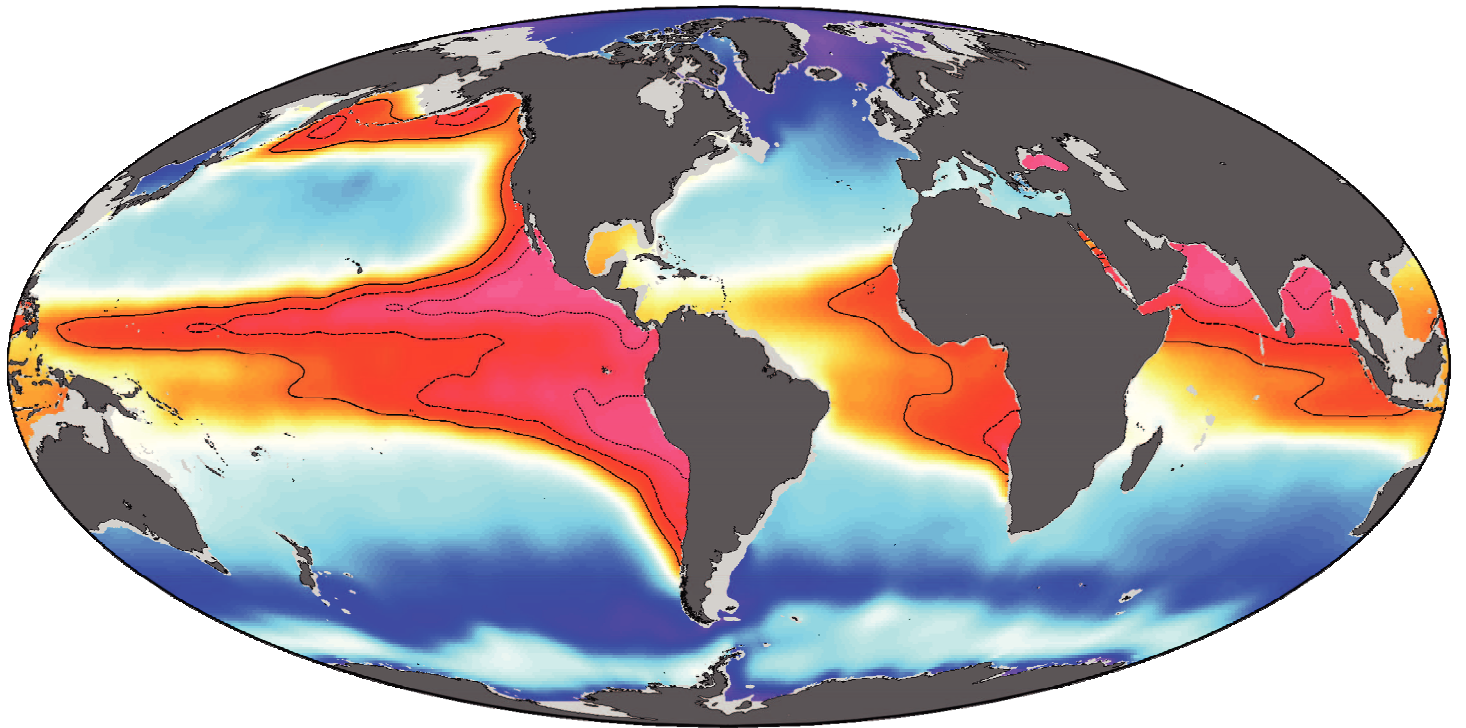


Nitrogen Losses and Nutrient Regeneration in Oxygen Minimum Zones



Tim Kalvelage

Nitrogen Losses and Nutrient Regeneration in Oxygen Minimum Zones

Dissertation zur Erlangung des
Doktorgrades der Naturwissenschaften

- Dr. rer. nat. -

dem Fachbereich Geowissenschaften
der Universität Bremen
vorgelegt von

Tim Kalvelage

Bremen, Juni 2012

Die vorliegende Arbeit wurde in der Zeit von April 2009 bis Juni 2012 am Max-Planck-Institut für Marine Mikrobiologie in Bremen angefertigt.

1. Gutachter: **Prof. Dr. Marcel M.M. Kuypers**

2. Gutachter: **Priv. Doz. Dr. Hermann W. Bange**

Datum des Promotionskolloquiums: 27. Juli 2012

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Abstract

In the tropical oceans, coastal upwelling of nutrient-rich deep waters fuels high surface productivity. The decomposition of sinking algal biomass results in the formation of large oxygen-deficient water bodies at mid depths (~100–1,000 m). Although, these oxygen minimum zones (OMZs) amount to less than 1% of the global ocean volume, they account for ~30-50% of total oceanic nitrogen (N) loss. Anammox, the anaerobic oxidation of ammonium with nitrite to gaseous dinitrogen is the major N-loss pathway in OMZs. The recirculation of N-deficient waters to the surface limits phytoplankton growth and thus carbon sequestration in large parts of the tropical oceans. Continuing ocean de-oxygenation is expected to result in significantly increasing N-losses, thereby reducing the ocean's capacity to attenuate rising atmospheric carbon dioxide.

This thesis aimed to determine regulatory effects of oxygen and organic matter availability on anammox and N-linked processes in OMZs. Moreover, microaerobic organic matter remineralization as a potential source of ammonium for anammox was investigated.

In the OMZs off Namibia and Peru, aerobic and anaerobic N-cycling processes co-occurred over a wide range of oxygen concentrations ($>0-20 \mu\text{mol L}^{-1}$). Aerobic ammonia oxidation and nitrate reduction appeared insensitive to non-detectable ($<1 \mu\text{mol L}^{-1}$) and elevated ($\sim 25 \mu\text{mol L}^{-1}$) oxygen concentrations, respectively. Anammox clearly decreased with increasing oxygen levels but remained active up to $\sim 20 \mu\text{mol L}^{-1}$. During a large-scale survey in the OMZ off Peru, export production was identified as a reliable predictor of N-loss via anammox. Pronounced N-loss did not coincide with accumulations of nitrite or large N-deficits in the offshore OMZ. Instead, high rates of organic matter remineralization fueled high anammox activity over the Peruvian shelf. Microaerobic respiration was the major remineralization pathway in the upper OMZs and provided most of the ammonium for anammox in this zone.

In summary, this thesis provides detailed insights into the regulation of complex N-cycling in OMZs. The obtained results will help to improve biogeochemical models and therewith to reliably assess impacts of future ocean deoxygenation on the ocean's N-inventory.

Kurzfassung

In Küstengebieten der tropischen Ozeane führt der Auftrieb von nährstoffreichem Tiefenwasser zu hoher Produktivität an der Oberfläche. Durch den Abbau absinkender Algenbiomasse entstehen in mittleren Tiefen (~100-1.000 m) große, sauerstoffarme Wasserkörper. Diese so genannten Sauerstoffminimumzonen (SMZs) machen weniger als 1% des gesamten Ozeanvolumens aus, sind aber für ~30-50% des Stickstoffverlusts im Meer verantwortlich. Anammox, die anaerobe Oxidation von Ammonium mit Nitrit zu Stickstoffgas, ist der wichtigste Stickstoffverlustprozess im Ozean. Die Rückströmung von stickstoffarmem Wasser an die Oberfläche limitiert Primärproduktion und somit die Speicherung von Kohlenstoff in großen Teilen der tropischen Ozeane. Die fortwährende Sauerstoffverarmung der Ozeane wird voraussichtlich zu deutlich erhöhtem Stickstoffverlust führen und somit die Kapazität der Ozeane reduzieren, den steigenden Kohlenstoffdioxidgehalt der Atmosphäre zu verringern.

Ziel dieser Dissertation war es zu bestimmen wie Sauerstoff sowie die Verfügbarkeit von organischem Material Anammox und daran gekoppelte Prozesse des Stickstoffkreislaufs regulieren. Außerdem wurde die mikroaerobe Remineralisierung von organischem Material untersucht, da diese eine potentielle Ammoniumquelle für Anammox in SMZs darstellt.

In den SMZs vor Namibia und Peru kamen aerobe und anaerobe Prozesse des Stickstoffkreislaufs über einen weiten Sauerstoffbereich gleichzeitig vor ($>0-20 \mu\text{mol L}^{-1}$). Aerobe Ammoniakoxidation und Nitratreduktion waren unempfindlich gegenüber nicht messbarem Sauerstoff ($<1 \mu\text{mol L}^{-1}$), bzw. erhöhtem Sauerstoffgehalt ($\sim 25 \mu\text{mol L}^{-1}$). Anammox nahm deutlich ab bei erhöhten Sauerstoffkonzentrationen, war aber bis zu einer Konzentration von $\sim 20 \mu\text{mol L}^{-1}$ Sauerstoff aktiv. Während einer groß angelegten Studie in der SMZ vor Peru wurde Exportproduktion als zuverlässiger Indikator für Stickstoffverlust durch Anammox identifiziert. Ausgeprägter Stickstoffverlust fiel nicht mit

Nitritakkumulationen oder großen Stickstoffdefiziten in der SMZ des offenen Ozeans zusammen. Stattdessen sorgte der starke Abbau von organischem Material über dem peruanischen Schelf für hohe Anammox-Raten. Mikroaerober Abbau von organischem Material war der dominierende Remineralisierungsprozess in der oberen SMZ und lieferte dort den größten Teil des für den Anammox-Prozess benötigten Ammoniums.

Zusammenfassend bietet diese Dissertation detaillierte Einblicke in die Regulation des komplexen Stickstoffkreislaufs in SMZs. Die erlangten Ergebnisse werden helfen, biogeochemische Modelle zu verbessern um so die zukünftigen Auswirkungen der Sauerstoffverarmung der Ozeane auf den marinen Stickstoffhaushalt zuverlässig abschätzen zu können.

Nutrient limitation of marine primary production

Carbon (C) fixation in the upper ocean by photosynthetic bacteria and algae (phytoplankton) accounts for ~50% of global annual primary production (Behrenfeld et al. 2001). Hence, these minute organisms play a vital role in regulating atmospheric levels of carbon dioxide (CO₂). The major part of phytoplankton-derived particulate matter (80%) is recycled in the ocean's upper mixed layer, but some of it (~20%), the so-called export production, sinks as marine snow to the deep ocean (Denman et al. 2007). While the exported particulate C is mostly converted back to DIC and only locked up in the deep ocean for ~500 y (Stuiver et al. 1983), a small fraction (<0.5%) escapes dissolution and remineralization and is permanently buried in marine sediments. This is referred to as the biological pump, which on long time scales (~10³-10⁴ y) is a key regulator of the earth's climate (McElroy 1983; Berner 2003; Falkowski 2012).

Biological C fixation in the oceans is largely determined by the availability of nitrogen (N) and phosphorus (P), which are essential building blocks of life. Phytoplankton biomass shows a remarkably constant elemental composition of ~16 moles N per mole P (Redfield 1934; Copin-Montegut and Copin-Montegut 1983). During the remineralization of phytoplankton-derived organic matter, N and P are proportionally released (Table 1), resulting in the deep-ocean nitrate (NO₃⁻) to phosphate (PO₄³⁻) ratio of 16:1, termed the Redfield ratio (Redfield et al. 1963). Eventually, these remineralized nutrients are re-circulated to the euphotic zone and drive primary production.

However, in tropical nutrient-depleted surface waters NO₃⁻ is often non-detectable despite a small residue of PO₄³⁻. This non-Redfield nutrient stoichiometry results in N-limitation of phytoplankton growth (Fig. 1) (McElroy 1983; Falkowski 1997; Tyrrell 1999). The depletion of NO₃⁻ relative to PO₄³⁻ owes to fundamental differences between N and P cycling in the oceans: P-availability is mainly controlled by riverine input and sedimentary

deposition (Froelich et al. 1982). In contrast, the (unperturbed) marine N-cycle is dominated by microbially-mediated processes. Fixation of gaseous dinitrogen (N_2) by diazotrophs in the surface waters is the main source of bioavailable N and subsurface microbial respiration of fixed inorganic N to N_2 is the main oceanic N-sink (Gruber and Galloway 2008). Microbial N_2 production drives upwelled deep waters to non-Redfield nutrient stoichiometries and hence limits phytoplankton growth in large parts of the tropical oceans (Fig. 2c).

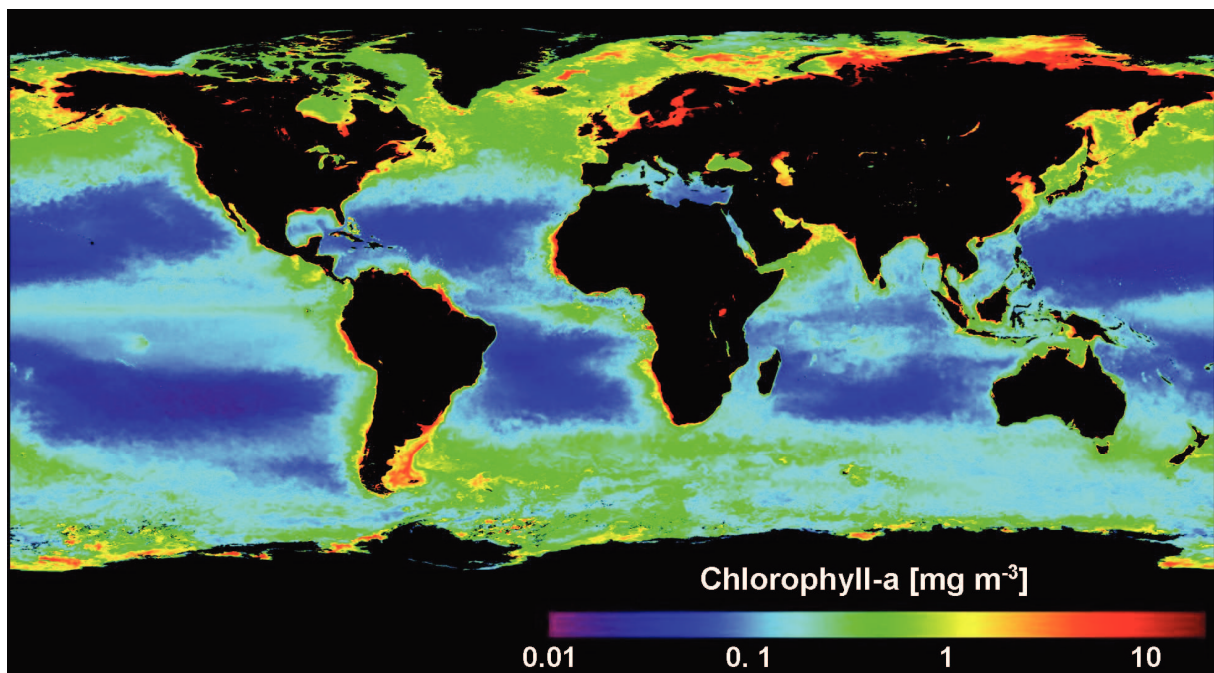


Figure 1 | Satellite-derived (MODIS) distribution of surface chlorophyll-a (2011 annual average).

Large parts of the (sub) tropical oceans are marked by low algal biomass. Often nitrogen is in short supply relative to phosphorus in these waters, thus limiting primary production. In contrast, high productivity at low latitudes is fuelled by upwelling of nutrient-rich deep waters, which primarily occurs at the western boundaries of the continents (off California/Mexico, Mauretania, Namibia and Peru/Chile) but seasonally also in other regions (e.g. off Oman and Western India). (Source: <http://oceancolor.gsfc.nasa.gov/cgi/l3>)

Oxygen minimum zones

At the eastern boundaries of the Atlantic and Pacific Ocean basins as well as in the northern Indian Ocean, wind-driven circulation results in coastal upwelling of nutrient-rich deep waters. The resultant high surface productivity leads to high export production and consequently

strong microbial respiration in the subsurface. Additionally, these regions are poorly ventilated, as they are largely unaffected by large-scale ocean circulation (Wyrski 1962; Karstensen et al. 2008). Combined, high O_2 consumption and a lack of O_2 replenishment result in the formation of large hypoxic to anoxic water bodies, typically at depths of 100-1,000 m (Fig. 2a,b) (Kamykowski and Zentara 1990; Helly and Levin 2004; Thamdrup et al. 2012).

In these so-called oxygen minimum zones (OMZs), NO_3^- (which energetically is the next favourable electron acceptor after O_2) is ultimately converted to N_2 by the microbial processes of anammox and denitrification. Conditions favouring NO_3^- respiration are found in the eastern tropical North and South Pacific (ETNP and ETSP, respectively), the eastern tropical South Atlantic (ETSA) as well as the Arabian Sea and Bay of Bengal. Except for the latter, all have been identified as major sites of oceanic N-loss. Although, OMZs amount to less than 1% of the global ocean volume, they are estimated to account for ~30-50% of N-loss in the world's oceans (Codispoti 2007; Gruber 2008) and thus play a key role in controlling the ocean's nutrient balance.

Nitrogen cycling in oxygen minimum zones

Nitrogen Loss via Anammox and Denitrification – Heterotrophic denitrification refers to the sequential reduction of NO_3^- via NO_2^- , NO and N_2O to N_2 during organic matter remineralization (Fig. 3; Table 1). Conventionally, accumulations of NO_2^- in OMZs, so-called secondary NO_2^- maxima, have been interpreted as signs of active denitrification (Cline and Richards 1972; Codispoti and Packard 1980; Naqvi 1987; Devol et al. 2006). Oxidation of organic matter results in the stoichiometric release of NH_4^+ . As aerobic NH_3 oxidation is impaired in O_2 -deficient waters, heterotrophic denitrification should result in the

accumulation of NH_4^+ . Hence for a long time, oceanographers were puzzled by the generally low to non-detectable NH_4^+ concentrations in OMZs. Already in the 1960's it was hypothesized that NH_4^+ might be oxidized anaerobically to N_2 using NO_x (NO_3^- and NO_2^-) as an electron acceptor (Richards et al. 1965). But it was not until 1995, that clear evidence for such pathway was found.

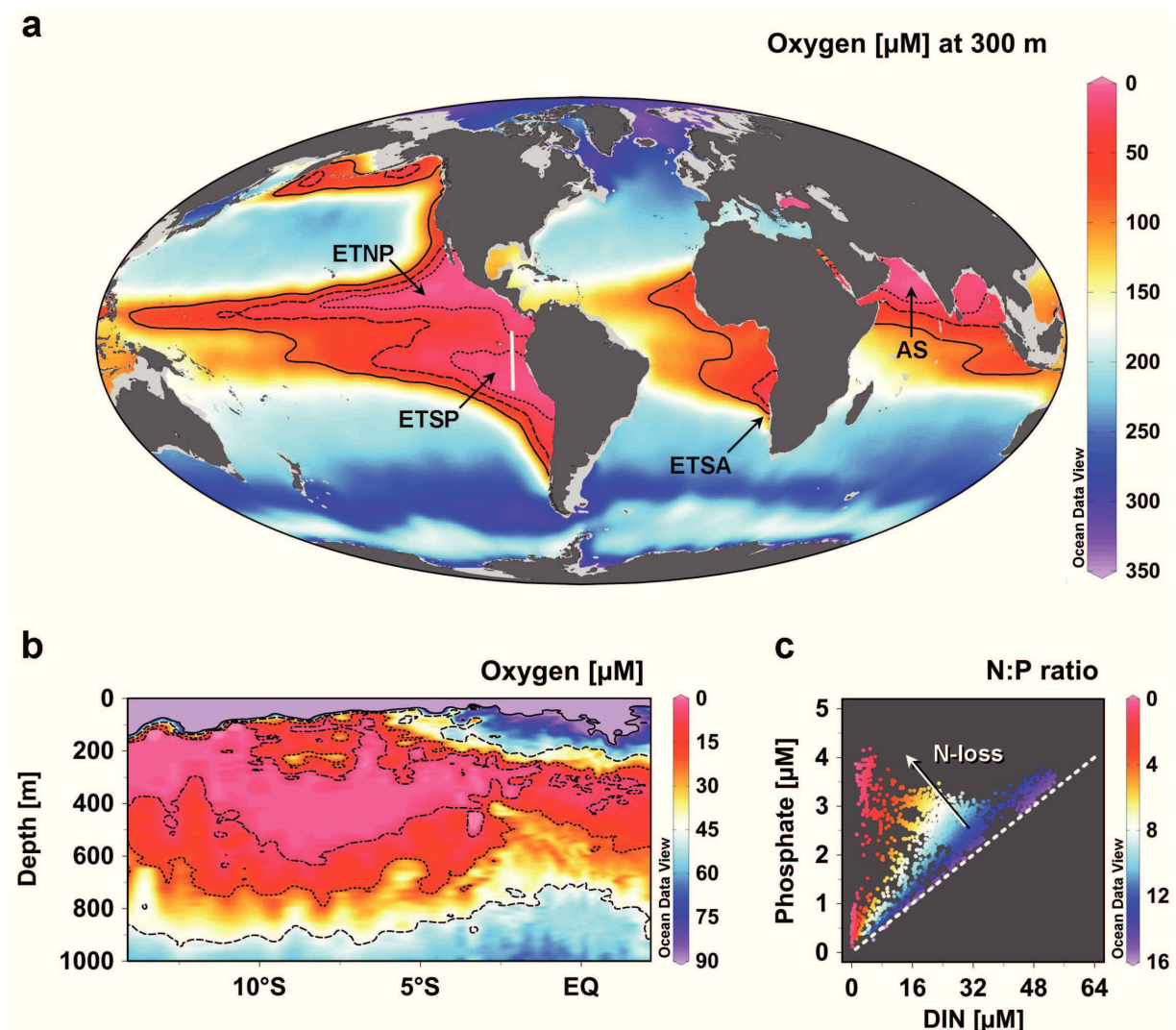


Figure 2 | Oxygen minimum zones (OMZs) and associated nitrogen (N) loss.

