_2\) concentrations up to 25 \(\mu\text{mol L}^{-1}\) \cite{19,20}. Off Peru/Chile the measured anammox rates were often the highest at the base of the oxycline and in the upper OMZ \cite{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 \(\text{O}_2\) concentrations as low as 1 \(\mu\text{mol L}^{-1}\) \cite{22}, marine anammox bacteria can tolerate \(\text{O}_2\) concentrations higher than the upper \(\text{O}_2\) limit (5 \(\mu\text{mol L}^{-1}\)) often used to restrict anaerobic processes in biogeochemical models \cite{23}. Recently, Jensen et al. \cite{24} investigated the \(\text{O}_2\) sensitivity of anammox in the near-anoxic zone of the Black Sea water column and showed that anammox bacteria remained active up to \(\sim 9\ \mu\text{mol L}^{-1}\) of \(\text{O}_2\). Still unknown is whether this relatively high \(\text{O}_2\) 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 \(\text{NO}_3^-\) and \(\text{NH}_4^+\), e.g. \(\text{NH}_4^+\) 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 \(\text{O}_2\) concentrations. Thus far, it remains unclear whether or not processes coupled to anammox can proceed in the same range of \(\text{O}_2\) as assumed for anammox \((0–20\ \mu\text{mol L}^{-1})\), or if they show different \(\text{O}_2\) sensitivities that might hence restrict N-loss to a narrower \(\text{O}_2\) 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 \cite{25}. In OMZ waters, secondary \(\text{NO}_2^-\) maxima are often interpreted as active \(\text{NO}_3^-\) reduction \cite{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 \(\text{NH}_4^+\) \cite{25,20}. Heterotrophic \(\text{NO}_3^-\) reduction to \(\text{NO}_2^-\) has been measured at high rates in the Peruvian OMZ \cite{29,30}, and has been estimated to account for approximately two thirds of the \(\text{NO}_2^-\) required for anammox in this region \cite{30}. At the same time, \(\text{NO}_3^-\) reduction also provides an important source of \(\text{NH}_4^+\) released from oxidized organic matter \cite{30,31}. Lipschultz et al. \cite{29} investigated the effect of varying \(\text{O}_2\) 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 \(\text{in situ}\) conditions (2.5 \(\mu\text{mol L}^{-1}\) of \(\text{O}_2\)), while rates decreased by \(\sim 75\%\) at 20 \(\mu\text{mol L}^{-1}\) of \(\text{O}_2\).

When \(\text{O}_2\) 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 \(\text{NH}_4^-\) oxidation are generally highest near the upper OMZ boundaries \cite{32,33}. In the Peruvian OMZ, this is also where anammox bacteria are most active \cite{10}. These bacteria are partly fueled by \(\text{NH}_3\) oxidation in this zone \cite{30}. A similarly tight coupling between anammox and \(\text{NH}_3\) oxidation was shown earlier for the Black Sea \cite{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 \(\text{O}_2\) (<1–2 \(\mu\text{mol L}^{-1}\)) in the core of OMZs \cite{30,33,35} and are thus obviously well adapted to near-anoxic \(\text{O}_2\) conditions. When Lipschultz et al. \cite{29} investigated the \(\text{O}_2\) 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 \(\text{O}_2\) (2.5 \(\mu\text{mol L}^{-1}\)), whereas no stimulation was achieved by an increase to \(\sim 20\ \mu\text{mol L}^{-1}\) of \(\text{O}_2\).

With anammox as well as \(\text{NO}_3^-\) reduction being apparently tolerant to relatively high \(\text{O}_2\) and \(\text{NH}_3\) oxidation being apparently able to cope with severe \(\text{O}_2\) 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 \(\text{CO}_2\). However, at present no study has systematically investigated the \(\text{O}_2\) 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 Peruvian upwelling systems, two of the most productive regions in the world’s oceans associated with massive N-loss, where we explored the effect of \(\text{O}_2\) 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 \(\sim 375\ \text{m}\) depth off the coast of Peru. The pump CTD system was equipped with a conventional ampereometric \(\text{O}_2\) micro-sensor to obtain vertical profiles of dissolved \(\text{O}_2\). In addition, the recently developed STOX (Switchable Trace amount \(\text{O}_2\)xygen) sensor \cite{6}, which allows high-accuracy \(\text{O}_2\) measurements in near-anoxic environments (detection limit: 50–
100 nmol L$^{-1}$ during our deployments), was deployed. At least five measuring cycles after $\pm$10 min sensor equilibration at a given sampling depth were used to calculate O$_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$_3^-$ 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 & Lube) in a shore-based laboratory. Detection limits for NH$_4^+$, NO$_3^-$, NO$_2^-$ 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 nmol L$^{-1}$) following Gruber and Sarmiento [8]: N$^* = [\text{NH}_4^+] + [\text{NO}_3^-] + [\text{NO}_2^-] - 16 \times [\text{PO}_4^{3-}] + 2.9$ nmol kg$^{-1}$ x density in kg L$^{-1}$.