(a) Oxygen (O_2) concentrations in the world's oceans at 300m depth. (b) Vertical distribution of O_2 along 85.83°W (white line in panel a). Isolines (a,b) mark O_2 concentrations of 5 (dashed-dotted) 20 (dotted), 45 (dashed) and $90 \mu\text{mol L}^{-1}$ (solid). (c) Dissolved inorganic nitrogen ($\text{DIN} = [\text{NO}_3^-] + [\text{NO}_2^-] + [\text{NH}_4^+]$) versus phosphate in the eastern tropical South Pacific (ETSP). N:P ratios significantly lower than the global average of 16:1 (Redfield ratio; white dashed line) indicate N-loss. Other areas of oceanic water column N-loss are the eastern tropical North Pacific (ETNP), the Namibian shelf in the eastern tropical South Atlantic (ETSA) and the Arabian Sea (AS). (Sources: (a) World Ocean Atlas 2009 (Garcia et al. 2010). (b,c) Data collected in the ETSP between December 2008 and February 2009 onboard R/V Meteor)

The anaerobic oxidation of ammonium (anammox) with NO_2^- to N_2 (Fig. 3; Table 1) is a chemolithoautotrophic process and was first discovered in a waste water treatment plant (Mulder et al. 1995; van de Graaf et al. 1995). Anammox bacteria belong to a monophyletic group in the phylum Planctomycetes. They are slow growing, with a doubling of ~ 14 d, and possess a unique cell compartment, the anammoxosome (Strous et al. 1999). The latter is the site of the anammox reaction and comprised of dense ladderane lipids to contain the highly toxic intermediate hydrazine (N_2H_2) (Kartal et al. 2008).

The discovery of an alternative N_2 -producing pathway inspired a number of studies investigating the occurrence and significance of anammox in the environment. Discrimination of N_2 production via anammox and denitrification was enabled by a modification of the ^{15}N -isotope pairing technique and anammox was detected in several O_2 -deficient environments, including coastal marine sediments, anoxic fjords and semi-enclosed basins (Thamdrup and Dalsgaard 2002; Dalsgaard et al. 2003; Kuypers et al. 2003). Recent studies have focused on the role played by anammox in oceanic OMZs where previously, N-loss had been solely attributed to denitrification. In fact, anammox turned out to be the dominant pathway for water column N-loss in the OMZs off Namibia, Peru/Chile and on the Omani shelf (Kuypers et al. 2005; Thamdrup et al. 2006; Hamersley et al. 2007; Galán et al. 2009; Jensen et al. 2011). There were generally little or no signs of active denitrification in these regions, apart from a single study carried out in the central Arabian Sea (Ward et al. 2009; Bulow et al. 2010). Anammox activity was generally found to be highest in productive shelf waters, particularly in the OMZ bottom waters. High anammox rates were also measured in the upper OMZs at O_2 concentrations of $\sim 10\text{-}20 \mu\text{mol L}^{-1}$ (Kuypers et al. 2005; Hamersley et al. 2007), even though cultured anammox bacteria are inhibited by $\sim 1 \mu\text{mol L}^{-1}$ of O_2 (Strous et al. 1997).

Ammonium and nitrite - sinks and sources – Heterotrophic denitrification has traditionally been regarded as the major remineralization pathway in OMZs. In the absence thereof, anammox activity must be sustained by alternative sources of NH_4^+ and NO_2^- .

A variety of micro-organisms are capable of NO_3^- respiration, but rarely beyond the level of NO_2^- (Zumft 1997; Gonzales et al. 2006). Nitrate reduction to NO_2^- as a heterotrophic process releases 16 moles of NH_4^+ per mole of organic matter remineralized (Table 1), thus is

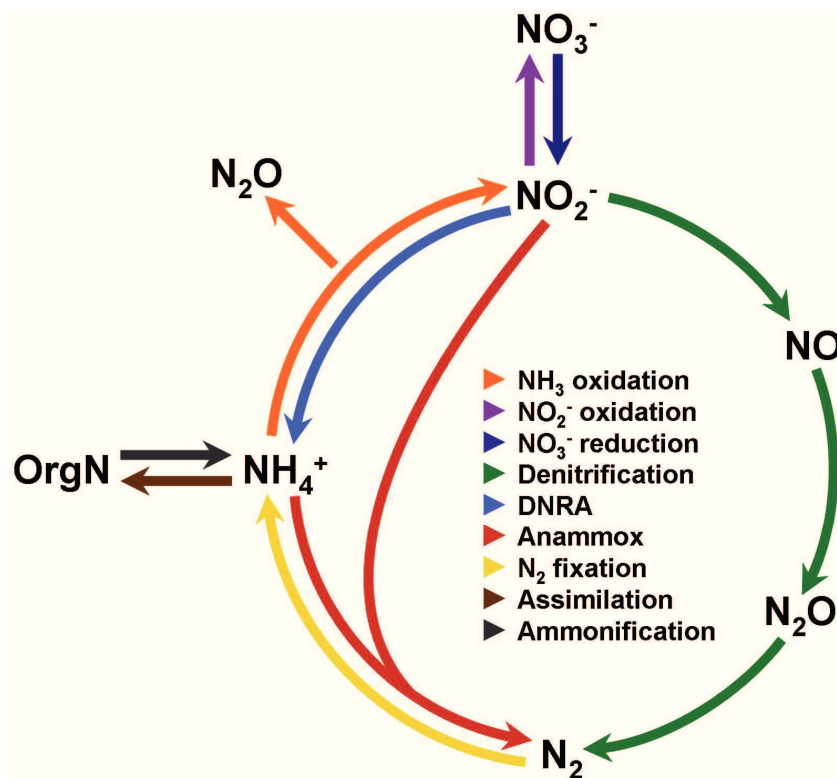


Figure 3 | Nitrogen (N) cycling in oxygen minimum zones (OMZs).

A complex N-cycle, including both oxidative and reductive microbial processes, is found in OMZs. Organically bound N (N_{org}) exported from the euphotic zone, is released as ammonium (NH_4^+) during degradation of organic matter (ammonification). Ammonium is readily incorporated into biomass (assimilation) by most micro-organisms. During autotrophic nitrification, NH_4^+ is aerobically oxidized to nitrate (NO_3^-) via nitrite (NO_2^-). If oxygen is scarce, NO_3^- is preferentially used as an alternative electron acceptor for the respiration of organic matter or reduced inorganic compounds, such as hydrogen sulfide. In a first step NO_3^- is reduced to NO_2^- . Subsequently, NO_2^- may be either fully reduced to NH_4^+ , in a process termed dissimilatory nitrate/nitrite reduction to ammonia (DNRA), or, during denitrification, successively converted to the gaseous N-species nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2). Nitrous oxide can also be produced by aerobic NH_3 oxidizers. The presumably major N_2 -forming pathway in OMZs is the anaerobic oxidation of ammonia (anammox) with NO_2^- to N_2 , an autotrophic process. A recent study indicates that N_2 fixation in OMZ waters may partially compensate for the loss of fixed inorganic N therein (Fernandez et al. 2011).

capable of supplying both NO_2^- and NH_4^+ in OMZs. Initially, NO_3^- -reducing activity in OMZs was inferred from commonly observed accumulations of NO_2^- (Brandhorst 1959; Cline and Richards 1972). Later, ^{15}N -labelling experiments provided direct evidence for NO_3^- reduction to NO_2^- as an important stand-alone process throughout the OMZs of the ETSP, ETSA and in the Arabian Sea (Lipschultz et al. 1990; Lam et al. 2009; Füssel et al. 2011; Lam et al. 2011). In the ETSP and in the Arabian Sea, NO_3^- reduction rates positively correlated with NO_2^- concentrations indicating that NO_3^- reduction is chiefly responsible for the formation of secondary NO_2^- maxima (Lipschultz et al. 1990; Lam et al. 2011).

Dissimilatory nitrate/nitrite reduction to ammonium (DNRA) coupled to the oxidation of organic matter, releases 69 moles of NH_4^+ per mole organic carbon (Fig. 3; Table 1). DNRA has recently been measured at substantial rates in the OMZs of the ETSP and the Arabian Sea (Lam et al. 2009; Jensen et al. 2011). Particularly on the Peruvian and Omani shelf, DNRA was highly active and was estimated to provide up to 100% of the NH_4^+ required for anammox. In contrast, DNRA remained largely undetectable in the Namibian OMZ (Füssel et al. 2011).

In the presence of O_2 , NH_4^+ can be oxidized to NO_2^- instead of N_2 (Fig. 3; Table 1). Ammonia (NH_3) oxidation to NO_2^- is the first step of nitrification, a chemolithoautotrophic process carried out by both NH_3 -oxidizing bacteria and archaea. Early studies with isolates of marine bacterial NH_3 oxidizers revealed their capability to nitrify under low- O_2 conditions ($<5 \mu\text{mol L}^{-1}$) (Gundersen 1966; Carlucci and McNally 1969). Accordingly, aerobic NH_3 oxidation has been detected in all major OMZs (Ward and Zafiriou 1988; Ward et al. 1989; Lipschultz, et al. 1990; Lam et al. 2009; Füssel et al. 2011; Lam et al. 2011). Rates of NH_3 oxidation were generally highest near the upper oxycline but were often measured at non-detectable O_2 concentrations.

In the second step of nitrification, NO_2^- is further oxidized to NO_3^- by NO_2^- -oxidizing bacteria (Fig. 3; Table 1). Relatively few studies have included rate measurements of NO_2^- oxidation in parallel with those of NH_3 oxidation in OMZs. Active NO_2^- oxidation has been found in the ETSP and ETSA OMZs. Here, rates of NO_2^- oxidation consistently exceeded those of NH_3 oxidation and, in contrast to the latter, revealed no clear trends throughout the OMZ (Ward et al. 1989; Lipschultz et al. 1990; Füssel et al. 2011).

Nitrogen loss as nitrous oxide – The production of nitrous oxide (N_2O) is only a minor oceanic N-sink (Bianchi et al. 2012). However, N_2O is a potent greenhouse gas with a 300-fold greater global warming potential than CO_2 . Nitrous oxide (N_2O) is an intermediate in heterotrophic denitrification and accumulates at low O_2 concentrations, inhibiting the N_2O reductase (Fig. 3) (Firestone and Tiedje 1979; Körner and Zumft 1989; Coyne and Tiedje 1990; McKenney et al. 1994). Additionally, aerobic NH_3 oxidizers can form N_2O as a by-product or during a process often termed nitrifier-denitrification (Ritchie and Nicholas 1972; Downes 1988; Ostrom et al. 2000). At reduced O_2 concentrations, the yield of N_2O relative to NO_2^- during bacterial nitrification may be as high as 10% (Goreau et al. 1980). Various lines of evidence indicate N_2O production by both nitrifiers and denitrifiers in the OMZs of the ETSP and the Arabian Sea (Codispoti and Christensen 1985; Fariás et al. 2007; Nicholls et al. 2007; Fariás et al. 2009; Lam et al. 2011; Ryabenko et al. 2012). Recently, low O_2 -induced N_2O production has been observed in the only cultured marine archaeon *Nitrosopumilus maritimus*. It has been suggested that most of the N_2O accumulating in OMZs may be produced by the highly abundant archaeal NH_3 oxidizers (Stewart et al. 2011; Loescher et al. 2012).

Table 1 | Major respiratory nitrogen cycling processes in oxygen minimum zones (OMZs).

In OMZs, micro-organisms use two principally different ways of harnessing energy: by oxidizing organic matter (organotrophy) or reduced inorganic compounds (chemolithotrophy). While organotrophs rely on organic matter as their source of carbon (heterotrophy), chemolithotrophs fix dissolved inorganic carbon (autotrophy). Note that not all reactions possible are listed here. For instance, both denitrification and DNRA use NO_2^- as electron acceptor. Denitrification may stop at the level of NO or N_2O while H_2S may be fully oxidized to SO_4^{2-} . Sulfide oxidation also proceeds via DNRA. Reaction stoichiometries are based on Redfield organic matter composition.

	Process	Idealized Stoichiometry
Organotrophy	Oxic respiration	$(\text{CH}_2\text{O})_{106}(\text{NH}_3)_{16}\text{H}_3\text{PO}_4 + 106 \text{O}_2 + 16 \text{H}^+ \rightarrow 106 \text{CO}_2 + 16 \text{NH}_4^+ + 106 \text{H}_2\text{O} + \text{H}_3\text{PO}_4$
	NO_3^- reduction to NO_2^-	$(\text{CH}_2\text{O})_{106}(\text{NH}_3)_{16}\text{H}_3\text{PO}_4 + 212 \text{NO}_3^- + 16 \text{H}^+ \rightarrow 106 \text{CO}_2 + 16 \text{NH}_4^+ + 212 \text{NO}_2^- + 106 \text{H}_2\text{O} + \text{H}_3\text{PO}_4$
	Denitrification	$(\text{CH}_2\text{O})_{106}(\text{NH}_3)_{16}\text{H}_3\text{PO}_4 + 84.8 \text{NO}_3^- + 16 \text{H}^+ \rightarrow 106 \text{CO}_2 + 16 \text{NH}_4^+ + 42.4 \text{N}_2 + 148.4 \text{H}_2\text{O} + \text{H}_3\text{PO}_4$
	DNRA	$(\text{CH}_2\text{O})_{106}(\text{NH}_3)_{16}\text{H}_3\text{PO}_4 + 53 \text{NO}_3^- + 122 \text{H}^+ \rightarrow 106 \text{CO}_2 + 69 \text{NH}_4^+ + 53 \text{H}_2\text{O} + \text{H}_3\text{PO}_4$
Chemolithotrophy	NH_3 oxidation	$\text{NH}_3 + 1.5 \text{O}_2 \rightarrow \text{HNO}_2 + \text{H}_2\text{O}$
	NO_2^- oxidation	$\text{NO}_2^- + 0.5 \text{O}_2 \rightarrow \text{NO}_3^-$
	Anammox	$\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + 2 \text{H}_2\text{O}$
	H_2S oxidation	$5 \text{HS}^- + 2 \text{NO}_3^- + 7 \text{H}^+ \rightarrow 5 \text{S}^0 + \text{N}_2 + 6 \text{H}_2\text{O}$

Chemolithotrophic Sulfide Oxidation – Occasionally, enhanced export production and a prolonged stagnant water column result in the depletion of not only O_2 but also other major alternative electron acceptors. Such conditions foster the reduction of sulfate (SO_4^{2-}) to toxic hydrogen sulfide (H_2S) (Brüchert et al. 2003). Alternatively, sulfidic events can be triggered by eruptive gas release from shelf sediments (Emeis et al. 2004). While H_2S inhibits anammox bacteria and NH_3 oxidizers (Joye and Hollibaugh 1995; Jensen et al. 2008), a number of micro-organisms flourish under euxinic conditions. These chemolithoautotrophs gain energy from denitrification or DNRA coupled to the oxidation of H_2S (Table 1) (Simon

2002; Ghosh and Dam 2009). During a recent sulfidic event on the Namibian shelf, ^{15}N -labelling experiments revealed high rates of denitrification coupled to the oxidation of H_2S . Both colloidal sulphur (S^0) and SO_4^{2-} were identified as oxidation products (Lavik et al. 2008). Accumulations of H_2S have also been noted on the Peruvian and Western Indian shelf (Dugdale et al. 1977; Naqvi et al. 2000). Off India, sulfidic conditions were associated with increased production of N_2O , likely due to chemolithoautotrophic denitrification.

Ocean de-oxygenation

The earth's climate, ecosystems and global element cycles have experienced significant changes since the beginning of the industrial revolution. To a large extent, they are no longer controlled by forces of nature but human activities, such that the initiation of a new geological era, the Anthropocene, has been argued (Crutzen and Stoermer 2000). Fossil fuel combustion, cement production and land-use change has led to an increase of atmospheric CO_2 from pre-industrial levels of ~ 280 ppm to ~ 400 ppm. Consequently, the global mean temperature has risen by $\sim 1^\circ\text{C}$ since 1850 (Trenberth et al. 2007). Anthropogenic N-fixation via N-fertilizer production, legume cultivation and fossil fuel combustion annually adds as much reactive N to the earth system as natural N-fixation (Falkowski et al. 2000; Gruber and Galloway 2008; Canfield et al. 2010). Mining annually releases three times more PO_4^{3-} than chemical weathering (Falkowski et al. 2000).

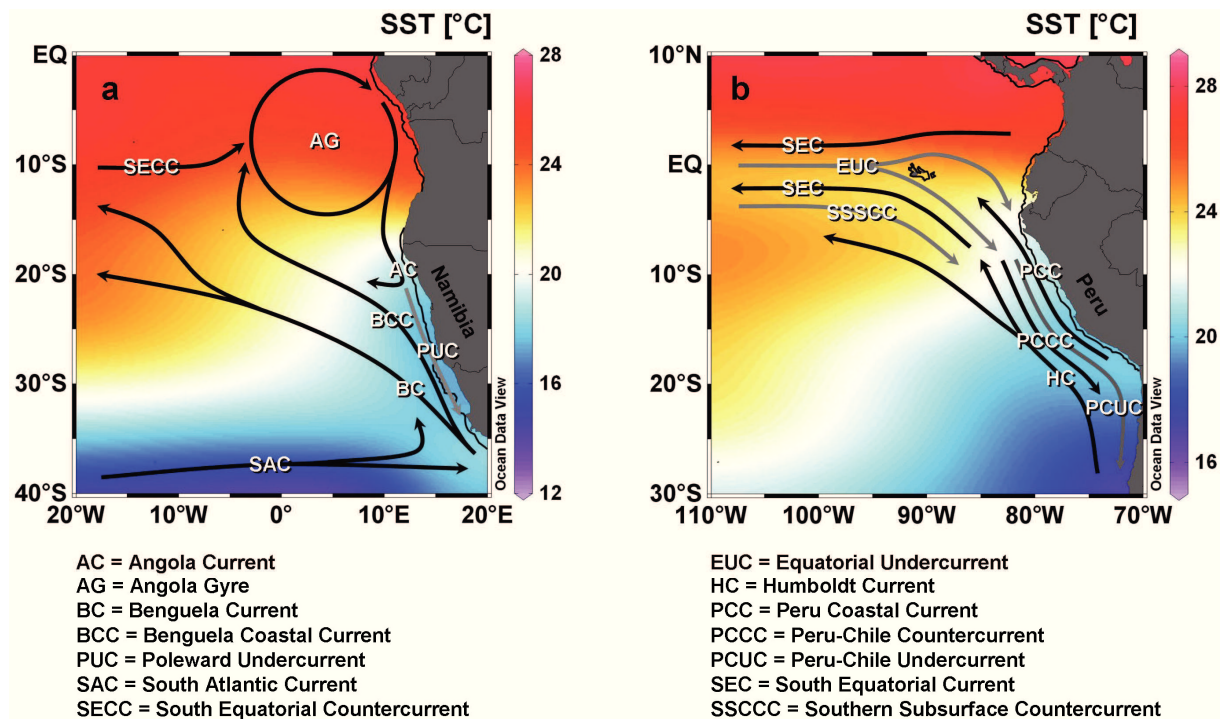
Due to physical and biological responses to these perturbations (reduced O_2 solubility, enhanced thermal stratification and strong O_2 consumption in eutrophied waters) dissolved O_2 concentrations in the oceans are decreasing (Keeling and Garcia 2002; Beman et al. 2005; Duce et al. 2008; Keeling et al. 2010). Coastal hypoxia and even anoxia is observed more frequently while oceanic OMZs are expanding globally, particularly in the Atlantic and

Pacific (Naqvi et al. 2000; Grantham et al. 2004; Naqvi et al. 2006; Diaz and Rosenberg 2008; Stramma et al. 2008; Rabalais et al. 2010). As these waters are often at or near thresholds for anaerobic processes, such as anammox and denitrification, expanding OMZs may result in significantly increasing N-losses via N_2 and N_2O production (Codispoti 2010; Deutsch et al. 2011).

Biogeochemical modelling has become increasingly important to assess the short- and long-term impacts of ocean de-oxygenation. Based on a simple model, Bianchi et al. (2012) estimated an increase in N-loss of 14 Tg N y^{-1} (equivalent to 10-20% of current water column N-loss) per $\mu\text{mol L}^{-1}$ of O_2 decrease, while a mean decrease of 3-12 $\mu\text{mol L}^{-1}$ of O_2 by 2100 is expected (Keeling et al. 2010). Coupled ocean biogeochemical-circulation models similarly predict a dramatic increase in water column N-loss (+50% and +300% by 2100 and 4000, respectively) in the course of global climate change (Oschlies et al. 2008; Schmittner, et al. 2008). However, such estimates are speculative at best as current biogeochemical models do not adequately reproduce the tropical OMZs and present-day patterns of N-loss (Matear and Hirst 2003; Moore and Doney 2007; Peña et al. 2010). To realistically assess the future ocean's nutrient balance, a better understanding of both biogeochemical and physical key processes associated with OMZs is required.

The Namibian and Peruvian upwelling system

In this thesis, two regions highly susceptible to ocean de-oxygenation were investigated: The OMZ in the ETSA and the OMZ in the ETSP, associated with the Namibian (Benguela) and Peruvian (Humboldt) upwelling, respectively. The naturally eutrophic waters off Namibia and Peru sustain some of the highest primary production rates in the ocean and support economically important fisheries (Carr 2002; Chavez and Messié 2009; Hutchings et al. 2009).



The ETSA OMZ is confined to a narrow zone along the Namibian continental margin. Here, surface productivity is fuelled by upwelling of nutrient-rich South Atlantic midwaters (Fig. 1). Although the upwelled waters are well-oxygenated, microbial organic matter decomposition results in severely O_2 -depleted and occasionally even anoxic bottom waters over large areas of the Namibian shelf (Chapman and Shannon 1985). In addition, low- O_2 waters are advected from the Angola Dome region and spread over the shelf (Fig. 4a) (Mohrholz 2008; Hutchings et al. 2009). The extent of the OMZ on the Namibian shelf shows high intra-annual variability and is largely determined by the southward and northward advancement of the low- O_2 Angola current waters and the O_2 -rich Benguela current waters, respectively (Chapman and Shannon

1985; Mohrholz et al. 2008; Hutchings et al. 2009). While the OMZ in the ETSA is rather negligible in terms of N-loss (~1% of global water column N-loss) (Kuypers et al. 2005), regular accumulations of toxic H₂S on the Namibian shelf can have devastating impacts on the local ecosystem (Hutchings et al. 2009; Lavik et al. 2008).

One of the largest OMZs globally is found in the ETSP. Off central Peru, the core of the ETSP OMZ ($O_2 \leq 20 \mu\text{mol L}^{-1}$) extends more than 1000 km into the open ocean and exhibits a thickness of up to ~700 m (Fuenzalida et al. 2009). Most of the OMZ waters originate near the equator as part of the eastward flowing Equatorial Undercurrent (EUC) (Fig. 4b). The EUC splits around Galapagos and forms two branches: One continues eastward, turns polewards near the coast off Ecuador and flows into the Peru-Chile Undercurrent (PCUC), while the other branch turns southeast and eventually forms the Peru-Chile Countercurrent (Strub et al. 1998; Fiedler and Talley 2006; Kessler 2006; Stramma et al. 2010). The PCUC source waters are well oxygenated, but O₂ rapidly declines due to strong respiration in the highly productive Peruvian upwelling region (Karstensen and Ulloa 2008). On their way south, the PCUC waters are enriched in NO₂⁻ but depleted overall in N, signifying N-loss (Wooster et al. 1965; Nelson and Neshyba 1979; Silva et al. 2009). In total, the ETSP OMZ is estimated to account for ~25% of global water column N-loss (Codispoti 2007; Bianchi et al. 2012). However, significant variability in primary production and N-loss have been observed in the ETSP in concert with inter-annual (El Niño-Southern Oscillation) and decadal (Pacific Decadal Oscillation) oscillations, which may be amplified in the course of global climate change (Codispoti and Packard 1980; Codispoti et al. 1986; Philander 1999; Chavez et al. 2003; Pennington et al. 2006; Martinez et al. 2009).