15N 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 15N-labeling experiments. The experimental procedure for 15N-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 15N-labeled N$_2$ in 15NH$_4^+$ (+15NO$_2^-$), 15NO$_3^-$ (+15NH$_4^+$) and 15NO$_3^-$ (+15NO$_2^-$) (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 15NO$_2^-$ production in 15NH$_4^+$+15NO$_2^-$ and 15NO$_3^-$+15NO$_2^-$ 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 15N-production as a function of time. Only significant and linear production of 15N-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 15N 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 29N$_2$ and 30N$_2$ [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
adjusted to ~3.5, 7.5 and 12 μmol L⁻¹ of O₂, 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 O₂. One sample, to which no air-saturated water was added, served as an anoxic control at all stations. After additions of either ¹⁵NH₄⁺ + ¹⁴NO₂⁻, ¹⁵NO₂⁻ + ¹⁴NH₄⁺ or ¹⁵NO₃⁻ + ¹⁴NO₂⁻, samples were transferred into replicate vials (Exetainers, Labco) for time-series incubations. Except for the incubations with only ¹⁵NO₃⁻, ¹⁵N-species were added to all experiments to exclude substrate limitation, which would otherwise complicate the interpretation of any O₂ effects on the processes of interest. Moreover, keeping the ¹⁵N-pool of the product of a certain reaction well above the expected concentrations produced from the added ¹⁵N-substrate could minimize any further conversion of the newly formed ¹⁵N-products by co-occurring processes. The rate measurements for the various processes were carried out as described above. To exclude formation of ²⁵NO₂ due to coupled nitrification-denitrification in incubations amended with ¹⁵NH₄⁺ we added allylthio-urea (ATU; final concentration 84 μmol L⁻¹) to an additional sample of the highest O₂ treatment (~11.5 μmol L⁻¹) at St. 206 and 252. ATU is a specific inhibitor of aerobic NH₃ 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 O₂. For the remaining stations, O₂ concentrations were determined only for the initial time-point in each ¹⁵N-incubation experiment. We used a custom-built, fast-responding O₂ micro-sensor (Clark-type; MPI Bremen) for most measurements (detection limit: ~0.5 μmol L⁻¹ of O₂), except at St. 206 where a STOX sensor was used for selected samples.

Table 1. Concentrations of O₂, NH₄⁺, NO₂⁻ and N-conversion rates in ¹⁵N-labeling experiments in the OMZs off Namibia and Peru.