Scope and framework

Previous studies have identified anammox as the major pathway for water column N-loss in the oceans. The expansion of tropical OMZs due to global warming may result in a significant increase in N-loss. However, the regulation of anammox and N-linked processes is so far poorly understood. Overall, this thesis aimed to characterize the factors controlling N-cycling in OMZs, thereby facilitating reliable model-based assessments of future ocean changes.

Oxygen sensitivity – Surprisingly, in the environment anammox bacteria have been found active at O_2 concentrations of $\sim 10\text{-}20 \mu\text{mol L}^{-1}$ (Kuypers et al. 2005; Hamersley et al. 2007; Jensen et al. 2008). NO_3^- reduction to NO_2^- appears to be similarly O_2 -tolerant (Lipschultz et al. 1990). At the same time, the aerobic processes of NH_3 and NO_2^- oxidation are obviously well adapted to very low O_2 concentrations ($< 1 \mu\text{mol L}^{-1}$) (Lipschultz et al. 1990; Lam et al. 2009; Füssel et al. 2011). These findings imply a broader O_2 window for the co-occurrence of aerobic and anaerobic pathways than generally defined in biogeochemical models (Paulmier et al. 2009). However, to date no study has systematically investigated the regulatory effect of O_2 on co-occurring N-cycling processes.

Here, the O_2 -sensitivities of anammox, NO_3^- reduction to NO_2^- and nitrification were determined in parallel ^{15}N -labeling experiments in the Namibian and Peruvian OMZ waters. Rate measurements were complemented by high-accuracy *in situ* O_2 measurements (detection limit: $\sim 50\text{-}100 \text{ nmol L}^{-1}$) with switchable trace amount oxygen (STOX) sensors (Revsbech et al. 2009).

Organic matter availability – Recent studies have observed pronounced auto- and heterotrophic N-cycling activities, including anammox, near the upper OMZ boundary (Thamdrup et al. 2006; Hamersley et al. 2007; Lam et al. 2009; Jensen et al. 2011). The high

activities have been attributed to enhanced organic matter respiration and thus NH_4^+ release in this zone. In accordance, high rates of N-loss have generally been observed in productive coastal waters, compared to those further offshore (Lam and Kuypers 2011). Hence, export production appears to largely regulate the vertical and lateral distributions of N-cycling processes in OMZs.

To explore the regulatory effect of organic matter availability, a large-scale survey of N-cycling activities, accompanied by extensive chlorophyll, nutrient and O_2 measurements, was conducted in the ETSP. In an area of $>1 \times 10^6 \text{ km}^2$ off Peru, where the ETSP OMZ is most pronounced, rates of anammox, denitrification, NO_3^- reduction to NO_2^- , DNRA and nitrification were determined in ^{15}N -labeling experiments. Simultaneously, export production was modelled to analyze the relation between N-cycling activity and organic matter availability.

Microaerobic respiration – Below $\sim 5 \mu\text{mol L}^{-1}$ of O_2 , NO_3^- is generally assumed to replace O_2 as the major electron acceptor in organic matter respiration (Devol 1978; Codispoti et al. 2001). However, active aerobic nitrification throughout the OMZs indicates the persistence of O_2 -dependent processes even at non-detectable concentrations of O_2 . The NH_4^+ demands of anammox cannot be entirely fulfilled by hitherto identified sources of NH_4^+ in OMZs (Lam et al. 2009). Therefore, so far neglected microaerobic organic matter respiration may be an important remineralization pathway in OMZs and consequently an important source of NH_4^+ for anammox.

The extent of microaerobic respiration as a potential source of NH_4^+ was investigated in the Namibian and Peruvian OMZs. A newly developed ^{18}O -tracer technique was applied to measure microaerobic respiration in parallel to the above-mentioned ^{15}N -labeling experiments. Direct rate measurements were combined with metagenomic analyses of functional genes encoding for terminal respiratory oxidases with high O_2 affinities.

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Overview of enclosed manuscripts

Oxygen sensitivity of anammox and coupled N-cycle processes in Oxygen Minimum Zones

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

Published in: *PLoS ONE* (2011), 6(12):e29299.

Organic matter export regulates N-cycling in the South Pacific Oxygen Minimum Zone

Kalvelage, Tim, Gaute Lavik, Sergio Contreras, Lionel Arteaga, Phyllis Lam, Caroline Löscher, Andreas Oschlies, Aurélien Paulmier, Lothar Stramma and Marcel M.M. Kuypers

Submitted to: *Nature Geoscience*

Microaerobic Respiration in Oxygen Minimum Zones

Kalvelage, Tim, Gaute Lavik, Marlene M. Jensen, Sergio Contreras, Niels Peter Revsbech, Aurelién Paulmier, Harald Schunk and Marcel M.M. Kuypers

In preparation

Oxygen sensitivity of anammox and coupled N-cycle processes in Oxygen Minimum Zones

Kalvelage, Tim^{1*}, Marlene M. Jensen^{1,4}, Sergio Contreras^{1,5}, Niels Peter Revsbech², Phyllis Lam¹, Marcel Günter¹, Julie LaRoche³, Gaute Lavik¹ and Marcel M.M. Kuypers¹

¹Max Planck Institute for Marine Microbiology, Bremen, Germany

²Department of Biological Sciences, University of Aarhus, Denmark

³GEOMAR | Helmholtz Centre for Ocean Research Kiel, Germany

⁴Current address: Technical University of Denmark, Kongens Lyngby, Denmark

⁵Current address: Large Lakes Observatory, University of Minnesota Duluth, USA

*Corresponding author. E-mail: tkalvela@mpi-bremen.de

Keywords: Oxygen minimum zone, ocean de-oxygenation, nitrogen loss, anammox, ammonia oxidation, nitrate reduction, oxygen sensitivity, STOX sensor.

Acknowledgements

We wish to thank the governments of Namibia and Peru for access to their territorial waters. We sincerely thank the cruise leaders Kay Emeis (M76-2) and Martin Frank (M77-3) as well as the crews of the cruises onboard R/V Meteor for their support at sea. We are grateful for the technical and analytical assistance of Gabriele Klockgether, Daniela Franzke, Inka Boosmann, Violeta Leon, Aurélien Paulmier, Moritz Holtappels, Andreas Ellrott, Volker Meyer, Philipp Hach and Michael Jünemann. We thank Gail Arnold and Rachel Foster for reading the early version of the manuscript and offering valuable comments to improve the article. This study was supported by the DFG-funded Sonderforschungsbereich 754 “Climate-Biogeochemistry Interactions in the Tropical Ocean” and the Max Planck Society. Additional funding came from the European Research Council, grant 267233, and the Danish Council for Independent Research: Natural Sciences, grant 10-083140.

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, NH₃ 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, NH₃ oxidation to NO₂⁻ and NO₃⁻ reduction to NO₂⁻ as the main NH₄⁺ and NO₂⁻ sources for anammox were only moderately affected by changing O₂ concentrations. Intriguingly, aerobic NH₃ 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 NH₃ 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 (O_2) is one of the key regulatory factors of major biogeochemical cycles in the marine environment (Falkowski 2008). 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) into 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 respiration of sinking organic matter by heterotrophic microorganisms consumes O_2 at maximal rates.

Subsurface regions of severely reduced O_2 concentrations ($O_2 \leq 5 \mu\text{mol L}^{-1}$), 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 (Wyrski 1962; Karstensen et al. 2008), this leads to permanently O_2 -depleted to anoxic conditions at mid-depths (Kamykowski and Zentara 1990; Helly and Levin 2004; Revsbech et al. 2009).

Although OMZs (if defined by $O_2 \leq 5 \mu\text{mol L}^{-1}$) account for only ~0.1% of the global ocean volume (Codispoti et al. 2001), 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 (Codispoti et al. 2001; Gruber and Sarmiento 1997). 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 (Kuypers et al. 2005; Thamdrup et al. 2006; Hamersley et al. 2007).

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 (Naqvi et al. 2000; Stramma et al. 2008). A continuing decline in dissolved O_2 due to reduced O_2 solubility and enhanced stratification (Keeling et al. 2010), as well as coastal and open ocean eutrophication (Diaz and Rosenberg 2008; Duce et al. 2008), 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. (Deutsch et al. 2011) calculated that a reduction of the mean upper ocean O_2 content by only 1% would mean a doubling of water masses with $O_2 \leq 5 \mu\text{mol L}^{-1}$, thus significantly enlarging the ocean volume potentially affected by N-loss.

However, the sensitivities of anammox and denitrification to changes in dissolved O_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 $20 \mu\text{mol L}^{-1}$ of O_2 (Smethie Jr 1987). 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 $20 \mu\text{mol L}^{-1}$ in the Namibian and Peruvian upwelling systems, respectively (Kuypers et al. 2005; Hamersley et al. 2007), and it has been

suggested that marine snow aggregates could provide suitable anoxic micro-niches at ambient O_2 concentrations up to $25 \mu\text{mol L}^{-1}$ (Ploug 2001; Woebken et al. 2007). Off Peru/Chile the measured anammox rates were often the highest at the base of the oxycline and in the upper OMZ (Thamdrup et al. 2006; Hamersley et al. 2007; Galán et al. 2009), 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 $1 \mu\text{mol L}^{-1}$ (Strous et al. 1997), marine anammox bacteria can tolerate O_2 concentrations higher than the upper O_2 limit ($5 \mu\text{mol L}^{-1}$) often used to restrict anaerobic processes in biogeochemical models (Paulmier et al. 2009). Recently, Jensen et al. (2008) 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 $\sim 9 \mu\text{mol L}^{-1}$ 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 ($0\text{-}20 \mu\text{mol L}^{-1}$), 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 (Zumft 1997). In OMZ waters, secondary NO_2^- maxima are often interpreted as active NO_3^- reduction (Cline and Richards 1972; Codispoti and Packard 1980). 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^+ (Zumft 1997; Gonzales et al. 2006). Heterotrophic NO_3^- reduction to NO_2^- has been measured at high rates in the Peruvian OMZ (Lipschultz et al. 1990; Lam et al. 2009), and has been estimated to account for approximately two thirds of the NO_2^- required for anammox in this region (Lam et al. 2009). At the same time, NO_3^- reduction also provides an important source of NH_4^+ released from oxidized organic matter (Dalsgaard et al. 2003; Lam et al. 2009). Lipschultz et al. (Lipschultz et al. 1990) 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 ($2.5 \mu\text{mol L}^{-1}$ of O_2), while rates decreased by $\sim 75\%$ at $20 \mu\text{mol L}^{-1}$ 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 (Ward and Zafiriou 1988; Ward et al. 1989). In the Peruvian OMZ, this is also where anammox bacteria are most active (Hamersley et al. 2007). These bacteria are partly fueled by NH_3 oxidation in this zone (Lam et al. 2009). A similarly tight coupling between anammox and NH_3 oxidation was shown earlier for the Black Sea (Lam et al. 2007). 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 ($<1\text{-}2 \mu\text{mol L}^{-1}$) in the core of OMZs (Lam et al. 2009; Ward et al. 1989; Molina et al. 2005) and are thus obviously well adapted to near-anoxic O_2 conditions. When Lipschultz et al. (1990) investigated the O_2 sensitivity of NH_3 oxidation in the Peruvian OMZ, the inferred de-oxygenation of the samples only caused a $\sim 50\%$ decrease in activity relative to ambient O_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 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 CO₂. However, at present no study has systematically investigated the O₂ 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 world's oceans associated with massive N-loss, where we explored the effect of O₂ on anammox, NH₃ oxidation and NO₃⁻ reduction throughout the OMZ.

Materials and Method

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 O₂ micro-sensor to obtain vertical profiles of dissolved O₂. In addition, the recently developed STOX (Switchable Trace amount OXYgen) sensor (Revsbech et al. 2009), which allows high-accuracy O₂ measurements in near-anoxic environments (detection limit: 50-100 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 O₂ concentrations. Water samples were taken with a depth resolution of 1-2 m for nutrient analyses. NH₄⁺ was measured fluorometrically (Holmes et al. 1999) and NO₂⁻ was analyzed spectrophotometrically (Grasshoff et al. 1999) on board. Water samples for

NO_3^- and PO_4^{3-} were stored frozen until spectrophotometric determination (Grasshoff et al. 1999) 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 $\mu\text{mol L}^{-1}$) following Gruber and Sarmiento (1997): $\text{N}^* = [\text{NH}_4^+] + [\text{NO}_2^-] + [\text{NO}_3^-] - 16 \times [\text{PO}_4^{3-}] + 2.9 \mu\text{mol kg}^{-1} \times \text{density in kg L}^{-1}$

^{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 (Dalsgaard 2003; Kuypers et al. 2005; Holtappels et al. 2011). Briefly, N-loss by either anammox or heterotrophic denitrification was measured as the production of ^{15}N -labeled N_2 in $^{15}\text{NH}_4^+$ (+ $^{14}\text{NO}_2^-$), $^{15}\text{NO}_2^-$ (+ $^{14}\text{NH}_4^+$) and $^{15}\text{NO}_3^-$ (+ $^{14}\text{NO}_2^-$) (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 $^{15}\text{NO}_2^-$ production in $^{15}\text{NH}_4^+$ + $^{14}\text{NO}_2^-$ and $^{15}\text{NO}_3^-$ + $^{14}\text{NO}_2^-$ incubations respectively. The N-isotopic composition of NO_2^- was determined by GC/IRMS after conversion to either nitrous oxide (N_2O) by sodium azide (McIlvin and Altabet 2005), or to N_2 by sulfamic acid (Granger et al. 2006; Füssel et al. 2011). 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 (Lam et al. 2009), 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 $^{29}\text{N}_2$ and $^{30}\text{N}_2$ (Holtappels et al. 2011). 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 $12 \mu\text{mol L}^{-1}$ 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 $24 \mu\text{mol L}^{-1}$ 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 $^{15}\text{NH}_4^+ + ^{14}\text{NO}_2^-$, $^{15}\text{NO}_2^- (+ ^{14}\text{NH}_4^+)$ or $^{15}\text{NO}_3^- + ^{14}\text{NO}_2^-$, samples were transferred into replicate vials (Exetainers, Labco) for time-series incubations. Except for the incubations with only $^{15}\text{NO}_2^-$, ^{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 $^{29}\text{N}_2$ due to coupled nitrification-denitrification in incubations amended with $^{15}\text{NH}_4^+$ we added allylthiourea (ATU; final concentration $84 \mu\text{mol L}^{-1}$) to an additional sample of the highest O_2 treatment ($\sim 11.5 \mu\text{mol L}^{-1}$) at St. 206 and 252. ATU is a specific inhibitor of aerobic NH_3 oxidation (Hall 1984; Hall and Jeffries 1984; Bedard and Knowles 1989) and does not affect anammox activity shown at least in sediments (Jensen et al. 2007). 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: $\sim 0.5 \mu\text{mol L}^{-1}$ 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 (Kemmer and Keller 2010).

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 $\sim 200 \mu\text{mol L}^{-1}$ in the surface waters to less than $10 \mu\text{mol L}^{-1}$ at ~ 80 m. STOX measurements at the incubation depths revealed O_2 concentrations as low as $0.60 \pm 0.11 \mu\text{mol L}^{-1}$ 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 $\mu\text{mol L}^{-1}$ in the oxic zone (<80 m) and decreased to 0.1-0.5 $\mu\text{mol L}^{-1}$ 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 $\mu\text{mol L}^{-1}$ (St. 252). Nitrite concentrations were fairly constant in the upper ~100 m (0.1-0.5 $\mu\text{mol L}^{-1}$) and increased to ~2 and ~4 $\mu\text{mol L}^{-1}$ 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 $\mu\text{mol L}^{-1}$ in the lowest sampling depths at both stations.

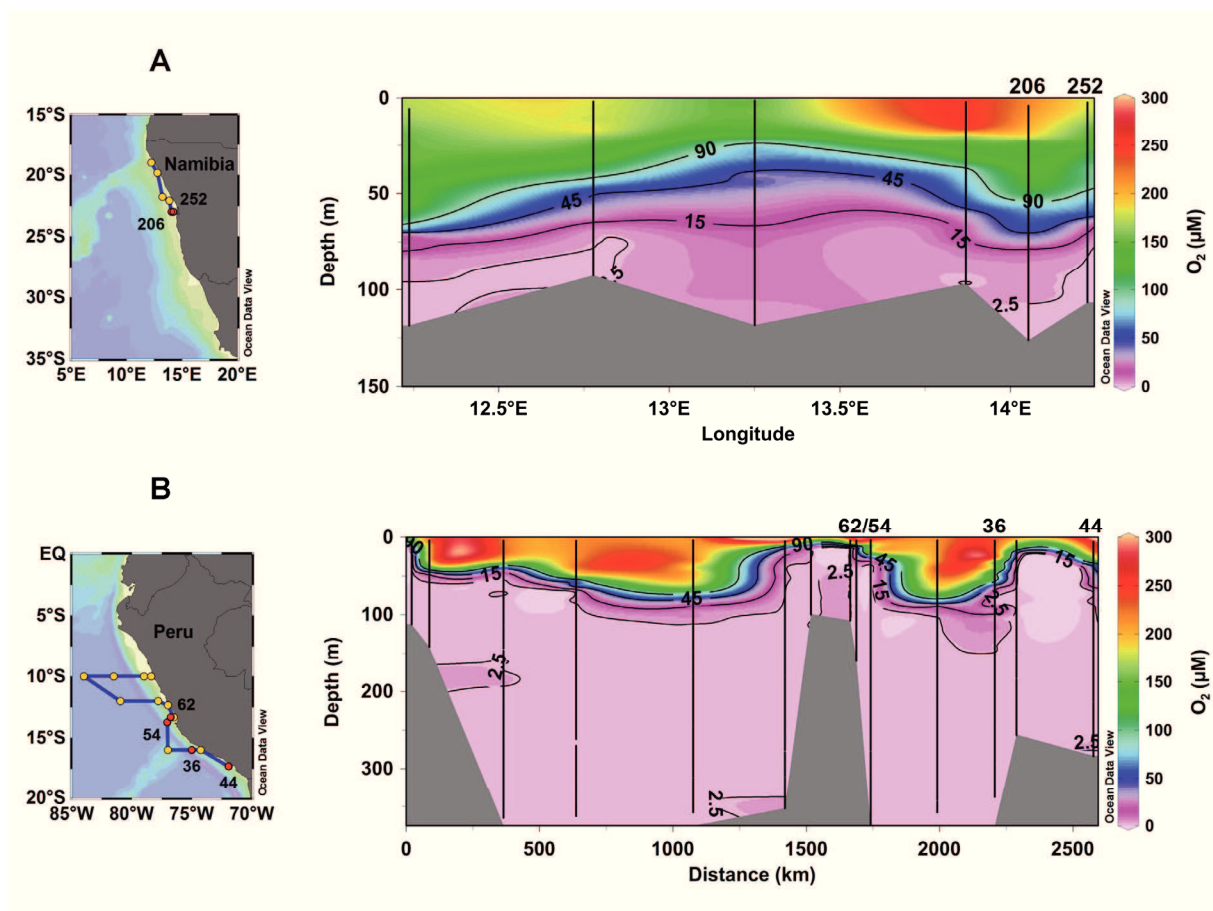


Figure 1 | Locations of the sampled stations and distribution of dissolved O_2 .

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: ~375m). 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.

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 (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 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 NO₂⁻ 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 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 ¹⁵N¹⁴N formation in all of our

$^{15}\text{NH}_4^+$ (+ $^{14}\text{NO}_2^-$) and $^{15}\text{NO}_2^-$ -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 $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_2^-$ experiments. As no increase in $^{15}\text{N}^{15}\text{N}$ was detectable in either $^{15}\text{NO}_2^-$ or $^{15}\text{NO}_3^-$ 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 $\text{N L}^{-1} \text{d}^{-1}$ at the base of the oxycline to 144 to 496 nmol $\text{N L}^{-1} \text{d}^{-1}$ in the central OMZ and were generally higher at St. 252.

In the OMZ off Peru, the N-deficit was strongest over the shelf ($\text{N}^* = -33 \mu\text{mol L}^{-1}$; St. 62) and less pronounced towards the open ocean ($\text{N}^* = 10 \mu\text{mol L}^{-1}$; St. 54), indicating the highest N-loss likely occurred near the coast. Six depths per station were sampled and $^{15}\text{N}^{14}\text{N}$ formation in $^{15}\text{NH}_4^+ + ^{14}\text{NO}_2^-$ and $^{15}\text{NO}_2^- + ^{14}\text{NH}_4^+$ 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 $^{29}\text{N}_2$ and there was no detectable increase in $^{15}\text{N}^{15}\text{N}$ over time detected in either $^{15}\text{NO}_2^-$ or $^{15}\text{NO}_3^-$ 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 $\text{N L}^{-1} \text{d}^{-1}$; 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 $\text{N L}^{-1} \text{d}^{-1}$; St. 54).

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

* No addition of ¹⁴N-species.§ In μmol L⁻¹.

‡ Determined with STOX sensor.

† In nmol N L⁻¹ d⁻¹.

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

Off Peru, NO_3^- reduction could be detected in 21 out of 23 $^{15}\text{NO}_3^- + ^{14}\text{NO}_2^-$ 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 $\text{nmol N L}^{-1} \text{d}^{-1}$) and decreased towards the open ocean (up to 48 $\text{nmol N L}^{-1} \text{d}^{-1}$).

Distribution of ammonia oxidation activity – Ammonia oxidation, measured as $^{15}\text{NO}_2^-$ production in $^{15}\text{NH}_4^+ + (^{14}\text{NO}_2^-)$ 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 ($<1 \mu\text{mol L}^{-1}$) at St. 252. Rates increased with depth at St. 206 (from 29 to 84 $\text{nmol N L}^{-1} \text{d}^{-1}$) but remained rather constant at St. 252 ($\sim 100 \text{nmol N L}^{-1} \text{d}^{-1}$).

Off Peru, NH_3 oxidation to NO_2^- was determined in $^{15}\text{NH}_4^+ + ^{14}\text{NO}_2^-$ 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 $\text{nmol N L}^{-1} \text{d}^{-1}$. 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 O_2 *in situ* (i.e. O_2 concentrations were below detection) shipboard experiments revealed NH_3 oxidation activity also at St. 54 at 75 m as well as in the central OMZ at St. 62 (1.7 to 5.0 $\text{nmol N L}^{-1} \text{d}^{-1}$).

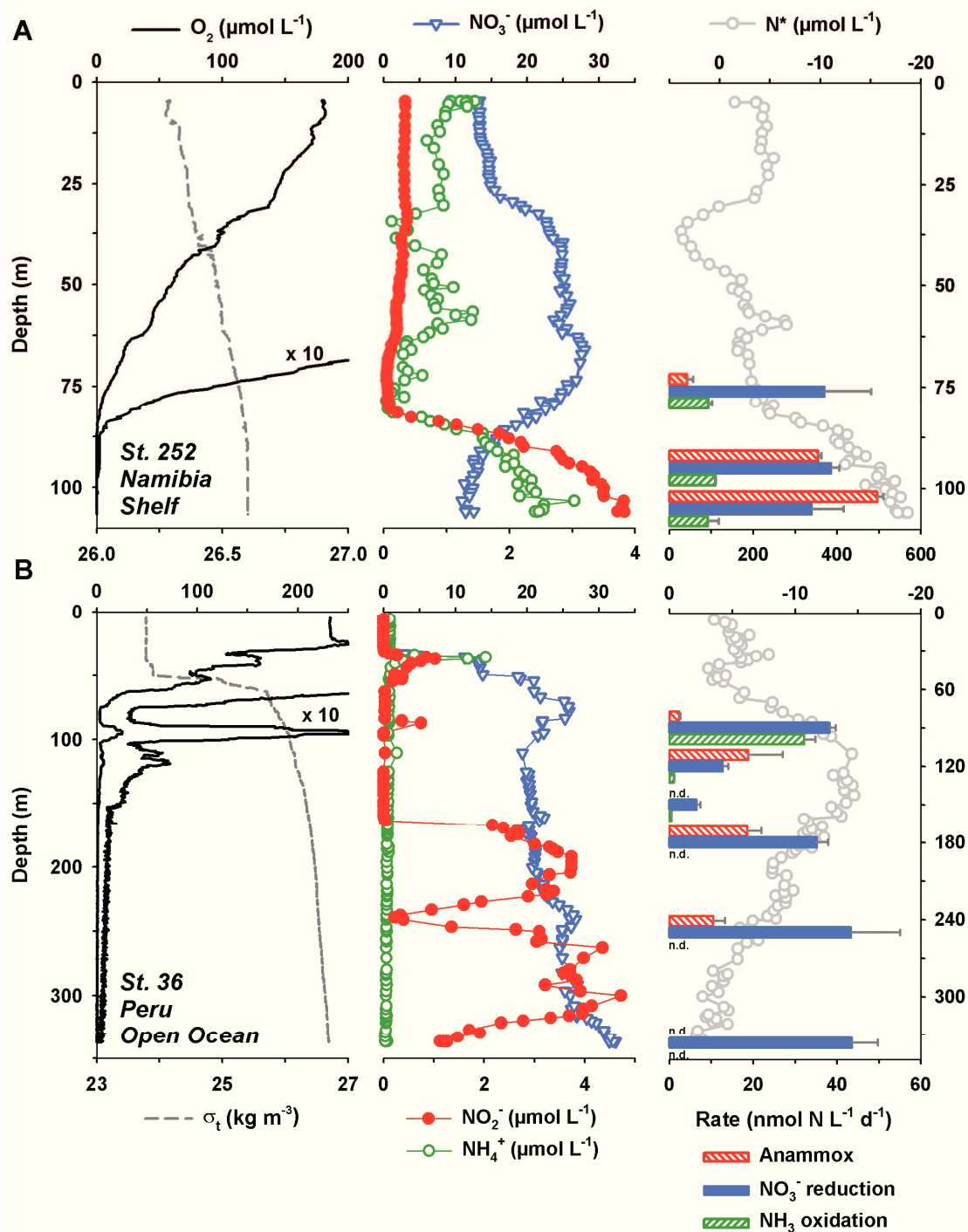


Figure 2 | Physicochemical zonation and N-conversion rates at selected stations.

Stations are plotted for cruises M76-2 and M77-3 to the OMZs off A) Namibia and B) Peru, respectively. Water depths were 111 m at St. 252 and 2845 m at St. 36. N^* was calculated from the fixed inorganic N- and PO_4^{3-} concentrations (data not shown). Anammox rates were determined in $^{15}\text{NH}_4^+$ (St. 252) and $^{15}\text{NO}_2^- + ^{14}\text{NH}_4^+$ incubations (St. 36). All rates are net rates corrected for the percentage of ^{15}N in the pool of the respective N-species. Error bars for rates are standard errors calculated from linear regression. n.d. = non detectable.

Oxygen sensitivity of anammox and coupled N-cycle processes

Oxygen sensitivity of anammox – Anammox activity, as indicated by $^{15}\text{N}^{14}\text{N}$ production from $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_2^-$, 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 $^{15}\text{NH}_4^+$ as well as $^{15}\text{NO}_2^-$ at St. 206 and the one with $^{15}\text{NH}_4^+$ 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 $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_2^-$ at both stations. Activity decreased with increasing O_2 and was, on average, $\sim 85\%$, $\sim 70\%$ and $\sim 50\%$ of the anoxic control at ~ 3.7 , ~ 8.1 and $\sim 11.3 \mu\text{mol L}^{-1}$ 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 ($\pm 0.44 \mu\text{mol L}^{-1}$ on average). No substantial difference in $^{15}\text{N}^{14}\text{N}$ production was observed between $^{15}\text{NH}_4^+$ -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 $11\text{-}12 \mu\text{mol L}^{-1}$ of dissolved O_2 .

In the OMZ off Peru, $^{15}\text{N}^{14}\text{N}$ production rates in $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_2^-$ 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 $14.4 \mu\text{mol L}^{-1}$ O_2 at St. 44 (Pearson $r = -0.99$, $P < 0.05$) and $10.9 \mu\text{mol L}^{-1}$ at St. 62 (Pearson $r = -0.96$, $P < 0.05$). No rates were detectable beyond $\sim 20 \mu\text{mol L}^{-1}$ 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, $\sim 30\%$ activity of the anoxic control experiment remained detectable when O_2 was increased from the in situ $\sim 1.2 \mu\text{mol L}^{-1}$ (measured by STOX) to $5.5 \mu\text{mol L}^{-1}$ of O_2 in the 120 m sample. In comparison, anammox was fully inhibited at $2.8 \mu\text{mol L}^{-1}$ of O_2 already in the 180 m sample, where O_2 was not detectable by

the STOX sensor *in situ*. A similarly strong 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 NO₃⁻ reduction rates to ~60% of the control experiment at St. 252 (Fig. 3B).

In the Peruvian OMZ, production of ¹⁵NO₂⁻ from ¹⁵NO₃⁻ was never fully inhibited by O₂, not even in the highest O₂ treatments (~25 μmol L⁻¹ of O₂). Nevertheless, NO₃⁻ reduction rates showed marked differences in their sensitivity towards elevated O₂ levels between and within our experimental stations. For example at St. 36, NO₃⁻ 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 NO₂⁻ 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, ¹⁵NO₂⁻ production in the ¹⁵NH₄⁺ 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 $\mu\text{mol L}^{-1}$ O_2) at St. 54 suggested a slightly lower NH_3 oxidation rate (-35 %) compared to the higher O_2 treatments (Fig. 3C).

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		NO_3^- reduction		Anammox			
		$^{15}\text{NH}_4^+ + ^{14}\text{NO}_2^-$		$^{15}\text{NO}_3^- + ^{14}\text{NO}_2^-$		$^{15}\text{NH}_4^+ + ^{14}\text{NO}_2^-$		$^{15}\text{NO}_2^- + ^{14}\text{NH}_4^+$	
		O_2 § ‡	Rate†	O_2 § ‡	Rate†	O_2 § ‡	Rate†	O_2 § ‡	Rate†
Namibian OMZ	M76-206	2.0	70±5	0.8	65±2	2.0	122±3	0.8	119±10 *
	(100m)	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 *
	(105m)	3.3	103±18	6.0	148±8	3.3	289±7	3.8	320±9 *
		7.7	89±24	10.1	123±7	7.7	246±7	7.4	267±5 *
		11.7	88±16	14.7	119±6	11.7	167±7	11.1	217±8 *
	+ ATU					10.9	179±7		
Peruvian OMZ	M77-36			1.4	22.3±2.5			0.6	10.1±1.2
	(120m)			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
	(180m)			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		
	(75m)	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		
	(75m)	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	
(50m)			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 $\mu\text{mol L}^{-1}$.

‡ Adjusted concentrations of O_2 , determined by μ -sensor measurements.

† In $\text{nmol N L}^{-1} \text{d}^{-1}$.

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 ¹⁵N-labeling experiments was anammox. This confirms the results from earlier studies, which detected N-loss due to anammox but not denitrification in these regions (Kuypers et al. 2005; Thamdrup et al. 2006; Hamersley et al. 2007). 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 (Carr 2002) as well as between Peruvian coastal and open-ocean waters, since organic matter transport ultimately fuels all processes delivering NH₄⁺ and NO₂⁻ for the anammox reaction (Dalsgaard et al. 2003; Lam et al. 2009). Anammox often showed the highest rates in the upper OMZ, as seen in previous studies (Thamdrup et al. 2006; Hamersley et al. 2007; Galán et al. 2009) probably in response to the high NH₄⁺ 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 NH₄⁺ diffusing out of the sediment (Kuypers et al. 2005; Lavik et al. 2008; Bohlen et al. 2011; 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 (Jensen et al. 2008). In comparison, anammox activity appeared increasingly sensitive to O₂ towards the open ocean and deeper in

the OMZ, where rates were not detectable above 2.8 to 5.5 $\mu\text{mol L}^{-1}$ 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 $\sim 20 \mu\text{mol L}^{-1}$ (Table 3; Fig. 3).

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 (Chapman and Shannon 1985). In the open-ocean off Peru, ventilation of the OMZ from above is hindered due to strong stratification (Fuenzalida et al. 2009). 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 (180m at St. 36). With O_2 concentrations consistently below 1-2 $\mu\text{mol L}^{-1}$, 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 $< 25 \mu\text{mol L}^{-1}$ (Kuypers et al. 2005; Woebken et al. 2007), while some anammox bacteria have been shown to be potentially particle-associated in the Namibian OMZ (Woebken et al. 2007). 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.

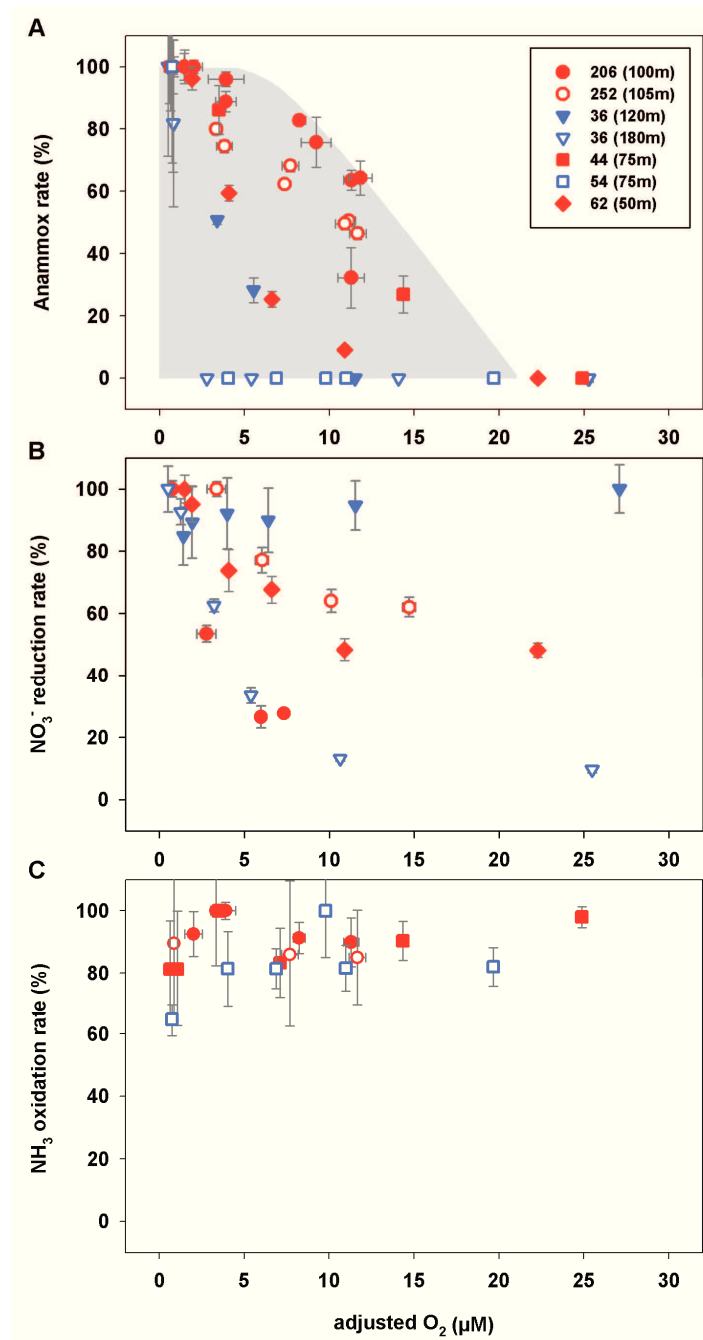


Figure 3 | N-cycle processes in Namibian and Peruvian OMZ waters with respect to dissolved oxygen.

(a) Anammox measured as $^{15}\text{N}^{14}\text{N}$ production in $^{15}\text{NO}_2^-$ (+ $^{14}\text{NH}_4^+$) and $^{15}\text{NH}_4^+$ + $^{14}\text{NO}_2^-$ incubations. (b) NO_3^- reduction measured as $^{15}\text{NO}_2^-$ production in $^{15}\text{NO}_3^-$ + $^{14}\text{NO}_2^-$ incubations. (c) NH_3 oxidation measured as $^{15}\text{NO}_2^-$ production in $^{15}\text{NH}_4^+$ + $^{14}\text{NO}_2^-$ incubations. N-conversion rates are given as percentages of the highest rate observed (= 100 %) for the different O₂ treatments at each incubation depth. Adjusted O₂ 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 O₂ sensitivity assays indicate an upper O₂ limit for N-loss due to anammox of ~20 μmol L⁻¹ (grey shading).

Table 3 | Overview of the response of NH₃ oxidation, NO₃⁻ reduction and anammox to changes in dissolved O₂.

Process	Region	Station	Sampled depth (m)	Substrate addition	Upper OMZ boundary (m) †	<i>in situ</i> 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 O₂ 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.

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 $360 \text{ nmol L}^{-1} \text{ d}^{-1}$) and decreased with increasing distance from the coast in the Peruvian OMZ (~ 10 to $50 \text{ nmol L}^{-1} \text{ d}^{-1}$ at St. 36). The rates measured off Peru are consistent with earlier results from ^{15}N -labeling experiments in the same region (Lipschultz et al. 1990; Lam et al. 2009) and a similar rate distribution was recently reported for the Arabian Sea OMZ (Nicholls et al. 2007; Lam et al. 2011).

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. (Lipschultz et al. 1990) 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 $17 \text{ } \mu\text{mol L}^{-1}$ 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 $\sim 50\%$ relative to the control already at $\sim 4 \text{ } \mu\text{mol L}^{-1}$ 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 (Robertson and Kuenen 1984; Robertson et al. 1995; Gao et al. 2009). 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 (Körner and Zumft 1989; Coyne and Tiedje 1990; McKenney et al. 1994). 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 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 (Lam et al. 2009; Hartsock and Shapleigh 2011).

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 (Ward et al. 1989; Lam et al. 2009; Molina and Fariás 2009).

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 $\mu\text{mol L}^{-1}$ of O_2) was achieved. A similar observation was made by Lipschultz et al. (1990), 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 $\sim 2 \mu\text{mol L}^{-1}$ (Gundersen 1966; Carlucci and McNally 1969; Goreau et al. 1980). The only cultured marine aerobic ammonia oxidizing archaea investigated so far appears to have a limited capacity to survive under near anoxic conditions (Martens-Habbena et al. 2009).

However, a higher O₂ affinity of archaeal 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) (Lam et al. 2009).

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. (2010). They demonstrated aerobic growth in a culture experiment at an O₂ concentration ≤3 nmol 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) (Schmidt and Bock 1997). 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 (Kuypers et al. 2005; Woebken et al. 2007).

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) (Strous et al. 1997) enlarges the global oceanic volume potentially affected by N-loss from the previously estimated 0.1% tenfold to ~1% (O₂ ≤20 μmol L⁻¹) (Lam and Kuypers 2011). In addition, recent reports show that OMZs have been expanding and intensifying worldwide, particularly in the tropical Atlantic and Pacific (Stramma et al. 2008). 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 $\sim 5 \mu\text{mol L}^{-1}$ of O₂ often implemented in models (Paulmier et al. 2009) 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 deoxygenation on N-cycling in OMZs and neighboring water masses, and hence global oceanic N-balance.

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Organic matter export regulates N-cycling in the South Pacific Oxygen Minimum Zone

Kalvelage, Tim^{1*}, Gaute Lavik¹, Sergio Contreras^{1,4}, Lionel Arteaga², Phyllis Lam¹, Carolin Löscher³, Andreas Oschlies², Aurélien Paulmier^{1,5}, Lothar Stramma² and Marcel M.M. Kuypers¹

¹Max Planck Institute for Marine Microbiology, Bremen, Germany

²GEOMAR | Helmholtz Centre for Ocean Research Kiel, Germany

³Institute for General Microbiology, Kiel, Germany

⁴Current address: Large Lakes Observatory, University of Minnesota Duluth, USA

⁵Current address: Laboratoire d'Etudes en Géophysique et Océanographie Spatiales, Toulouse, France

*Corresponding author. E-mail: tkalvela@mpi-bremen.de

Keywords: marine nitrogen cycle, export production, organic matter, nitrogen loss, N-deficit, anammox, secondary nitrite maximum, oxygen minimum zone, eastern tropical South Pacific, Peruvian upwelling system.

Acknowledgements

We wish to thank the Peruvian government for access to their territorial waters. We sincerely thank cruise leader Martin Frank as well as the crews of the cruises M77-3 and 4 onboard R/V Meteor for their support at sea. We are grateful for the technical and analytical assistance of Gabriele Klockgether, Inka Boosmann, Violeta Leon, Imke Grefe and Andreas Ellrott. We thank Niels Peter Revsbech, who generously provided the STOX sensors and Tim Ferdelmann for offering valuable comments to improve the article. This study was supported by the DFG-funded Sonderforschungsbereich 754 “Climate-Biogeochemistry Interactions in the Tropical Ocean” and the Max Planck Society.

Abstract

Oxygen minimum zones (OMZs) are major sites of oceanic nitrogen loss, which have been expanding in the last decades. Global biogeochemical models predict that future ocean de-oxygenation will significantly alter the ocean's nutrient balance and possibly hamper biological carbon sequestration. However, given the discrepancy between modeled and observed global patterns of nitrogen loss, such forecasts remain speculative and require a better understanding of the factors controlling pelagic nitrogen loss. Here we show that nitrogen loss and general nitrogen cycling activity in OMZs is tightly linked to the export of organic matter. We examined microbial nitrogen cycling processes and nutrient distributions in the Eastern Tropical South Pacific OMZ, covering an area of $>1 \times 10^6$ km². Contrary to the general belief, high nitrogen loss did not coincide with the prominent secondary nitrite maxima offshore. Instead, nitrogen loss due to anammox was most pronounced over the productive Peruvian shelf, driven by high rates of sinking organic matter as well as benthic ammonium release. We conclude that the offshore nitrogen deficits largely result from lateral advection of nitrogen-deficient shelf waters combined with a long ventilation time. Our results highlight the importance of coastal OMZs for the ocean's nutrient budget and may help to reconcile biogeochemical models with observations, to realistically assess the effects of climate change on oceanic nitrogen and carbon cycling.

Introduction

Coastal upwelling of nutrient-rich deep water fuels high rates of surface productivity at the eastern boundaries of (sub) tropical oceans. The resultant export of organic matter stimulates strong microbial respiration in the subsurface. Combined with the lack of oxygen (O₂) replenishment in these poorly ventilated areas, permanently O₂-deficient water masses are formed at mid-depths (Kamykowski and Zentara 1990; Karstensen et al. 2008). These so-called oxygen minimum zones (OMZs) have been expanding globally and a continuing decline in dissolved O₂ is expected as anthropogenic pressures on the marine environment are growing (Diaz and Rosenberg 2008; Stramma et al. 2008; Keeling et al. 2010).

Although representing only ~1% (O₂ ≤ 20 μmol kg⁻¹) of the global ocean volume (Lam and Kuypers 2011), OMZs have a profound impact on the ocean's nitrogen (N) balance as they account for ~30% of the oceanic N-loss (Gruber 2004). Ocean de-oxygenation might significantly enlarge the ocean volume affected by N-loss (Deutsch et al. 2011), therewith exacerbate N-limitation of phytoplankton and reduce the ocean's capacity to attenuate rising atmospheric carbon dioxide (CO₂). Assessing the effects of expanding OMZs on the future ocean's nutrient balance, however, remains speculative, as biogeochemical models do not reproduce present-day global patterns of N-loss (Moore and Doney 2007; Schmittner et al. 2008; Somes et al. 2010). A major deficit of those models appears to be the coarse resolution near the coast. Recent studies indicate that N-loss in shelf OMZs (Kuypers et al. 2005; Hamersley et al. 2007; Jensen et al. 2011), coastal-offshore OMZ water mass exchange (Lam et al. 2011) and OMZ-sediment interactions (Bohlen et al. 2011) play an important role on the overall N-budget.

Based on the observed accumulations of nitrite (NO₂⁻) and associated N-deficits in OMZ waters, N-losses have traditionally been attributed to heterotrophic denitrification (Cline and Richards 1972; Codispoti and Packard 1980; Naqvi 1987), the stepwise reduction of

nitrate (NO_3^-) to gaseous dinitrogen (N_2). Recent studies have however failed to detect significant denitrifying activity in OMZs; rather, anammox, the anaerobic oxidation of ammonium (NH_4^+) with NO_2^- , has been identified as a major N_2 -forming pathway in these environments (Kuypers et al. 2005; Thamdrup et al. 2006; Hamersley et al. 2007; Jensen et al. 2011).