| Station (water depth) | Depth (m) | in situ O₂ | NH₄⁺ | NO₂⁻ | NH₄⁺ oxidation† | NO₂⁻ reduction† | Anammox† |
|-----------------------|-----------|------------|------|------|-----------------|----------------|----------|
| Namibian M76-206 (131 m) | 90 | 3.39±0.15 | 0.01 | 0.21 | 29±2* | 81±9* | 36±1* | 13±2* |
| OMZ [23.01°S/14.15°E] | 100 | 2.14±0.10 | 0.02 | 0.60 | 44±1* | 103±19* | 107±2* | 149±5* |
| M76-252 (111 m) | 110 | 0.6±0.11 | 2.01 | 0.90 | 84±5* | 97±23* | 144±10* | 153±4* |
| [23.00°S/14.23°E] | 76 | 1.11±0.25 | 0.12 | 0.14 | 93±9 | 370±111 | 42±15 | 43±8 |
| Peruvian M77-36 (2845 m) | 95 | 0.00±0.10 | 2.24 | 3.43 | 110±1 | 385±21 | 355±8 | 399±4* |
| OMZ [16.00°S/75.00°W] | 105 | 0.00±0.10 | 2.51 | 3.83 | 92±26 | 339±77 | 496±15 | 462±32* |
| M77-44 (281 m) | 10 | 1.49±0.11 | 0.05 | 0.12 | 35±3 | 42±2 | 2.3±0.4 |
| [17.34°S/71.94°W] | 75 | 0.73±0.09 | 0.14 | 0.01 | 19±4 | no data | 5.1±0.3 |
| M77-54 (1893 m) | 87 | 0.75±0.10 | 0.09 | 0.01 | 21±2 | 166±15 | 18±2 |
| [13.75°S/77.03°W] | 125 | 0.02±0.04 | 0.07 | 0.28 | 0.8±0.1 | 126±8 | 14±2 |
| [13.75°S/76.75°W] | 150 | 0.02±0.03 | 0.07 | 0.33 | 0.0 | 19±5 | 23±2 |
| 280 | 0.01±0.04 | 0.07 | 0.50 | 0.0 | 145±32 | 7.8±0.6 |
| M77-62 (160 m) | 75 | 0.00±0.05 | 0.03 | 0.93 | 5.0±0.4 | 71±1 | 6.3±2 |
| [13.35°S/76.75°W] | 100 | 0.00±0.04 | 0.04 | 4.01 | 0.0 | 71±8 | 3.0±0.2 |
| 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 |

*No addition of ¹⁴N-species.
†In μmol L⁻¹.
Determined with STOX sensor.
1 In mmol N L⁻¹ d⁻¹.

doi:10.1371/journal.pone.0029299.t001
Data analysis

We applied least-squares fitting to each set of samples of the O2 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 O2 and inorganic N-species (Fig. 2A). At both stations O2 declined gradually with depth, from ~200 μmol L−1 in the surface waters to less than 10 μmol L−1 at ~90 m. STOX measurements at the incubation depths revealed O2 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 O2 during M76-2). Ammonium concentrations were typically in the range of 1–3 μmol L−1 in the oxic zone (<30 m) and decreased to 0.1–0.5 μmol L−1 at the base of the oxycline (Fig. 2B). Towards the sediment-water interface NH4+ 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 NO2− and NH4+ in the lower OMZ was accompanied by a sharp decrease in NO3− 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 O2 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 (Fig. 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 O2 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 O2 in the central OMZ at best; mostly here O2 concentrations remained below the detection limit of the STOX sensor (~50 nmol L−1 of O2 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 NH4+ 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) NH4+ maxima of ~2 μmol L−1 were measured at 20 and 35 m, which coincided with NO2− maxima (up to 1 μmol L−1). In general, NO2− concentrations in the surface waters remained below 0.5 μmol L−1, whereas NO3− 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 offshore less pronounced NO3− 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 15N2-N formation in all of our 15NH4+ (+14NO3−) and 15NO2−-incubations at the three depths sampled per station (Table 1). Corrected for the labeling percentage (i.e. the mole fraction of 15N in the respective N-substrate pool), rates were comparable in 15NH4+ and 15NO2− experiments. As no increase in 15N2-N was detectable in either 15NO2− or 15NO3− incubations, the formation of 15N-labeled N2 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 15N14N formation in 15NH4++14NO3− and 15NO2−+14NH4+ was measured in 22 out of 24 incubation depths (Table 1). No formation of 15N-labeled N2 was detectable at 150 and 337 m at St. 36. As for the Namibian OMZ, whenever N2 formation occurred all of the 15N-labeled N2 was recovered as 15N2 and there was no detectable increase in 15N15N over time detected in either 15NO2− or 15NO3− incubations. Thus, anammox was the only detectable active N2-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 15NO3− production in all 15NO3−+14NO2− 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, NO3− reduction could be detected in 21 out of 23 15NO3−+14NO2− incubation experiments. The vertical distribution of NO3− reducing activity was slightly variable and high NO3− reduction rates did not always coincide with a noticeable accumulation of NO2−. Similar to anammox activity, maximum rates of NO3− 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 ammonium oxidation activity.