Nevertheless, the regulation of N-loss activity including anammox is not fully understood. Anammox requires both NH_4^+ and NO_2^- . Various sinks and sources of both compounds have been identified in OMZs and include NH_3 and NO_2^- oxidation, NO_3^- reduction to NO_2^- and dissimilatory $\text{NO}_3^-/\text{NO}_2^-$ reduction to NH_4^+ (DNRA) (Lam et al. 2009; Füssel et al. 2011; Jensen et al. 2011; Lam et al. 2011). Surprisingly, aerobic and anaerobic N-linked processes appear to co-occur over a large range of O_2 concentrations ($>0\text{-}20 \mu\text{mol L}^{-1}$) in OMZ waters and hence do not seem to be primarily controlled by O_2 (Lipschultz et al. 1990; Kalvelage et al. 2011). Instead, the vertical distributions of anammox and associated N-cycling processes indicate that their activities might be tightly coupled to the availability of organic matter. For instance, enhanced auto- and heterotrophic N-cycling activity near the upper OMZ boundary may be fueled by sinking organic matter and remineralized NH_4^+ (Thamdrup et al. 2006; Hamersley et al. 2007; Lam et al. 2009; Jensen et al. 2011). Moreover, high anammox rates have generally been measured in productive coastal waters, compared to the offshore OMZs (Lam and Kuypers 2011), indicating that N-loss might be ultimately regulated by primary production.

To test this hypothesis, we conducted a large-scale survey of N-cycling rates, N-functional gene abundances, as well as chlorophyll, nutrient and O_2 concentrations throughout the eastern tropical South Pacific (ETSP). The ETSP OMZ is one of the largest globally, the core of which extends >1000 km westward off the coast of Peru (Fuenzalida et al. 2009). Previous studies in this area were mainly confined to the shelf and continental slope area, thus the lateral distribution of N-cycling activity (i.e. from shelf to offshore) has been poorly

constrained. Here, we present by far the most comprehensive N-budget assessment including all major N-fluxes for this major OMZ. We show that N-loss due to anammox and N-cycling activity overall is highest over the Peruvian shelf and rapidly decreases towards the open ocean. We further modeled the export fluxes of organic matter from measured surface chlorophyll-a and compare them with N-cycling activity distribution, in order to illustrate how organic matter is likely a key determinant to N-cycling activity in the OMZs.

Materials & Methods

Physico-chemical and N-cycling rate measurements – Large-scale distributions of chemical and biological variables were determined during the cruises M77-3 and 4 from December 2008 to February 2009 onboard R/V Meteor. Sea water was collected with either a conductivity-temperature-depth (CTD) rosette system fitted with 10-L Niskin bottles or a pump-CTD system (depth range: ~375 m). Continuous vertical profiles of chlorophyll-a were obtained fluorometrically and calibrated against discrete values derived from acetone extraction. Oxygen was measured with a Seabird sensor and a highly sensitive STOX (Switchable Trace amount Oxygen) sensor (Revsbech et al. 2009) (detection limit: 50 nmol L⁻¹). Fixed inorganic N- and PO₄³⁻ concentrations were analyzed using standard protocols (Grasshoff et al. 1999; Holmes et al. 1999). Nitrogen deficits were calculated as N* following Gruber & Sarmiento (1997). Rates of microbial N-cycling were determined in short-term ¹⁵N-incubation experiments, as described in Füssel et al. (2011) and Holtapples et al. (2011).

Molecular ecological analyses – Nucleic acid samples were collected onto polyethersulfone membrane filters (0.2 µm; Millipore) and immediately frozen at -80°C until further analysis. Nucleic acids were extracted using a Qiagen DNA/RNA All prep Kit following the

manufacturers protocol with minor modifications (Loescher et al. 2012). Functional genes for archaeal and bacterial NH_3 oxidation (arch-*amoA* and β -/ γ -*amoA*, respectively), anammox (*hzo1* and 2), denitrification (denitrifier-*nirS*) and DNRA (*nrfA*) were PCR-amplified as described in L scher et al. (Loescher et al. 2012). Standards for quantitative PCRs were obtained from: *Nitrosococcus oceani* NC10 and *Nitrosomonas marina* NM22 and NM51 (β - and γ -*amoA*, respectively), an environmental clone (GenBank accession number JF796147; arch-*amoA*), *Scalindua* sp. (*hzo1* and 2), *Pseudomonas aeruginosa* PAO1 (denitrifier-*nirS*) and *Escherichia coli* K12 (*nrfA*).

Modeling of export production – Export production was calculated from estimates of net primary production and the ratio of export production to total primary production (*ef*-ratio). Net primary production (NPP) at the time and location of our experimental stations was computed from measured chlorophyll-a concentrations and satellite-based (MODIS ocean color data) estimates of photosynthetic available radiation using the Vertically Generalized Production model (Behrenfeld and Falkowski 1997). *Ef*-ratios were calculated from NPP and measured surface temperatures after Laws et al. (Laws et al. 2000).

Results & Discussion

Nutrient distributions – Consistent with past observations in the ETSP (Codispoti and Packard 1980; Codispoti and Christensen 1985), pronounced secondary NO_2^- maxima ($\geq 1 \mu\text{mol L}^{-1}$) were found in the offshore OMZ between 10°S and 18°S (supplementary Fig. 1). These extended from the continental slope up to several hundred kilometers westward, with maximum concentrations up to $\sim 11 \mu\text{mol L}^{-1}$ (16°S). Integrated over the thickness of the OMZ ($\text{O}_2 \leq 15 \mu\text{mol L}^{-1}$; Fig. 1b; supplementary Fig. 2), NO_2^- concentrations reached $>2 \text{ mol}$

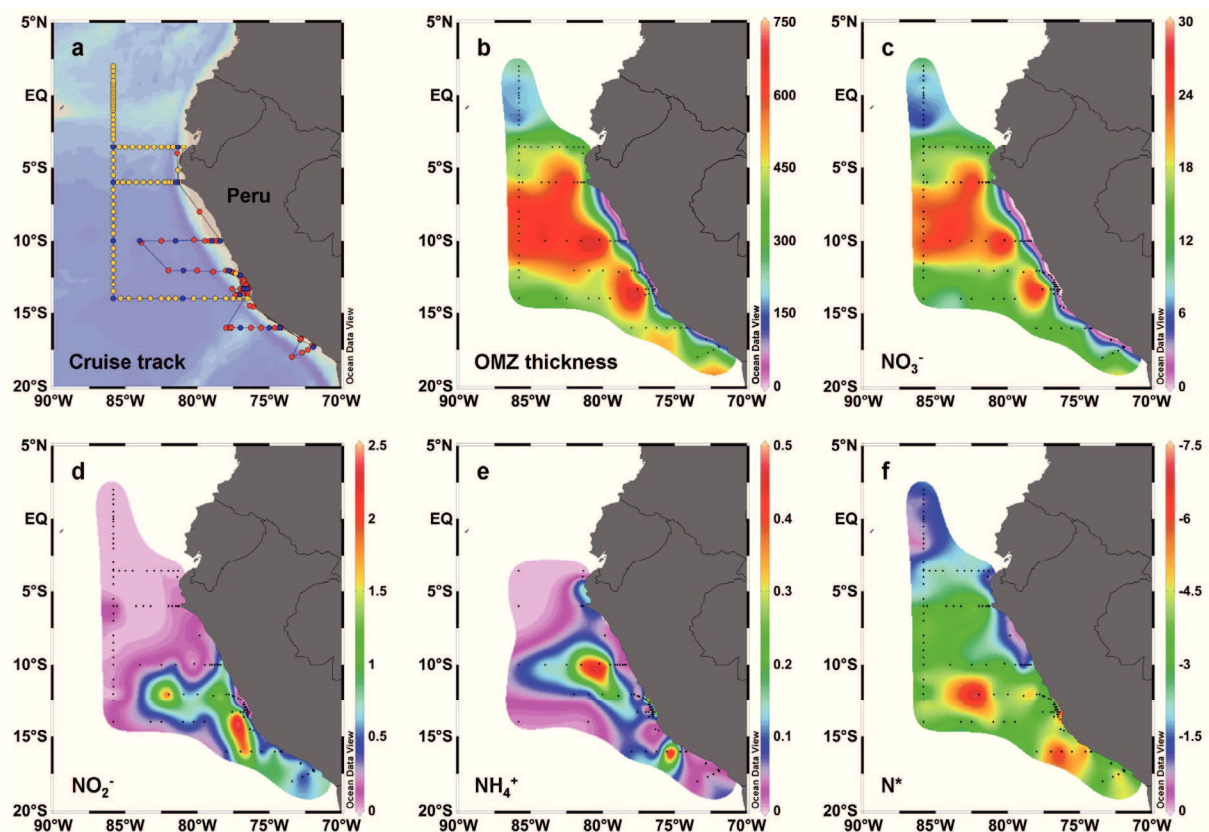


Figure 1 | Cruise map and nutrient distributions in the ETSP OMZ.

(a) Sampling sites during M77-3 (●) and M77-4 (●) and ^{15}N -experimental stations (●). (b) Vertical extent of the OMZ (in m) as defined by $\text{O}_2 \leq 15 \mu\text{mol L}^{-1}$. (c-f) Concentrations of NO_3^- , NO_2^- , NH_4^+ and N^* (in mol m^{-2}) integrated over the thickness of the OMZ.

m^{-2} in the offshore region (Fig. 1d). On the shelf, NO_2^- concentrations were as high as $\sim 12 \mu\text{mol L}^{-1}$ (10°S). Associated with the accumulation of NO_2^- in the offshore expansion of the OMZ were distinct N^* minima, that ranged from $-8 \mu\text{mol N L}^{-1}$ at 3.58°S down to $-32 \mu\text{mol N L}^{-1}$ at 16°S . Depth-integrated values of N^* (Fig. 1e) and NO_2^- were significantly correlated (Spearman $R = -0.61$, $p \leq 0.001$). Southward intensification of both NO_2^- maxima and N^* minima likely reflects the accumulation of time-integrated microbial activity in the OMZ waters, which has been transported poleward along the South American continental slope by the Peru-Chile Undercurrent (Wooster et al. 1965; Nelson and Neshyba 1979).

Concentrations of NH_4^+ were mostly below $0.25 \mu\text{mol L}^{-1}$ throughout the OMZ. Noticeably higher values ($\geq 0.5 \mu\text{mol L}^{-1}$) were sometimes detected over the shelf as well as

near the upper OMZ boundary further offshore (10-18°S). Deeper in the offshore OMZ, plumes of elevated NH_4^+ concentrations (up to $\sim 3 \mu\text{mol L}^{-1}$) were detected on the 10°S, 12°S and at 16°S transects (supplementary Fig. 1), resulting in depth-integrated concentrations of as much as $\sim 0.5 \text{ mol m}^{-2}$ (Fig. 1e).

Over the Peruvian shelf between 12°S and 14°S, N^* minima down to $\sim 60 \mu\text{mol L}^{-1}$ were detected from non-detectable levels of NO_x^- ($\text{NO}_3^- + \text{NO}_2^-$) yet PO_4^{3-} concentrations up to $\sim 4 \mu\text{mol L}^{-1}$ (supplementary Fig. 1). These extreme N-deficits coincided with the presence of hydrogen sulfide (H_2S). Stations with measurable H_2S are – unless indicated otherwise – excluded from the results presented in the following, as the community composition and microbial processes in the H_2S -bearing waters were profoundly different from the typical OMZ scenario (Schunck et al. submitted.).

Sources of NO_2^- – Nitrite is generated by aerobic NH_3 oxidation, in the first step of nitrification. Ammonia oxidation has been identified as an important source of NO_2^- in the Peruvian OMZ and has been found active in near-anoxic conditions (Ward et al. 1989; Lipschultz et al. 1990; Lam et al. 2009). Our measured rates of NH_3 oxidation were generally highest at the base of the oxycline (up to $\sim 90 \text{ nmol N L}^{-1} \text{ d}^{-1}$ at St. 805), decreased to detection limit at the stations furthest offshore (Table 1) and were not measurable at the core of the OMZ. The detection of archaeal and bacterial functional genes, which encode for the membrane-bound ammonia monooxygenase subunit A (arch-*amoA* and β -/ γ -*amoA*, respectively) (Table 1) support previous findings that both NH_3 -oxidizing archaea and bacteria are present in the Peruvian OMZ (Lam et al. 2009).

Integrated over the OMZ, production rates of NO_2^- via NH_3 oxidation ranged from undetectable at the westernmost stations to as much as $4.7 \text{ mmol NO}_2^- \text{ m}^{-2} \text{ d}^{-1}$ near the coast (Fig. 2a; supplementary Table 1). In total, we estimate a NO_2^- production of $\sim 3.8 \text{ Tg N y}^{-1}$ by aerobic NH_3 oxidation for the OMZ volume sampled during this study ($\sim 5.5 \times 10^5 \text{ km}^3$), with

24% generated in the coastal OMZ (≤ 600 m) and 76% offshore (> 600 m) (Fig. 3). Significant rates have also been reported for the surface mixed layer in the ETSP (Fernández et al. 2009), but are not accounted for in our current OMZ budget.

Nitrate is the next preferred electron acceptor after O_2 for the oxidation of organic matter and can be used by a wide variety of micro-organisms. The reduction of NO_3^- to NO_2^- is the first step in both denitrification and DNRA. In the OMZs off Peru, Namibia and in the Arabian Sea, the reduction of NO_3^- to NO_2^- produces most of the NO_2^- needed by anammox (Lam et al. 2009; Füssel et al. 2011; Lam et al. 2011). We detected NO_3^- reduction to NO_2^- throughout the OMZ at all investigated stations with rates of up to $\sim 1 \mu\text{mol N L}^{-1} \text{d}^{-1}$ on the central shelf (St. 807); while they were on the order of $\sim 10 \text{ nmol N L}^{-1} \text{d}^{-1}$ at the furthest offshore stations (Table 1).

Depth-integrated rates of NO_3^- reduction ranged from $\sim 77 \text{ mmol N m}^{-2} \text{d}^{-1}$ on the shelf to $\sim 3 \text{ mmol N m}^{-2} \text{d}^{-1}$ offshore (Fig. 2c; supplementary Table 1). Integrated for the whole region, we estimate an annual NO_3^- reduction of $\sim 49 \text{ Tg N}$, of which 29% occurs in the coastal OMZ and 71% offshore (Fig. 3). Depth-integrated rates were significantly correlated with depth-integrated NO_2^- concentrations (Spearman $R = 0.71$, $p \leq 0.001$) (Table 2). A positive correlation between NO_2^- distributions and NO_3^- reducing activity has been observed previously in the Peruvian (Lipschultz et al. 1990) and the Arabian Sea (Lam et al. 2011) OMZs, indicating a major contribution of NO_3^- reduction to the formation of secondary NO_2^- maxima.

Table 1 | Abundance of selected N-functional genes and N-conversion rates in the ETSP during cruise M77-3.

Functional genes and N-conversion rates were not always determined at the same station and/or depths but with a comparable latitudinal and longitudinal as well as vertical resolution.

		N-functional gene abundances (10^2 copies mL ⁻¹)							N-conversion rates (nmol N L ⁻¹ d ⁻¹)					
		arch- <i>amoA</i>	β - <i>amoA</i>	γ - <i>amoA</i>	<i>hzo1</i>	<i>hzo2</i>	den- <i>nirS</i> *	<i>nrfA</i>	NH ₃ ox.	Anamox	Denitrification	DNRA	NO ₂ ⁻ ox.	NO ₃ ⁻ red.
Coastal OMZ (≤600 m)	N:	63 (64)	8 (49)	0 (64)	8 (64)	42 (64)	0 (63)	0 (64)	27 (33)	33 (33)	3 (33)	7 (33)	27 (32)	27 (32)
	Range:	0.16-2773	0.05-1056	-	0.05-0.09	0.14-12.8	-	-	0.22-48.8	2.84-227	2.21-5.42	0.48-1.74	8.48-928	3.79-1010
	Mean:	676	135	-	0.07	4.45	-	-	8.24	43.4	4.19	1.14	172	203
	Median:	90	5.0	-	0.06	3.77	-	-	3.40	21.2	4.94	1.10	65.4	101
Offshore OMZ (>600 m)	N:	67 (71)	2 (33)	0 (72)	4 (71)	43 (72)	2 (72)	1 (72)	17 (40)	33 (40)	0 (40)	3 (40)	27 (40)	25 (34)
	Range:	0.04-2332	0.15-1.36	-	0.01-0.09	0.06-14.7	0.06-1.98	0.11	0.51-88.8	0.71-46.9	-	0.33-1.31	4.58-186	4.53-77.4
	Mean:	352	0.75	-	0.08	3.15	1.02	0.11	20.9	6.14	-	0.82	40.6	32.1
	Median:	89.5	0.75	-	0.08	1.51	1.02	0.11	5.79	3.01	-	0.83	30.2	22.3

N = number of samples in which N-functional genes/N-processes were detected; in parenthesis: number of samples analyzed.

*denitrifier-*nirS*.

Sinks of NO₂⁻ – Nitrite oxidation, the second step in nitrification, was found most active in the upper OMZ throughout the ETSP. However, compared to NH₃ oxidation, its activity remained detectable deeper into the OMZ core, consistent with earlier reports (Lipschultz et al. 1990; Ward et al. 1989). While NO₂⁻ oxidation rates were at most ~20 nmol N L⁻¹ d⁻¹ or sometimes not detectable along the section furthest offshore (85.83° W), the highest rates of up to ~900 nmol N L⁻¹ d⁻¹ (Table 1) were measured over the Peruvian shelf (St. 38) under low O₂ conditions.

Although NO₂⁻ oxidation is believed to require O₂, this process has been detected where O₂ levels fell below 1-2 μmol L⁻¹ in the Peruvian (Lipschultz et al. 1990; Ward et al. 1989) and Namibian OMZs (Füssel et al. 2011). In our study, O₂ sensitivity assays (~1-25 μmol L⁻¹) at two stations revealed only a moderate effect of reduced O₂ concentrations on NO₂⁻ oxidation (~50% activity at 1 μmol L⁻¹) (supplementary Fig. 3), which is consistent with recent observations in Namibian shelf waters (Füssel et al. 2011). Clearly, NO₂⁻ oxidizers are well adapted to O₂-deficient environments, though their possible activity at absolute anoxia remains to be assessed.

Rates of NO₂⁻ oxidation are generally thought to be limited by NO₂⁻ formation via NH₃ oxidation. Thus, nitrification has often been regarded as a single process that oxidizes NH₃ to NO₃⁻ (e.g. Fernández et al. 2009). Indeed, depth-integrated rates of NH₃ and NO₂⁻ oxidation were significantly correlated (Spearman R = 0.73, p ≤ 0.001) (Table 2). However, NO₂⁻ oxidation rates exceeded those of NH₃ oxidation, often by more than tenfold (Fig. 2a,b; supplementary Table 1). Similar observations have previously been made in the OMZs off Namibia (Füssel et al. 2011) and Peru (Ward et al. 1989; Lipschultz et al. 1990), suggesting a decoupling of the two steps of nitrification in O₂-deficient systems. This is most likely attributed to an alternative source of NO₂⁻ provided by NO₃⁻ reduction, particularly deeper in the OMZ. Overall, NH₃ oxidation accounted for only ~7% of the total NO₂⁻ production, while the majority came from NO₃⁻ reduction. Based on modeled N-fluxes in the ETSP, Anderson et

al. (1982) proposed a NO_2^- “shunt” in which 45-74% of the NO_3^- reduced to NO_2^- by “denitrifying” micro-organisms is re-oxidized by aerobic NO_2^- oxidizers. In agreement, our annual rates of NO_2^- oxidation to NO_3^- for the coastal (7 Tg N y^{-1}) and offshore OMZ (23 Tg N y^{-1}) are equivalent to 51% and 65%, respectively, of NO_3^- reduction to NO_2^- (Fig. 3). The tight correlation of depth-integrated rates measured for the two processes (Spearman $R = 0.75$, $p \leq 0.001$) (Table 2) strongly indicates a close coupling between NO_2^- oxidation and NO_3^- reduction in the ETSP OMZ.

Meanwhile, only sporadic and low rates of DNRA ($\leq 1.3 \text{ nmol L}^{-1} \text{ d}^{-1}$) were detected during the period of this study (Table 1). This is consistent with a general lack of detectable *nrfA*, a key functional gene encoding for the cytochrome *c* NO_2^- reductase, in this study. DNRA appears to exhibit a high degree of spatio-temporal variability. For instance, similarly low rates were measured on the Namibian shelf (Füssel et al. 2011), whereas tenfold greater rates of DNRA and *nrfA* gene abundance were found earlier in the Peruvian OMZ (Lam et al. 2009) as well as on the Omani shelf (Jensen et al. 2011). Hence, we cannot exclude DNRA as a significant sink for NO_x^- and source of NH_4^+ in the ETSP at other times.

N-losses – Anammox and denitrification convert dissolved inorganic N to N_2 , which is not bio-available to most micro-organisms. A minor portion ($< 1.5\%$) of oceanic N-loss can also occur via the formation of the potent greenhouse gas nitrous oxide (N_2O) (Gruber 2004), but is not further considered here.

At the time of our sampling, denitrification, as the production of $^{30}\text{N}_2$ from $^{15}\text{NO}_x$, was generally non-detectable. Low rates of denitrification ($\sim 2\text{-}5 \text{ nmol L}^{-1} \text{ d}^{-1}$) were measured in three samples from the Peruvian shelf OMZ (Table 1). Substantially higher rates were only detected in samples containing measurable amounts of H_2S (Fig. 2e; supplementary Table 1). Denitrification likely coupled to the oxidation of H_2S (Lavik et al. 2008; Schunck et al.

submitted.) was, however, of minor importance ($\ll 1\%$ total N-loss) for the overall N-budget in the ETSP OMZ, according to our estimates (supplementary information).

Corroborative of previous studies in the ETSP (Hamersley et al. 2007; Thamdrup et al. 2006; Ward et al. 2009), N_2 production attributed to anammox was detected at all stations except the two furthest offshore. Anammox activity was often enhanced in the upper OMZ and markedly elevated in the bottom waters over the shelf and upper continental slope. The highest rates, up to $\sim 225 \text{ nmol N L}^{-1} \text{ d}^{-1}$ (St. 807), were measured on the central shelf (10°S - 16°S). Further north and south, anammox rates decreased and were lowest ($\sim 5 \text{ nmol N L}^{-1} \text{ d}^{-1}$) at the westernmost stations (Table 1).