Ammonia oxidation, measured as 15NO2− production in 15NH4++15NO3− incubation experiments, was detected at all incubation depths (Table 1). At St. 206 15N-labeling experiments were carried out under anoxic conditions, whereas samples were incubated at in situ O2 (<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, NH3 oxidation to NO2− was determined in 15NH4++15NO3− incubations under anoxic conditions (St. 44 and 54) or at in situ O2 levels (St. 36 and 62). Maximum NH3 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 O2 was still measurable.
However, despite an apparent lack of O$_2$ in situ (i.e. O$_2$ concentrations were below detection) shipboard experiments revealed NH$_4^+$ oxidation activity also at St. 54 at 75 m as well as in the central OMZ at St. 62 (1.7 to 5.0 mmol 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 O$_2$ manipulation experiments without lag phase at the Namibian shelf stations (Table 2). Oxygen concentration and N$_2$ 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 O$_2$ were observed for the incubations amended with $^{15}$NH$_4^+$ and $^{15}$NO$_2^-$ at both stations.

Activity decreased with increasing O$_2$ and was, on average, ~85%, ~70% and ~50% of the anoxic control at ~3.7, ~8.1 and ~11.3 mmol L$^{-1}$ of oxygen, respectively (Fig. 3A). Over the course of the incubation (0–48 h) O$_2$ concentrations in the $^{15}$N-labeling experiments did not vary significantly (~0.44 mmol 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 N$_2$ at 11–12 mmol L$^{-1}$ of dissolved O$_2$.

In the OMZ off Peru, $^{15}$N-$^{14}$N production rates in $^{15}$NH$_4^+$ and $^{15}$NO$_2^-$ incubations decreased with increasing O$_2$ concentrations in all O$_2$ manipulation experiments. However, substantial differences in the O$_2$ sensitivity of anammox were found between stations. Over the Peruvian shelf, adjusted O$_2$ levels and N$_2$ production were linearly and negatively correlated up to 14.4 mmol L$^{-1}$ O$_2$ at St. 44 (Pearson $r = -0.99$, $P<0.05$) and 10.9 mmol L$^{-1}$ at St. 62 (Pearson $r = -0.96$, $P<0.05$). No rates were detectable beyond ~20 mmol L$^{-1}$ of O$_2$. At the open-ocean stations in the Peruvian OMZ, anammox activity appeared to be more sensitive to the added O$_2$ (Fig. 3A). At St. 36, ~30% activity of the anoxic control experiment remained detectable when O$_2$ was increased from the in situ ~1.2 mmol L$^{-1}$ (measured by STOX) to 5.5 mmol L$^{-1}$ of O$_2$ in the 120 m sample. In comparison, anammox was fully inhibited at 18.6 mmol L$^{-1}$ of O$_2$ already in the 180 m sample, where O$_2$ was not detectable by the STOX sensor in situ. A similarly strong O$_2$ response was seen at St. 54, where rates dropped to zero at 4.0 mmol L$^{-1}$ of O$_2$ in the 75 m incubation experiment.

**Oxygen sensitivity of nitrate reduction to nitrite.** Nitrate reduction rates in the O$_2$ sensitivity assay carried out for the Namibian OMZ waters, decreased with increasing O$_2$ concentrations (Table 2). The incubation experiments at St. 206 revealed a stronger negative response to elevated O$_2$ levels than those performed at St. 252. Activity at St. 206 was reduced to ~30% of the anoxic control in the highest O$_2$ treatment (7.3 mmol L$^{-1}$), whereas a doubling of the O$_2$ concentration (14.7 mmol 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$_3^-$ from $^{15}$NO$_2^-$ was never fully inhibited by O$_2$, not even in the highest O$_2$ treatments (~25 mmol L$^{-1}$ of O$_2$). Nevertheless, NO$_3^-$ reduction rates showed marked differences in their sensitivity towards elevated O$_2$ 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 O$_2$ treatments (1.4 to 27.1 mmol L$^{-1}$ of O$_2$), while activity decreased to ~10% of the control experiment in samples taken deeper (180 m) in the OMZ when adjusted to 25.5 mmol L$^{-1}$ of O$_2$ (Fig. 3B).