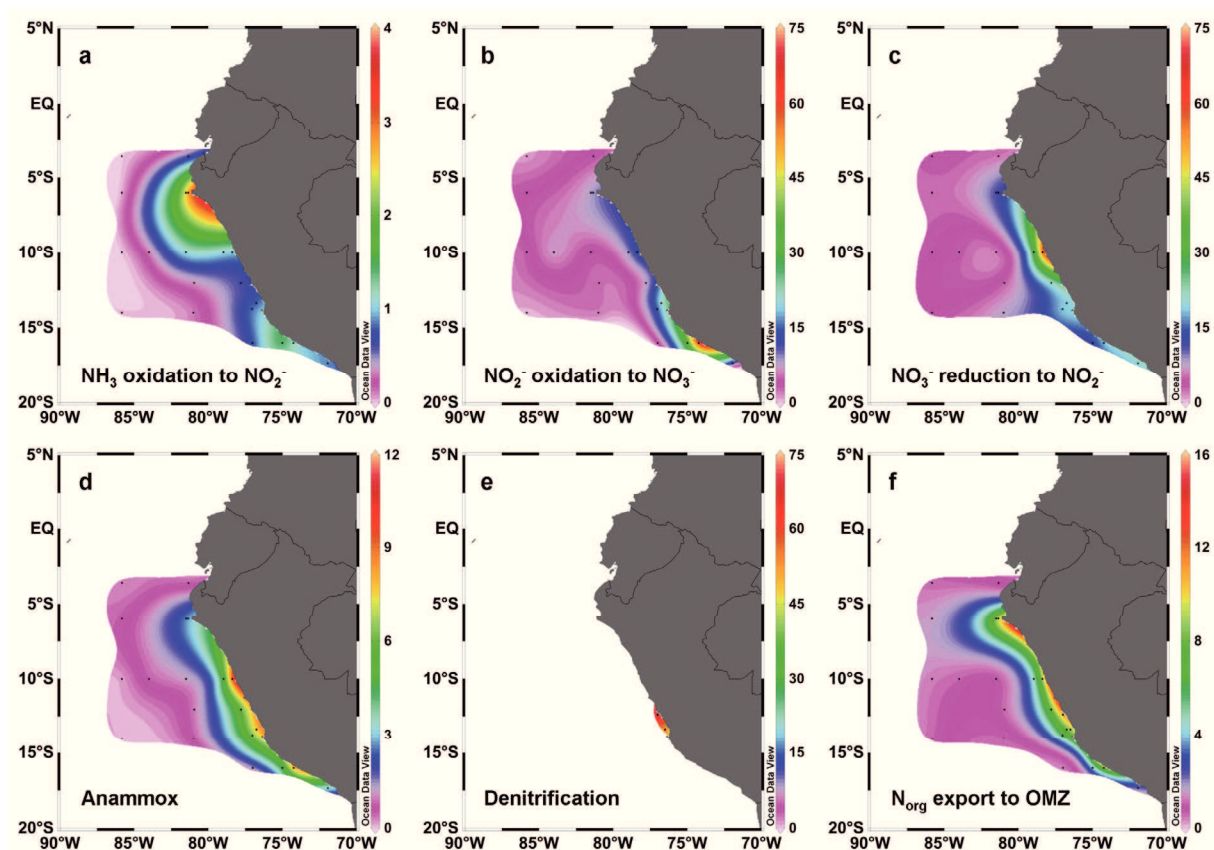


Figure 2 | Depth-integrated N-cycling rates in the ETSP OMZ.

(a,b) The two steps of the aerobic nitrification, NH_3 oxidation and NO_2^- oxidation. (c) NO_3^- reduction to NO_2^- . (d,e) N-loss due to anammox as well as denitrification coupled to the oxidation of H_2S during a sulfidic event on the Peruvian shelf. (f) Modelled export of organic N from the euphotic zone to the OMZ. All rates in $\text{mmol N m}^{-2} \text{ d}^{-1}$.

In accordance with the ^{15}N incubations, genes encoding for the hydrazine (N_2H_4) oxidoreductase (*hzol* and 2) were detected throughout the OMZ, whereas denitrifier-*nirS*, encoding for the cytochrome *cd1*-containing NO_2^- reductase, was generally not detected (Table 2). Our results are in good agreement with an earlier study, in which anammox (*scalindua*)-specific *nirS* were found to be more abundant than denitrifier-*nirS*, along with high anammox activity but non-detectable denitrification in the Peruvian OMZ (Lam et al. 2009).

Depth-integrated anammox rates were $>10 \text{ mmol N m}^{-2} \text{ d}^{-1}$ on the central shelf, similar to those reported by Hamersley et al. (2007), and $<1 \text{ mmol N m}^{-2} \text{ d}^{-1}$ at the furthest offshore stations (Fig. 2d; supplementary Table 1). Altogether, anammox accounts for an annual N-loss of $\sim 10 \text{ Tg}$ in an area of $1.2 \times 10^6 \text{ km}^2$, which is at the lower end of earlier estimates for the ETSP ($9\text{-}26 \text{ Tg N y}^{-1}$) (Codispoti and Packard 1980; Lipschultz et al. 1990; Deutsch et al. 2001; Hamersley et al. 2007).

Sediments underlying the OMZ are additional sites of N-loss, which are evidenced by flux measurements of DIN and N_2 made just prior to our sampling (Bohlen et al. 2011). Combined with reaction-diffusion modeling, these results revealed that anammox and denitrification were N-sinks in the Peruvian coastal sediments. Based on the reported sedimentary NO_x^- fluxes and NO_x^- partitioning between anammox, denitrification, and DNRA, we estimate a loss of 1 Tg N y^{-1} from sediments in contact with the OMZ bottom waters (Fig. 3).

Conventionally, the accumulation of NO_2^- in OMZ waters has been interpreted as signs of active N-loss (Cline and Richards 1972; Codispoti and Packard 1980; Naqvi 1987). For the ETSP, Codispoti and Packard (1980) suggested that most of the water-column N-loss ($\sim 83\%$) occurs within the offshore secondary NO_2^- maxima below 10°S . Minor N-loss ($\sim 17\%$) was attributed to the shallower coastal area and the northern Peruvian OMZ, where NO_2^-

accumulates less (Fig. 1d; supplementary Fig. 1). In contrast, our data do not support the idea of increased NO_2^- levels as an indicator for higher N-loss rates. Unlike NO_2^- , depth integrated anammox rates did not reveal any north-south trend, but decreased from shelf to offshore. Admittedly, depth-integrated anammox rates and NO_2^- showed a significant correlation (Spearman $R = 0.64$, $p < 0.001$) (Table 2), yet for the volumetric rates/concentrations this was only true on the shelf (Spearman $R = 0.72$, $p < 0.001$) and not offshore (Spearman $p > 0.5$). We interpret the offshore NO_2^- accumulations as a greater persistence of NO_3^- reduction to NO_2^- compared to other NO_2^- consuming processes in this area, where the net NO_2^- flux was about five times higher compared to the coastal OMZ (11.4 and 2.2 Tg N y^{-1} , respectively).

Additionally, ongoing water column N-loss cannot be deduced simply from the intensity of N^* minima, as shown by the lack of significant correlation (Spearman $p > 0.05$) between anammox activity and measured N^* (Table 2). While the N-deficit is largest (most negative N^*) offshore, anammox activity is highest over the shelf and upper continental slope. Though comprising only 10% of the area covered and merely 4% of the sampled OMZ volume, coastal OMZ waters contribute as much as 30% of the total N-loss detected (Fig. 3). Meanwhile, N-deficits in these waters amount to only 5% (4 Tg N) of the total N-deficit (71 Tg N). Hence, the large N-deficit offshore most likely results from horizontal advection of N-deficient shelf waters (Lam et al. 2009) that accumulate due to a long mean residence time in the offshore OMZ (~10 y based on N^* and measured N-loss).

Analogous observations were recently made in the Arabian Sea, where offshore NO_2^- accumulations presumably reflect long-term integrated N-loss within the OMZ (Lam et al. 2011), as indicated by low to non-detectable N_2 production rates in the central basin in contrast to rapid N-loss over the productive Omani shelf (Jensen et al. 2011). Further assessment on the water exchange and thus shelf-offshore DIN fluxes would be necessary to more accurately determine the relative importance of shelf versus offshore N-loss.

Sources of NH_4^+ – N-loss driven by anammox requires NH_4^+ , which usually does not accumulate in OMZs, nonetheless. Ammonium concentrations can be kept low by a tight coupling between NH_4^+ production and consumption processes, while the NH_4^+ released at the reported remineralization rates may already be sufficient to fuel anammox. Major sources of NH_4^+ are water-column organic matter remineralization and sedimentary NH_4^+ release.

DNRA and organic matter ammonification are active benthic NH_4^+ sources off the coast of Peru (Bohlen et al. 2011). During two preceding cruises (M77-1 and 2) to the ETSP, large NH_4^+ fluxes ($\sim 0.5\text{-}4 \text{ mmol m}^{-2} \text{ d}^{-1}$) from the sediments into the overlying OMZ waters were measured on a cross-shelf transect at 11°S . The often enhanced anammox activity in the coastal OMZ bottom waters suggests a strong influence from NH_4^+ diffusing out of the sediments (Dalsgaard et al. 2003; Hamersley et al. 2007). Assuming an average benthic NH_4^+ flux of $\sim 2 \text{ mmol m}^{-2} \text{ d}^{-1}$ and a typical anammox rate of $\sim 4 \text{ mmol NH}_4^+ \text{ m}^{-2} \text{ d}^{-1}$ for the Peruvian coastal waters, the underlying sediments could supply $\sim 50\%$ of the NH_4^+ needed for the anammox rates observed therein.

While sediments in contact with the OMZ are an important source of NH_4^+ , they cannot fully satisfy the NH_4^+ requirements of anammox in the coastal area. Moreover, the offshore OMZ waters are spatially decoupled from the sediments. Although there could be some lateral NH_4^+ advection off the shelf (supplementary Fig 1), anammox has to rely mostly on alternative NH_4^+ sources offshore.

Based on our results, ammonification of Redfield organic matter during NO_3^- reduction to NO_2^- generates 65% and 73% of the NH_4^+ needed for anammox in the coastal and offshore OMZ, respectively (Fig. 3). This might be an underestimate, considering the observed preferential N-degradation of organic matter via NO_3^- respiration under suboxic conditions (Van Mooy et al. 2002). Whether the reduction of NO_3^- is directly coupled to the oxidation of organic matter, or indirectly via a recently proposed cryptic sulfur cycle (Canfield et al. 2010), could not be discerned at this point.

Table 2 | Spearman rank correlation of depth-integrated nutrients and N-cycling rates as well as modelled export production.

	NH₃ oxidation	NO₂⁻ oxidation	NO₃⁻ reduction	Anammox	Export Production
NH₄⁺	0.51*	0.31	0.08	0.30	0.29
NO₂⁻	0.46*	0.49*	0.71***	0.64**	0.10
N*	-0.08	-0.20	-0.05	-0.02	-0.02
Export Production	0.56*	0.60**	0.75***	0.79***	
Anammox	0.75***	0.86***	0.88***		
NO₃⁻ reduction	0.49*	0.75***			
NO₂⁻ oxidation	0.73***				

Presented values are correlation coefficients with significant values denoted by * ($p \leq 0.05$), ** ($p \leq 0.01$) and *** ($p \leq 0.001$).

Near the upper OMZ boundary, remineralization of sinking organic matter and subsequent NH₄⁺ release is enhanced, consistent with the high anammox and NH₃ oxidation activity observed in this zone (Lipschultz et al. 1990; Thamdrup 2006; Hamersley et al. 2007; Lam et al. 2009; Jensen et al. 2011; Lam et al. 2011). Here, on average ~40% of their combined NH₄⁺ demands are supplied by NO₃⁻ reduction, with the remainder possibly coming from microaerobic organic matter remineralization²⁰. Though chemolithotrophic, the activity of O₂-dependent nitrification at non-detectable O₂ concentrations indicates microaerobic respiration to proceed even at nanomolar O₂ levels in OMZs. Microaerobic respiration in the upper Peruvian OMZ with an apparent half-saturation coefficient of <20 nmol L⁻¹ has been reported (Revsbech et al. 2009). In accordance, high O₂ consumption rates, mainly attributable to heterotrophic respiration, and genes encoding for terminal respiratory oxidases with high O₂-affinities were detected in the ETSP during our cruises (Chapter 4). While it has been suggested that O₂ is efficiently depleted to the limits of aerobic respiration in the OMZ core (Thamdrup et al. 2012), regular intrusions of more oxygenated surface waters or mixing events, such as related to eddy activity (Bertrand et al. 2010), may sustain aerobic microbial activity in the upper OMZ.

Linking surface productivity and subsurface N-cycling – Depth-integrated anammox rates correlated strikingly well with NO_3^- reduction, NO_2^- oxidation and NH_3 oxidation (Spearman $R = 0.88, 0.86$ and 0.75 , respectively; $p \leq 0.001$), indicating a common controlling factor for their concerted activity.

We estimated the export of organic matter at the base of the euphotic zone from net primary production (NPP) (Behrenfeld and Falkowski 1997) and the ratio of export- to total primary production (*ef*-ratio) (Laws et al. 2000). At the time of our sampling, NPP was up to $\sim 3 \text{ g organic C (C}_{\text{org}}) \text{ m}^{-2} \text{ d}^{-1}$ near the coast and decreased to $< 0.5 \text{ g C}_{\text{org}} \text{ m}^{-2} \text{ d}^{-1}$ further offshore, values typical for the Peruvian upwelling system (Pennington et al. 2006). Computed *ef*-ratios ranged from 0.16 (low-NPP sites) to 0.42 (high-NPP sites). The resulting N-export production rates (converted from measured C:N = 7.2 of surface particulate organic matter) were $> 10 \text{ mmol organic N (N}_{\text{org}}) \text{ m}^{-2} \text{ d}^{-1}$ over the shelf and on the order of $\sim 1 \text{ mmol N}_{\text{org}} \text{ m}^{-2} \text{ d}^{-1}$ at the stations furthest offshore (Fig. 2f; supplementary Table 1). Export production was highly correlated to depth-integrated rates of anammox, NO_3^- reduction and NO_2^- oxidation (Spearman $R = 0.79, 0.75$ and 0.60 , respectively, $p \leq 0.001$) as well as NH_3 oxidation (Spearman $R = 0.56$, $p \leq 0.01$) (Table 2). This suggests that the lateral distribution N-cycling activity, including anammox, is mainly determined by the export of organic matter, which is the ultimate source of the required reactive substrates NH_4^+ and NO_2^- , into the OMZ.

Overall, we estimate NPP of 12 and 47 Tg N y^{-1} in the coastal and offshore surface waters, respectively, which appear reasonable at a net lateral supply of 88 $\text{Tg NO}_3^- \text{ y}^{-1}$ to the upwelling region (Fig. 3). The corresponding export fluxes are 4.4 and 9.9 $\text{Tg N}_{\text{org}} \text{ y}^{-1}$, respectively. Taking into account organic matter sedimentation and export to the deep ocean, these export productions as N-sources in the OMZ are sufficient to support the measured N-fluxes reported here.

The tight coupling of N-cycling activity and N_{org} availability in the ETSP OMZ has consequences for N-loss estimates derived from microbial activities: primary production in

the Peruvian upwelling system is characterized by strong seasonality (Pennington 2006), thus the period of sampling is likely critical when extrapolating data acquired over a relatively short time span. Moreover, intra-annual variability, such as that related to El Niño Southern Oscillation, significantly affects surface productivity and thereby N-loss. In fact, Codispoti & Packard (1980) interpreted their upper estimate of 25 Tg N y⁻¹ as a result of unusually high export production during the mid-seventies “regime shift” in the Pacific (Chavez et al. 2003) and suggested a more typical “denitrification” rate of 10 Tg N y⁻¹ for the ETSP.

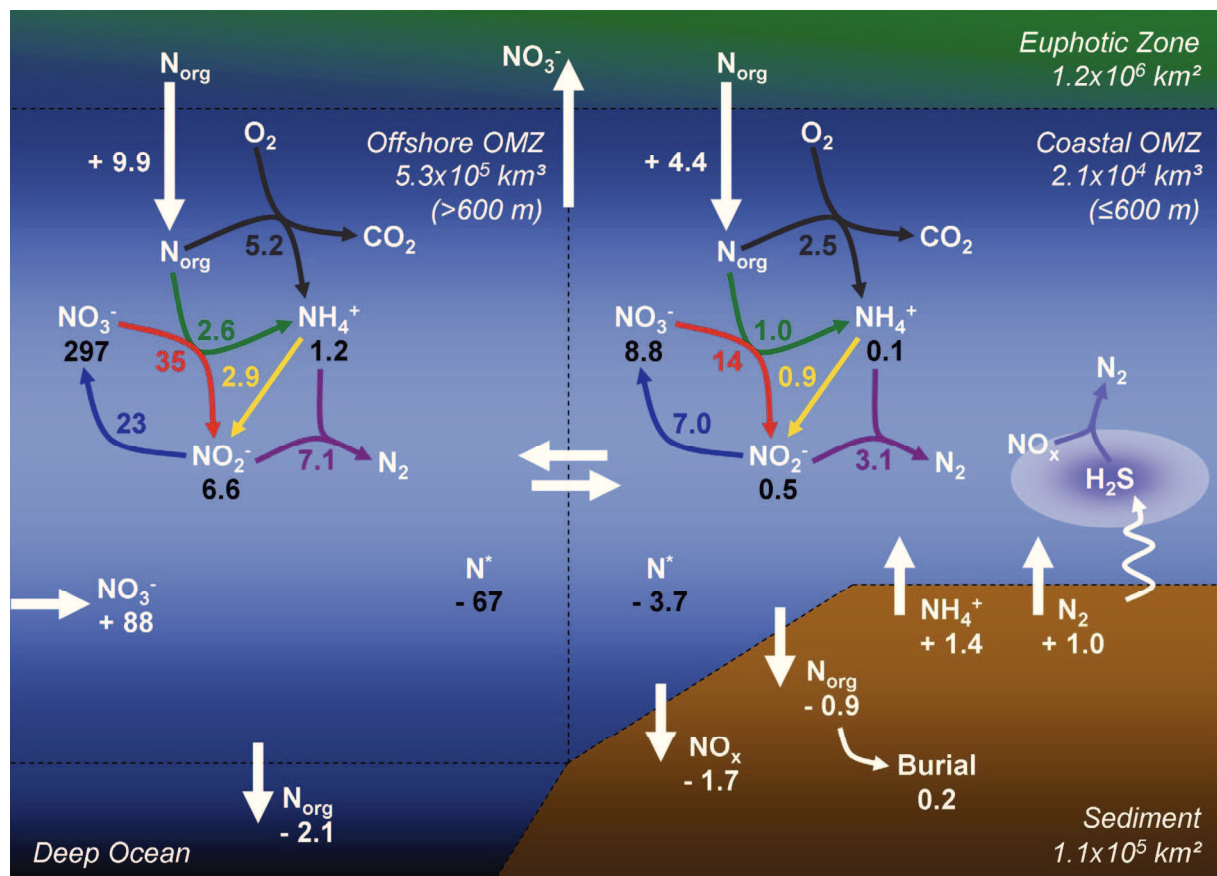


Figure 3 | N-fluxes and nutrient inventory of the ETSP OMZ.

Black numbers indicate DIN inventories (in Tg N). Fluxes (in Tg N y⁻¹) within the OMZ or across its boundaries are given in colour and white, respectively. A detailed description of the flux calculations is included in the supplementary information.

In summary, extensive sampling and experimentation throughout the ETSP OMZ shows that, contrary to the long-held assumption, high N-loss is spatially decoupled from the offshore secondary NO_2^- maxima and associated N-deficits. Instead, we provide direct evidence that the activity of anammox and N-linked processes is highly correlated with export production. High productivity over the shelf and upper slope, as well as sedimentary NH_4^+ release, drive high rates of tightly coupled N-cycling processes and thus N-loss via anammox in the shallow coastal OMZ compared to the offshore OMZ. This also highlights the important connection between pelagic and benthic processes in the OMZ, as well as the possible export of N-deficit signals from shelf to offshore.

While the globally expanding OMZs might increase the oceanic volume conducive to N-loss, N-loss would only continue to rise as long as there is sufficient nutrient supply for primary production in the euphotic zone, and nutrient supply is not hampered by intensified stratification (i.e. reduced upwelling) due to ocean warming. These positive and negative feedbacks are important considerations for biogeochemical models, which at present do not adequately reproduce the observed spatial patterns of N-loss in OMZs. In light of our results, the activities of N-loss via anammox may be directly linked to export production rates in biogeochemical models using the following empirical relationship: $\text{anammox} = 0.705 \times N_{\text{org}} \text{ export}$ (supplementary Fig. 4). This may facilitate a realistic assessment of the short- and long-term impacts of ocean de-oxygenation and changing productivity on N-cycling in OMZs, as well as their effects on neighboring water masses.

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Supplementary Information

Data interpolation – Depth-integrated nutrient concentrations and N-cycling rates (calculated from mid-point averages) and modelled export production rates were interpolated using Ocean Data View (Schlitzer 2011). Bathymetry data were obtained from the National Geophysical Data Center (<http://www.ngdc.noaa.gov/mgg/bathymetry/relief.html>).

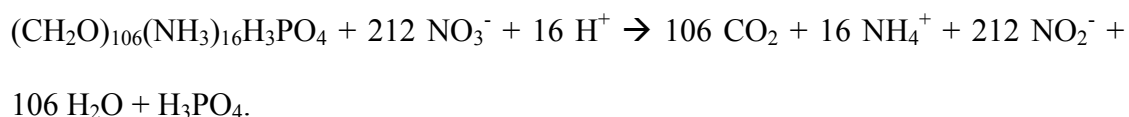
Fluxes of N_{org} – The export of N_{org} out of the OMZ to the deep ocean (depth >600 m) was calculated from modelled export production using a “Martin-curve” (Martin et al. 1987):

$$F = F_{100} \times (z/100)^b.$$

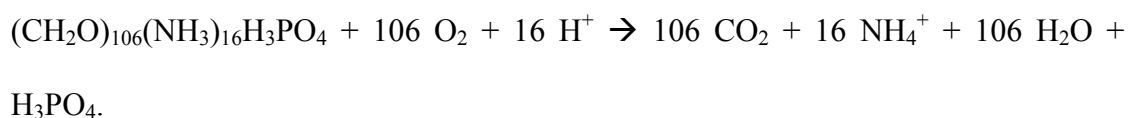
Here F is the flux of organic matter at the lower OMZ boundary (z) and F_{100} is the flux at 100 m, which was assumed to equal the modelled export production rates. We adopted an attenuation coefficient (b) of 0.82 (Berelson 2001).

Benthic N_{org} -flux and -burial rates (depth ≤ 600 m) were derived from modelled NPP and estimates of organic matter sedimentation for the Peruvian upwelling system (Suess and Killingley 1987). The benthic N_{org} -flux (N_{org} -burial) was calculated as 22% (6%) and 11% (2%) of NPP for ≤ 300 m and 301-600 m water depth, respectively.

Remineralization of N_{org} – Ammonification of N_{org} in the OMZ via NO_3^- reduction to NO_2^- was calculated using the following stoichiometry:



Any N_{org} remineralization that could not be attributed to NO_3^- reduction was assumed to occur via aerobic respiration:



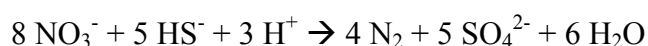
For our sampled area ($\sim 1.2 \times 10^6$ km²) we estimate aerobic remineralization of 5.2 Tg N_{org} y⁻¹. If we conservatively assume aerobic organic matter degradation to be restricted to the upper 10 m of the OMZ, heterotrophic oxic respiration of on average 0.58 $\mu\text{mol L}^{-1} \text{d}^{-1}$ would be sufficient to remineralize those 5.2 Tg N_{org} y⁻¹. In accordance, Revsbech et al. (2009) recently measured oxic microbial respiration of $\sim 2 \mu\text{mol L}^{-1} \text{d}^{-1}$ (which can be largely attributed to heterotrophic activity) in the upper Peruvian OMZ. Oxygen consumption rates on the same order of magnitude were determined during M77-3 (Chapter 4). We note that the amount of N_{org} available for aerobic respiration in our budget is sensitive to the choice of the attenuation coefficient. Others have suggested a smaller b-value of ~ 0.36 for O₂-deficient systems (Van Mooy et al. 2002; Hartnett and Devol 2003), which would result in an increased export of organic matter out of the OMZ and reduce our estimate of oxic N_{org} remineralization in the OMZ by $\sim 50\%$.

Benthic N-flux estimates – Sedimentary NH₄⁺, NO_x and N₂ fluxes for the shelf and upper continental slope sediments (≤ 600 m) were calculated from benthic NO_x fluxes measured off the coast of Peru and estimates of NO_x partitioning between sedimentary denitrification, anammox and DNRA (Bohlen et al. 2011). Using reaction-diffusion modelling, Bohlen et al. (2011) estimated roughly the following relative contributions to benthic NO_x consumption in the shallow shelf (≤ 300 m) and upper slope (301-600 m) sediments, respectively, at 11°S: DNRA: 70% and 30%; denitrification: 30% and 65%; anammox: 0% and 5%. The sediments from ~ 10 -16°S are particularly C_{org}-rich (Reimers and Suess 1983), which favour SO₄²⁻ reduction and thus DNRA by filamentous large sulphur bacteria (Bohlen et al. 2011). Hence, DNRA is likely of less importance in the sediments to the north and south of there. For the wider region, we assumed an average contribution of 50% (≤ 300 m) and 20% (301-600 m) of DNRA to benthic NO_x consumption and increased benthic denitrification accordingly. We adopted a flux of 3 mmol NO_x m⁻² d⁻¹ (≤ 300 m) and 2 mmol NO_x m⁻² d⁻¹ (301-600 m) into the

sediment to calculate the net release of NH_4^+ (via DNRA and N_{org} ammonification) and N_2 -production due to anammox and denitrification. We recognize that our estimates bear large uncertainties and further investigation on the north-south variability of benthic NO_x uptake and the partitioning between anammox, denitrification and DNRA is required to better constrain sedimentary N-fluxes off the coast of Peru. Nevertheless, our calculated sedimentary NO_x flux of 1.7 Tg y^{-1} compares well with an earlier estimate (2 Tg N y^{-1}) for a somewhat larger coastal area ($0\text{-}25^\circ\text{S}$) (Codispoti and Packard 1980).

Lateral NO_3^- supply – The lateral net transport of NO_3^- into the OMZ volume sampled was calculated from measured NO_3^- concentrations and shipboard ADCP (Acoustic Doppler Current Profiler) data obtained for the 3.58°S , 14°S and 85.83°W sections.

Water-column denitrification – The sulfidic event on the Peruvian shelf, covered an area of $\sim 10,000 \text{ km}^2$ at the time of our sampling. Chemolithotrophic denitrifiers achieve maximum energy yield when oxidizing H_2S all the way to sulphate (SO_4^{2-}) with NO_3^- :



Assuming a 100 m-thick H_2S -containing layer above the shelf and a H_2S concentration of on average $1 \mu\text{mol L}^{-1}$, results in a loss of $\sim 0.02 \text{ Tg N}$ due to chemolithotrophic denitrification. As part of the H_2S is only partially oxidized (e.g. to S^0) or oxidized with NO_2^- , O_2 and via DNRA (Schunck et al. in prep.), this should be considered an upper estimate. Even if we did not sample the prime of this sulfidic event and considering that accumulations of H_2S remain often undetected, the associated annual N-losses are likely only a fraction of those due to anammox activity in the ETSP.

Following pages:

Figure S1a-i | Vertical distributions of O_2 , nutrients and N-deficits.

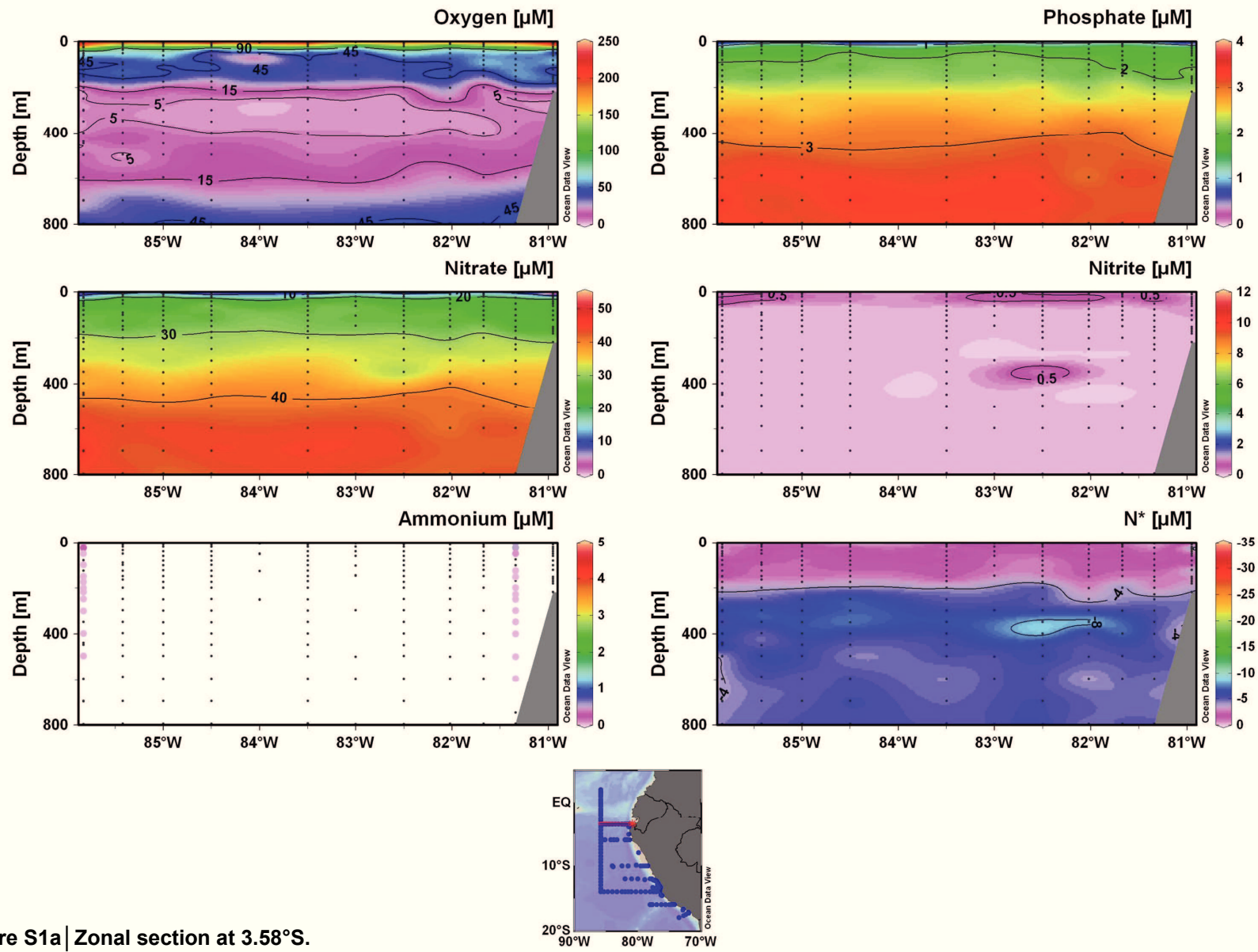


Figure S1a | Zonal section at 3.58°S.

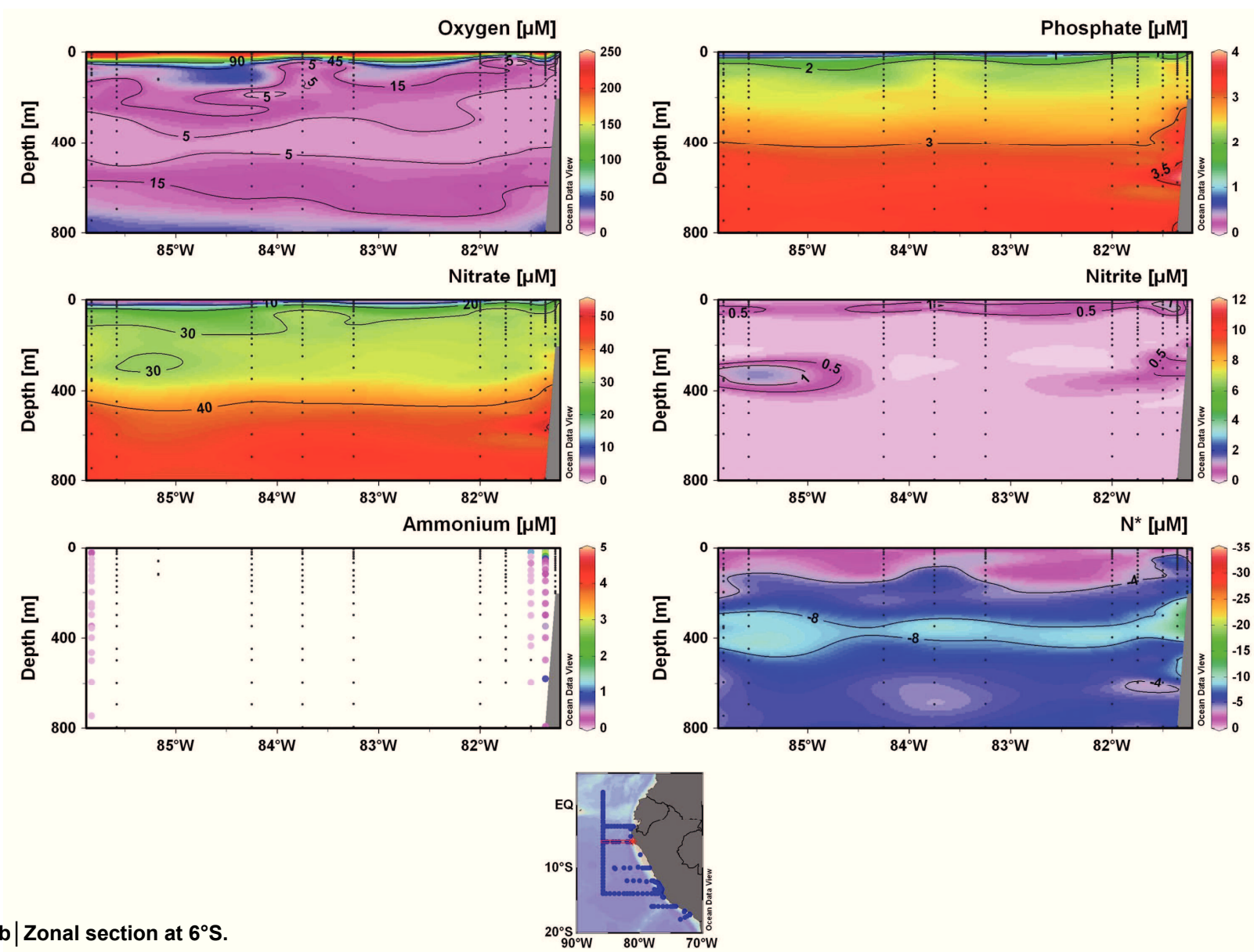


Figure S1b | Zonal section at 6°S.

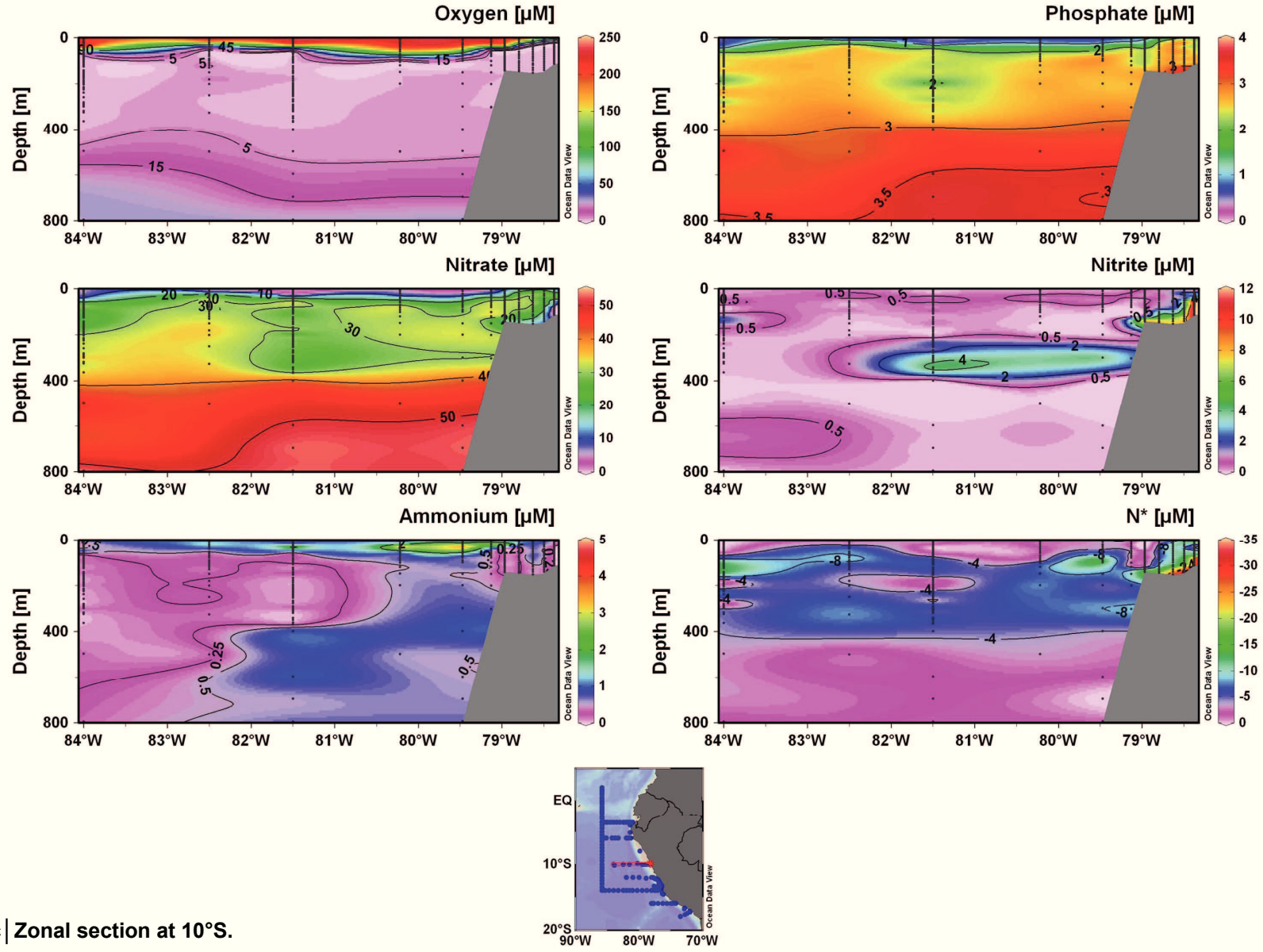


Figure S1c | Zonal section at 10°S.

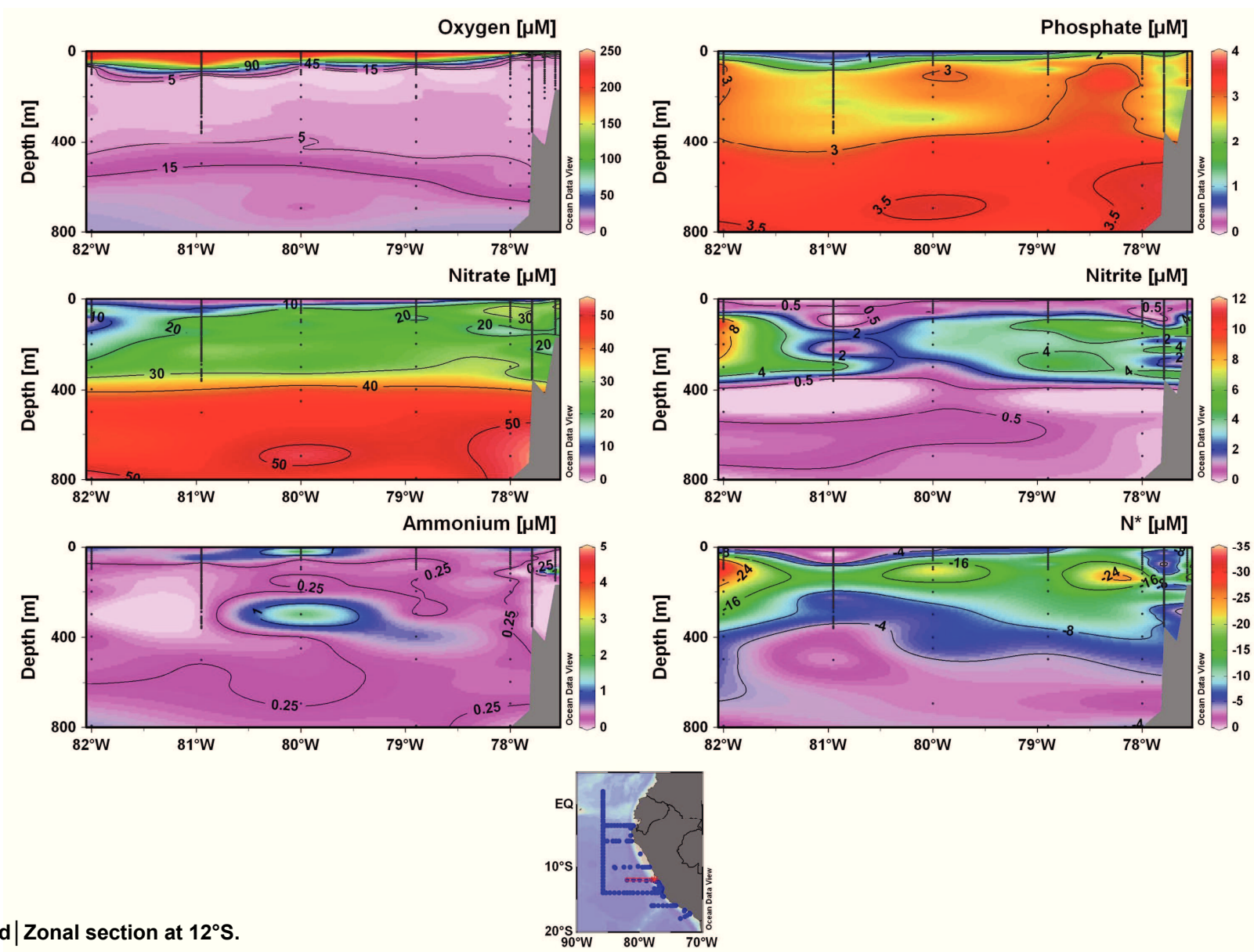


Figure S1d | Zonal section at 12°S.