**Oxygen sensitivity of ammonia oxidation.** Rates of NH$_3$ oxidation to NO$_2^-$ showed no significant difference over the range of the applied O$_2$ concentrations (~1–12 mmol L$^{-1}$) in the Namibian OMZ samples (Table 2). Activity varied by a maximum of ~15% among the different O$_2$ 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 O$_2$ treatments (~1–25 mmol L$^{-1}$). Only the control experiment (0.8 mmol L$^{-1}$ O$_2$) at St. 54 suggested a slightly lower NH$_3$ oxidation rate (~35%) compared to the higher O$_2$ 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$_2$-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 mmol N L$^{-1}$ d$^{-1}$) were measured in the Namibian shelf waters. Off Peru, rates declined from ~50 mmol N L$^{-1}$ d$^{-1}$ over the shelf to <10 mmol 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 O$_2$ tolerance assays, N-loss due to anammox was in fact detectable at O$_2$ levels significantly higher (up to ~15 mmol L$^{-1}$) than that generally used to define OMZs (~5 mmol L$^{-1}$ of O$_2$). Anammox activity in samples taken at the shallow sites appeared the least affected by increasing O$_2$. The rates therein remained measurable even at adjusted O$_2$ concentrations of 10 to 15 mmol L$^{-1}$. These are almost twice as high as the anammox O$_2$-tolerance level previously determined in the Black Sea suboxic zone [24]. In comparison, anammox activity appeared increas-
ingly sensitive to O₂ towards the open ocean and deeper in the OMZ, where rates were not detectable above 2.8 to 5.5 μmol L⁻¹ of O₂ (St. 36 and 54). Based on the observed negative linear correlation between the measured rates and adjusted O₂ levels, the upper O₂ limit for anammox to proceed in the OMZs is estimated to be ~20 μmol L⁻¹ (Table 3 & Fig. 3).

### Table 2. Rates of NH₃ oxidation, NO₃⁻ reduction and anammox measured at varying concentrations of dissolved O₂.

| Substrate additions: | NH₃ oxidation | | NO₃⁻ reduction | | Anammox | |
|----------------------|----------------|----------------|------------------|----------------|------------------|----------------|
|                      | O₂ ↓↓ Rate↑    | O₂ ↓↓ Rate↑    | O₂ ↓↓ Rate↑      | O₂ ↓↓ Rate↑      | O₂ ↓↓ Rate↑      | O₂ ↓↓ Rate↑      |
| Namibian M76-206 (100 m) | 2.0 70±5        | 0.8 65±2        | 2.0 122±3         | 0.8 119±10 *    | 2.0 122±3         | 0.8 119±10 *    |
| +ATU                 | 11.8 78±7       | 11.8 78±7       | 11.8 78±7         | 11.8 78±7       | 11.8 78±7         | 11.8 78±7       |
| Peruvian M77-36 (120 m) | 1.4 22.3±2.5    | 0.6 10.1±1.2    | 1.4 22.3±2.5      | 0.6 10.1±1.2    | 1.4 22.3±2.5      | 0.6 10.1±1.2    |
| +ATU                 | 10.9 179±7      | 10.9 179±7      | 10.9 179±7        | 10.9 179±7      | 10.9 179±7        | 10.9 179±7      |
| M77-44 (75 m)        | 0.6 12.0±2.3    | 0.6 12.0±2.3    | 0.6 12.0±2.3      | 0.6 12.0±2.3    | 0.6 12.0±2.3      | 0.6 12.0±2.3    |
|                      | 1.1 12.0±2.7    | 1.1 12.0±2.7    | 1.1 12.0±2.7      | 1.1 12.0±2.7    | 1.1 12.0±2.7      | 1.1 12.0±2.7    |
|                      | 3.5 14.7±0.2    | 3.5 14.7±0.2    | 3.5 14.7±0.2      | 3.5 14.7±0.2    | 3.5 14.7±0.2      | 3.5 14.7±0.2    |
|                      | 7.1 12.3±1.6    | 7.1 12.3±1.6    | 7.1 12.3±1.6      | 7.1 12.3±1.6    | 7.1 12.3±1.6      | 7.1 12.3±1.6    |
|                      | 14.4 13.3±0.9   | 14.4 13.3±0.9   | 14.4 13.3±0.9     | 14.4 13.3±0.9   | 14.4 13.3±0.9     | 14.4 13.3±0.9   |
|                      | 24.9 14.5±0.5   | 24.9 14.5±0.5   | 24.9 14.5±0.5     | 24.9 14.5±0.5   | 24.9 14.5±0.5     | 24.9 14.5±0.5   |
| M77-54 (75 m)        | 0.8 5.6±0.4     | 0.8 5.6±0.4     | 0.8 5.6±0.4       | 0.8 5.6±0.4     | 0.8 5.6±0.4       | 0.8 5.6±0.4     |
|                      | 4.0 6.3±0.9     | 4.0 6.3±0.9     | 4.0 6.3±0.9       | 4.0 6.3±0.9     | 4.0 6.3±0.9       | 4.0 6.3±0.9     |
|                      | 6.9 6.3±0.5     | 6.9 6.3±0.5     | 6.9 6.3±0.5       | 6.9 6.3±0.5     | 6.9 6.3±0.5       | 6.9 6.3±0.5     |
|                      | 9.8 7.8±1.2     | 9.8 7.8±1.2     | 9.8 7.8±1.2       | 9.8 7.8±1.2     | 9.8 7.8±1.2       | 9.8 7.8±1.2     |
|                      | 11.0 6.3±0.6    | 11.0 6.3±0.6    | 11.0 6.3±0.6      | 11.0 6.3±0.6    | 11.0 6.3±0.6      | 11.0 6.3±0.6    |
|                      | 19.7 6.4±0.5    | 19.7 6.4±0.5    | 19.7 6.4±0.5      | 19.7 6.4±0.5    | 19.7 6.4±0.5      | 19.7 6.4±0.5    |
| M77-62 (50 m)        | 1.5 105±5       | 1.5 105±5       | 1.5 105±5         | 1.5 105±5       | 1.5 105±5         | 1.5 105±5       |
|                      | 1.9 100±6       | 1.9 100±6       | 1.9 100±6         | 1.9 100±6       | 1.9 100±6         | 1.9 100±6       |
|                      | 4.1 77±7        | 4.1 77±7        | 4.1 77±7          | 4.1 77±7        | 4.1 77±7          | 4.1 77±7        |
|                      | 6.6 71±4        | 6.6 71±4        | 6.6 71±4          | 6.6 71±4        | 6.6 71±4          | 6.6 71±4        |
|                      | 10.9 51±4       | 10.9 51±4       | 10.9 51±4         | 10.9 51±4       | 10.9 51±4         | 10.9 51±4       |
|                      | 22.3 51±2       | 22.3 51±2       | 22.3 51±2         | 22.3 51±2       | 22.3 51±2         | 22.3 51±2       |

*No addition of ¹⁴N-species.

1In μmol L⁻¹.

2Adjusted concentrations of O₂ determined by μ-sensor measurements.

3In nmol N L⁻¹ d⁻¹.

doi:10.1371/journal.pone.0029299.t002
stations are represented by red and blue symbols, respectively. The O2
the Peruvian and Namibian stations, respectively. Shelf and open ocean
shown. Station numbers with double digits and triple digits represent
legend indicate the corresponding sampling depths at each station
were verified by micro-sensor measurements. Parentheses in figure
explained by an adaptation of anammox bacteria to fluctuations in
O2 treatments at each incubation depth. Adjusted O2 concentrations
as percentages of the highest rate observed ( = 100%) for the different
incubations. B) NO3
production in
incubations. C) NH3 oxidation measured as 15NO2
2
14NO2
2
reduction measured as 15NO2
2
14NH4
2
14NO2
2
reduction activity remained
2
respiration is
1
reduction was only moderately affected by increasing O2. About 50% of NO3
reduction activity remained when O2 was adjusted to ~14 to 17 μmol L
in our above-
mentioned samples (Table 3). More pronounced sensitivity to O2
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
of O2.

The observation, that in general NO3
reduction activity was
only moderately affected by increasing concentrations of O2 may at
first seem at odds with the fact that NO3
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 NO3
1 to N 2O are known to have variable
2
content is perhaps most stable within the core of the OMZ, where
the highest O2 sensitivity of anammox was measured in our
current study (180 m at St. 36). With O2 concentrations consistently
below 1–2 μmol L
1, anammox bacteria thriving therein are unlikely to have adapted to higher O2 levels compared
to their counterparts in more dynamic environments.

Alternatively, marine snow particles have been speculated to
provide “anoxic” micro-environments in which O2 is sufficiently
deputed to favor N-loss at ambient O2 levels <25 μmol L
[9,20], while some anammox bacteria have been shown to be
to a 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 O2 tolerance by anammox bacteria near the

Oxygen sensitivity of nitrate reduction in OMZ waters

The reduction of NO3
1 to NO2
1, 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
15N-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 NO3
1 to NO2
1 showed a high degree of
variability in O2 sensitivity amongst stations. No effect of
increasing O2 on NO3
1 reduction was observed in the 120 m
incubations at St. 36. At the remaining stations, the correlation
between activity and adjusted O2 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 NO3
1 reduction was only moderately affected by
increasing O2. About 50% of NO3
1 reduction activity remained
when O2 was adjusted to ~14 to 17 μmol L
in our above-
mentioned samples (Table 3). More pronounced sensitivity to O2
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
of O2.

The apparently higher O2 tolerance at the shelf stations may be
explained by an adaptation of anammox bacteria to fluctuations in
dissolved O2 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 O2 decline over the Namibian shelf, where
the level of dissolved O2 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 O2
content is perhaps most stable within the core of the OMZ, where
the highest O2 sensitivity of anammox was measured in our
current study (180 m at St. 36). With O2 concentrations consistently
below 1–2 μmol L
, anammox bacteria thriving therein are unlikely to have adapted to higher O2 levels compared
to their counterparts in more dynamic environments.
at 120 m at St. 36 remains puzzling. One possible explanation might be the high phylogenetic diversity and thus variable physiology of the NO₃⁻ 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 NO₂⁻ production rates. Off Peru, nitrifying activity peaked at the base of the oxycline, where the highest NH₄⁺ release due to remineralization of sinking organic matter can be expected. Though O₂ was not always detectable *in situ*, NH₃ oxidation rates could be detected at these upper OMZ depths, consistent with previous studies [30,33,35].

In the O₂ sensitivity assays, NH₃ oxidation at most decreased slightly in the anoxic control (St. 54) when compared to the higher O₂ treatments. No stimulation at higher O₂ levels (20 to 25 μmol L⁻¹ of O₂) 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 O₂ affinity of aerobic NH₃ oxidizers in both OMZs investigated. It has been shown that cultured bacterial NH₃ oxidizers, including marine nitrifiers, are, in principle, able to cope with very low O₂ concentrations down to at least ~2 μmol L⁻¹ [61–63]. The only cultured marine aerobic ammonia oxidizing archaia investigated so far appears to have a limited capacity to survive under near anoxic conditions [64]. However, a higher O₂ affinity of archaean NH₃ oxidizers in the environment is indicated by results from the Peruvian OMZ, which suggest that both bacterial and archaeal NH₃ oxidizers are active at undetectable *in situ* O₂ levels (<1.5–2 μmol L⁻¹) [30].

Based on our findings, the minimum O₂ concentration for NH₃ oxidizer to be active in OMZ waters is most likely in the nanomolar range. An adaptation of aerobic micro-organisms to extremely low O₂ has been shown in a recent study by Stolper et al. [65]. They demonstrated aerobic growth in a culture experiment at an O₂ concentration ≤3 μmol L⁻¹. Alternatively, when O₂ is scarce, NH₃ oxidizer may also grow anaerobically via the oxidation of NH₃ with gaseous nitrogen dioxide (NO₂) or tetraoxide (N₂O₄) [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 O₂ is a major controlling factor for anammox activity in OMZ waters. Based on our O₂ assays we estimate the upper limit for anammox to be ~20 μmol L⁻¹ O₂, which is significantly higher than previously shown for the Black Sea (Table 3 & Fig. 3). In contrast, NH₃ oxidation and NO₃⁻ reduction as the main NH₄⁺ and NO₂⁻ sources for anammox were little or only moderately affected by changing concentrations of dissolved O₂. Intriguingly, aerobic NH₃ oxidation was active at non-detectable O₂ concentrations, while NO₃⁻ reduction to NO₂⁻, which is generally considered to be an anaerobic process, was fully active up to at least 25 μmol L⁻¹ O₂. Hence, aerobic and anaerobic N-cycle pathways in OMZs can co-occur over a larger range of O₂ concentrations...
than previously assumed. The zone where N-loss can occur is primarily controlled by the O2-sensitivity of anammox and not by the O2-sensitivity of the tightly coupled aerobic NH3 oxidation and anaerobic NO3 reduction.

Additionally, our results indicate that N-loss and other N-cycling processes within such O2 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 NO3 reduction and anammox rates despite the ideal O2 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 NO2- and NH4+ for anammox and it may also provide suitable anoxic micro-environments for anammox bacteria in zones of higher ambient O2 [9,20].

The fact that anammox in the marine environment can proceed at O2 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% (O2 ≤ 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 O2 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 O2 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 O2 sensitivities.

In light of the above presented results, the simple switching from aerobic to anaerobic respiration at ~5 μmol L−1 of O2 often implemented in models [23] appears not realistic. The current study provides the first robust estimates of O2 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 Emee (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 Klöckgeller, Daniela Franzke, Inka Boosmann, Violeta Leon, Aurelien Paulmier, Moritz Holtappels, Andreas Elliott, Volker Meyer, Philipp Hach and Michael Junemann. 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 MMK. Performed the experiments: TK MMJ SC GL MMMK. Analyzed the data: TK MMJ JL GL. Contributed reagents/materials/analysis tools: NPR. Wrote the paper: TK PL GL MMK.

References

1. Falkowski PG (2008) Earth’s Biogeochemical Cycles. Science 320: 1034–1039.
2. Wyrtyk K (1962) Circulation and Water Masses in the Eastern Equatorial Pacific Ocean. Deep-Sea Research. pp 11–13.
3. Karsten J, Stramma L, Visbeck M (2008) Oxygen minimum zones in the eastern tropical Atlantic and Pacific oceans. Progress in Oceanography 77: 331–350.
4. Kamayawa D, Zentara S (1990) Hypoxia in the world ocean as recorded in the historical data set. Deep-Sea Research 37(12): 1861–1874.
5. Henson JJ, Levin LA (2004) Global distribution of naturally occurring marine hypoxia on continental margins. Deep-Sea Research 51: 1159–1168.
6. 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: 371–391.
7. Codispoti LA, Bruland 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? Science 293: 85–105.
8. Graber N, Sarmiento JL (1997) Global Patterns of Marine Nitrogen Fixation and Denitrification. Global Biogeochemical Cycles 11: 235–266.
9. Kuyper MM, Lavik G, Woebken D, Schmid M, Buch 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.
10. Hamersley MR, Lavik G, Woebken D, Ratnay KE, Lam P, et al. (2007) Anaerobic ammonium oxidation in the Peruian oxygen minimum zone. Limnology and Oceanography 52(3): 925–933.
11. 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(3): 2143–2156.
12. Naqvi SW, Jayakumar DA, Narvekar PV, Naik H, Sarmee VV, et al. (2000) Increased marine production of N2O due to intensifying anoxia on the Indian continental shelf. Nature 408(6810): 346–349.
13. Speranza L, Johnstone GC, Sippel J, Mohrholz V (2008) Expanding Oxygen-Minimum Zones in the Tropical Oceans. Science 320(3876): 655–658.
14. Keeling RF, Kortzinger A, Graber N (2009) Ocean Deoxygenation in a Warming World. Annual Review of Marine Science. pp 463–493.
15. Diaz RJ, Rosenberg R (2008) Spreading Dead Zones and Consequences for Marine Ecosystems. Science 321(5891): 926–929.
16. Duce RA, LaRoche J, Alteri K, Arrojo KR, Baker AR, et al. (2008) Impacts of Atmospheric Anthropogenic Nitrogen on the Open Ocean. Science 320(3876): 893–897.
17. Deutsch C, Brix H, Ito T, Frenzel H, Thompson L (2011) Climate-Forced Variability of Ocean Hypoxia. Science 333: 336–339.
18. Smetacek V, Wargo WE (1987) Nutrient regeneration and denitrification in low oxygen waters. Deep-Sea Research 34(6): 983–1006.
19. Ploug H (2001) Small-scale oxygen fluxes and remineralization in sinking aggregates. Limnology and Oceanography 46(7): 1624–1631.
20. 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.
21. 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.
22. Strous M, Van Gerven E, Kuenen JJ, Jetten M (1997) Effects of Aerobic and Microaerobic Conditions on Anaerobic Ammonium-Oxidizing (Anammox) Sludge. Applied and Environmental Microbiology 63(6): 2446–2441.
23. Paulmier A, Kriest I, Oschlies A (2009) Stoichiometries of remineralisation and denitrification in global biogeochemical ocean models. Biogeosciences 6(1): 2539–2566.
24. Jensen MM, Kuyper 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.
25. Zumft WG (1997) Cell Biology and Molecular Basis of Denitrification. Microbiology and Molecular Biology Reviews 61(4): 533–616.
26. 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.
27. Codispoti LA, Packard TT (1980) Denitrification Rates in the Eastern Tropical North Pacific Ocean. Limnology and Oceanography 17(6): 885–900.