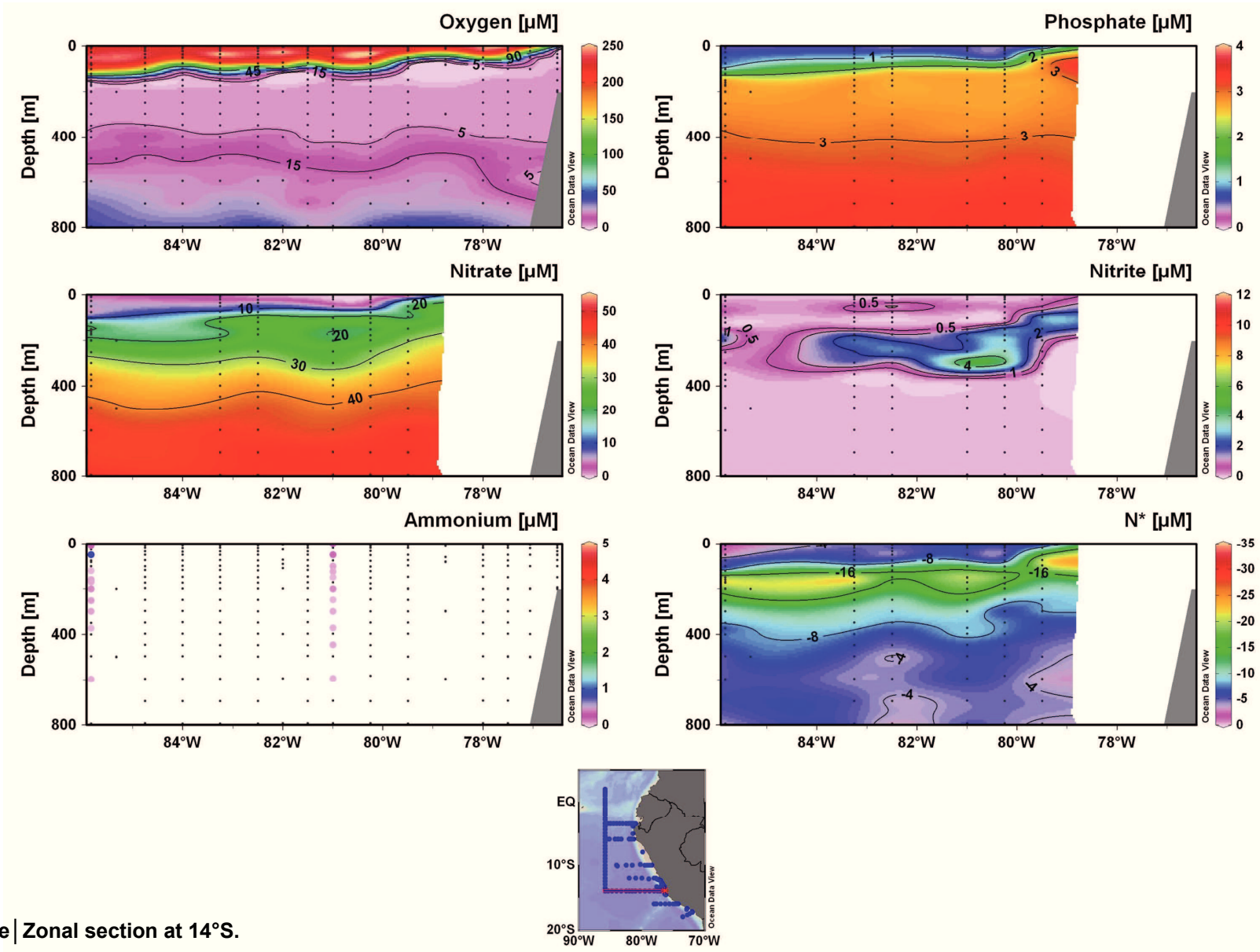


Figure S1e | Zonal section at 14°S.

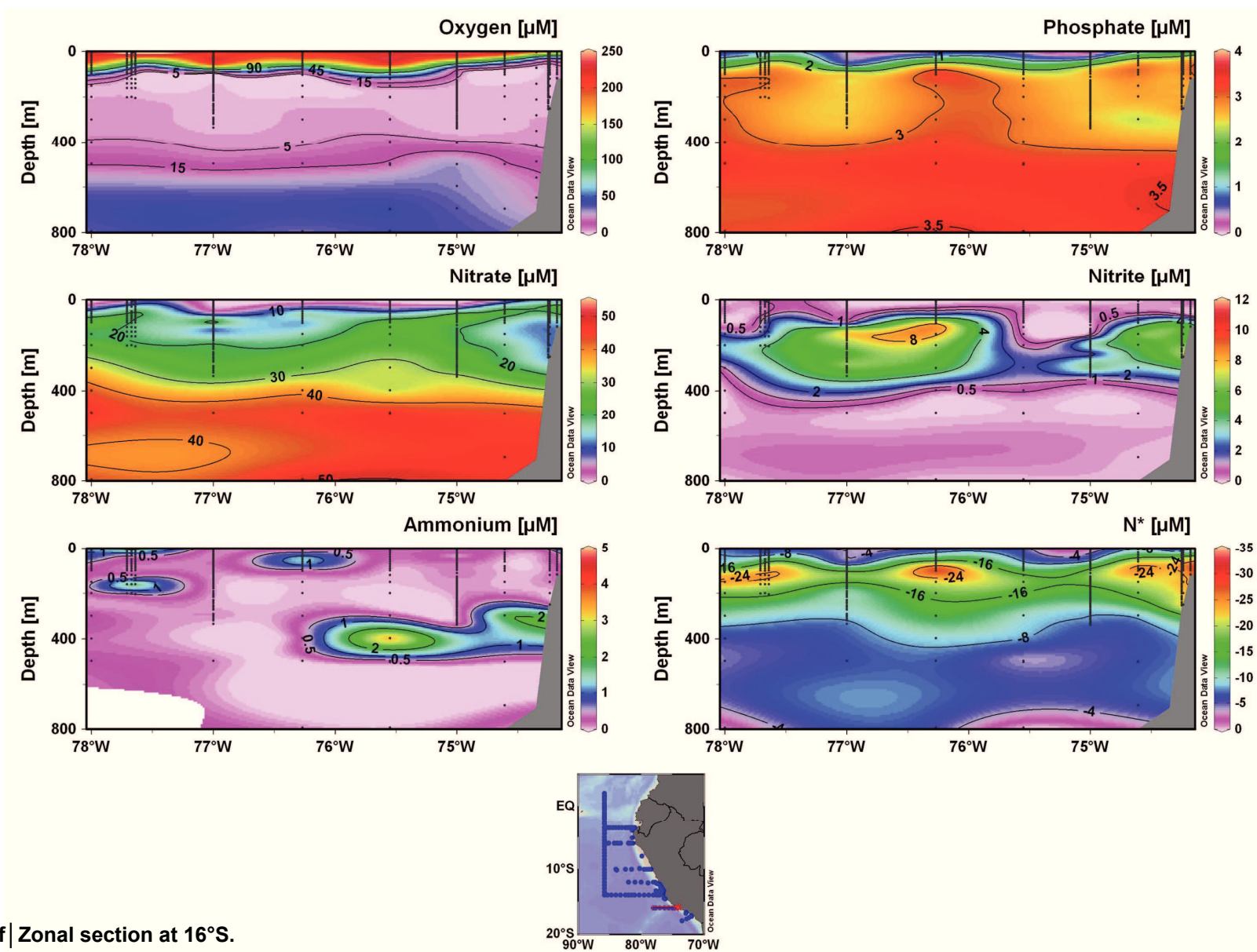


Figure S1f | Zonal section at 16°S.

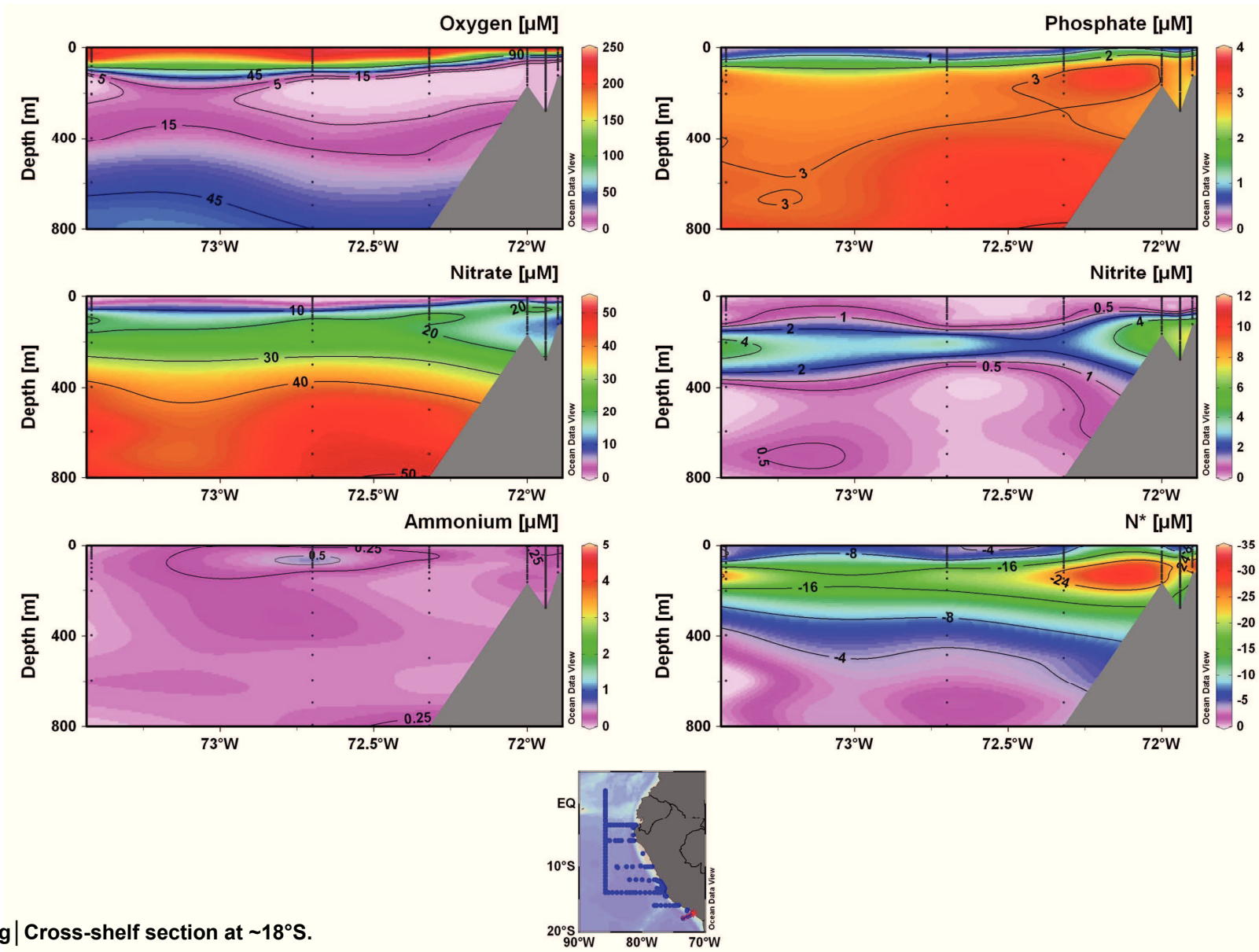


Figure S1g | Cross-shelf section at ~18°S.

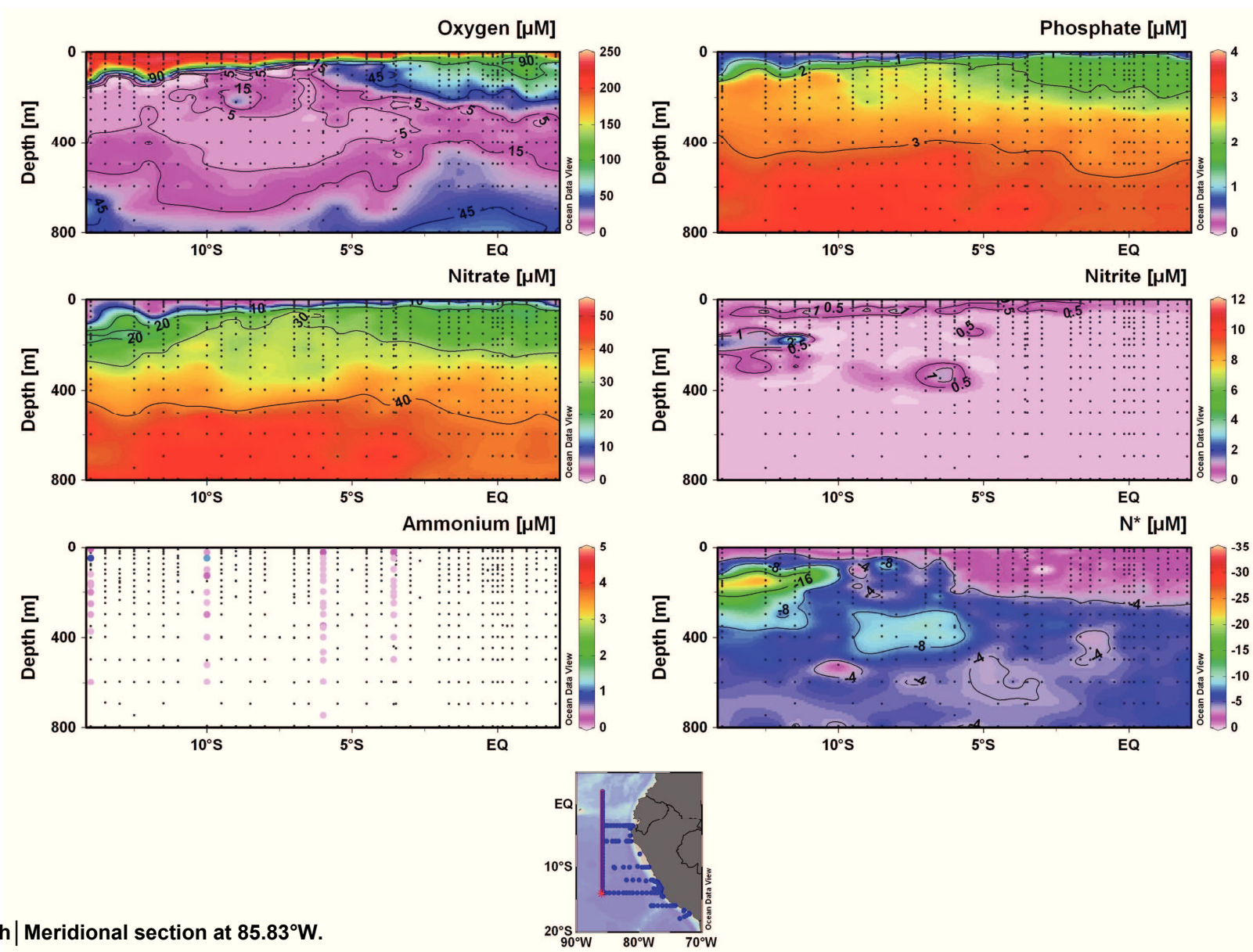


Figure S1h | Meridional section at 85.83°W.

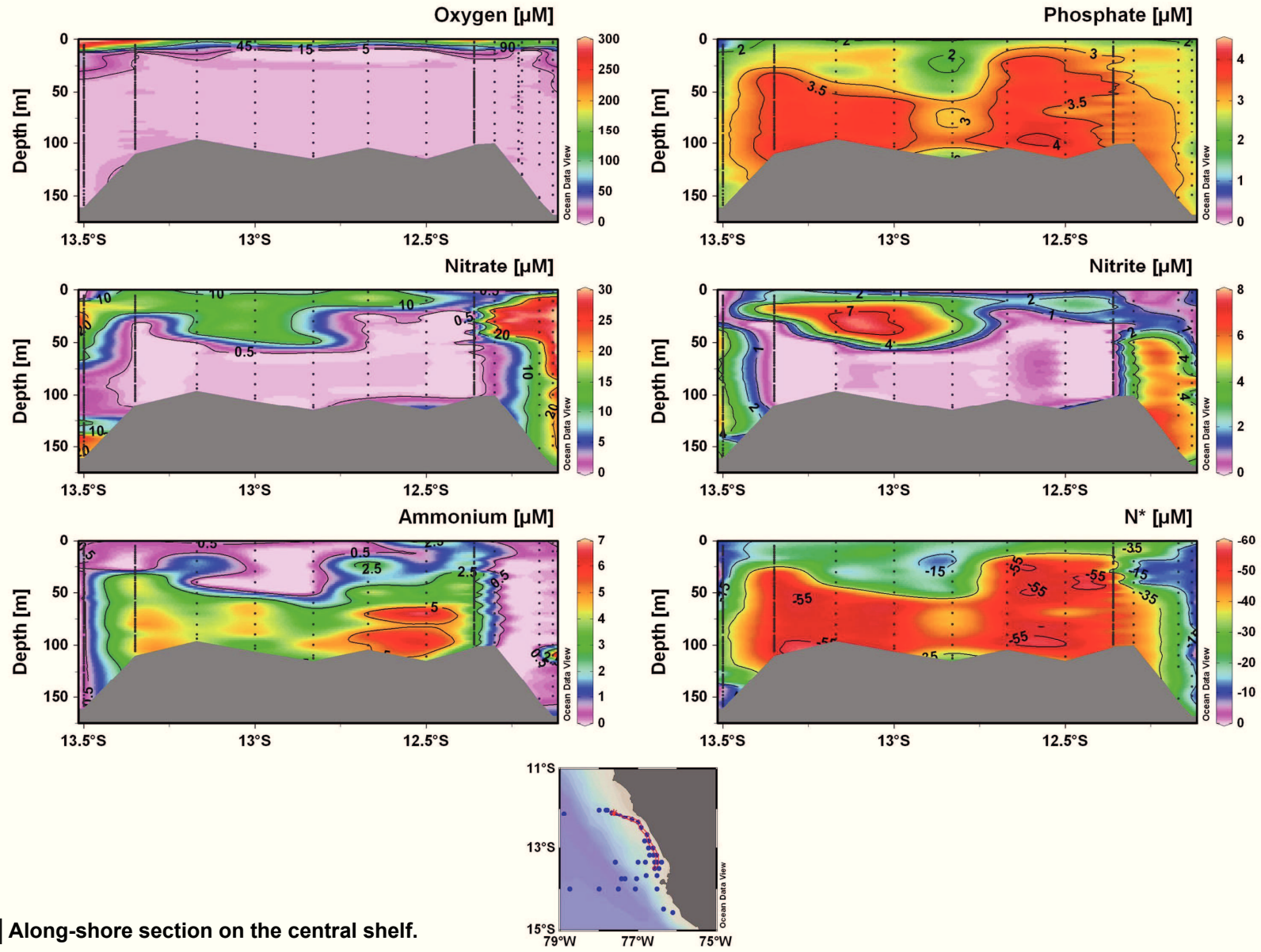


Figure S1i | Along-shore section on the central shelf.

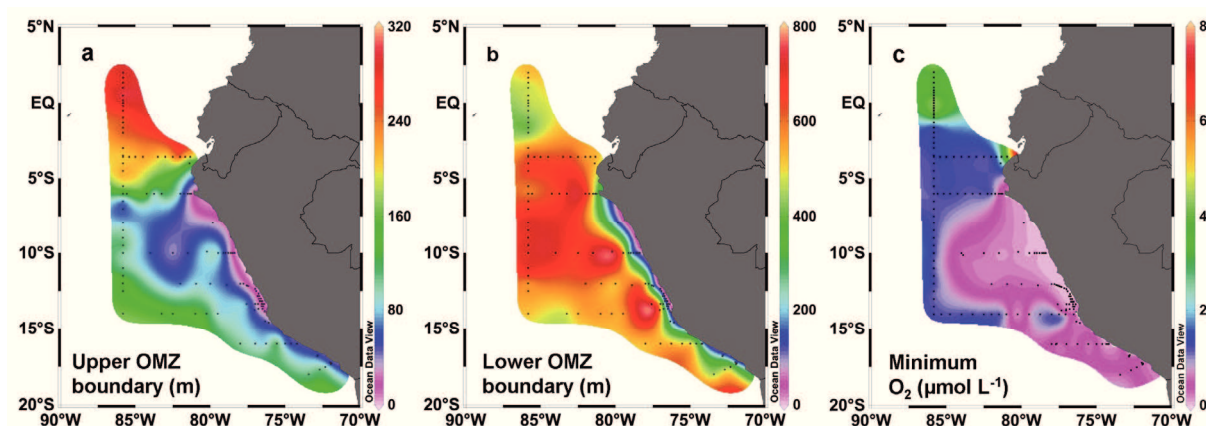


Figure S2 | Extent and intensity of the ETSP OMZ.

(a,b) Depth of upper and lower OMZ boundary (defined by $O_2 \leq 15 \mu\text{mol L}^{-1}$). (c) Minimum O_2 concentrations measured throughout the cruise (Seabird O_2 sensor data corrected for an observed offset of $\sim 2 \mu\text{mol L}^{-1}$ between Seabird- and STOX sensor measurements). Over the Peruvian shelf, O_2 concentrations $\leq 15 \mu\text{mol L}^{-1}$ were detected at depths as shallow as ~ 10 m. In the offshore region south of 3°S , the upper and lower OMZ boundaries were located at 50-200 m ($\sigma_t \sim 26.25 \text{ kg m}^{-3}$) and 500-800 m ($\sigma_t \sim 27.1 \text{ kg m}^{-3}$), respectively. To the north of this region, the OMZ was found deeper and less pronounced (~ 200 m thickness). Here, a depression of the upper OMZ boundary is likely caused by the eastward flowing Equatorial Undercurrent (Helly and Levin 2004). Oxygen was undetectable ($\leq 50 \text{ nmol L}^{-1}$) in the subsurface shelf waters as well as in the core of the offshore OMZ by STOX measurements (Kalvelage et al. 2011). Minimum O_2 concentrations were generally below $1.5 \mu\text{mol L}^{-1}$ south of 1°S , centered on a density (σ_t) of $\sim 26.6 \text{ kg m}^{-3}$. Higher minimum O_2 concentrations ($>3 \mu\text{mol L}^{-1}$) were measured at the northernmost stations as well as a few stations off the northwestern tip of the Peruvian coast.

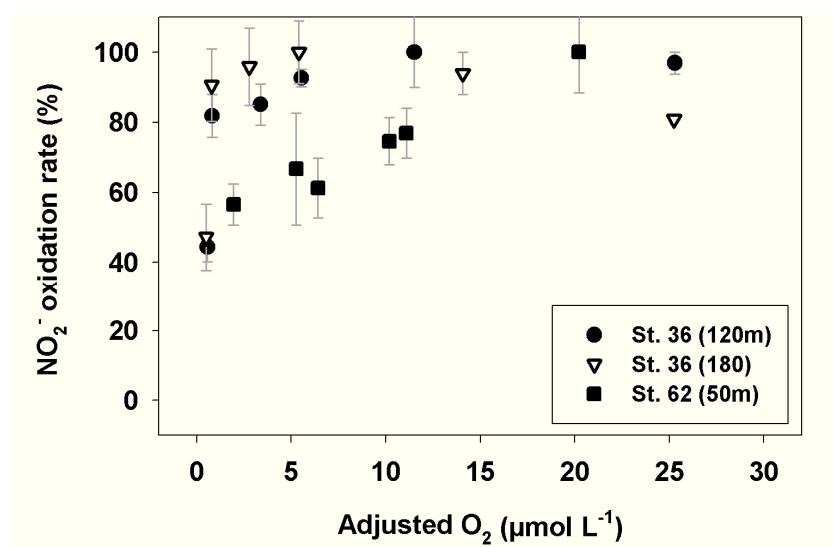


Figure S3 | Oxygen sensitivity of NO_2^- oxidation to NO_3^- .

Rates of NO_2^- oxidation rates were only moderately affected by decreasing O_2 concentrations. Compared to incubations at $>10 \mu\text{mol L}^{-1}$ of O_2 , still 44-57% activity was measured at $\leq 1 \mu\text{mol L}^{-1}$. Similar O_2 sensitivities of NO_2^- oxidation (36-59% activity at $O_2 \leq 1 \mu\text{mol L}^{-1}$) were recently reported for the Namibian OMZ (Füssel et al. 2011). The experimental procedure of the O_2 -sensitivity essay is described in Kalvelage et al. (2011).

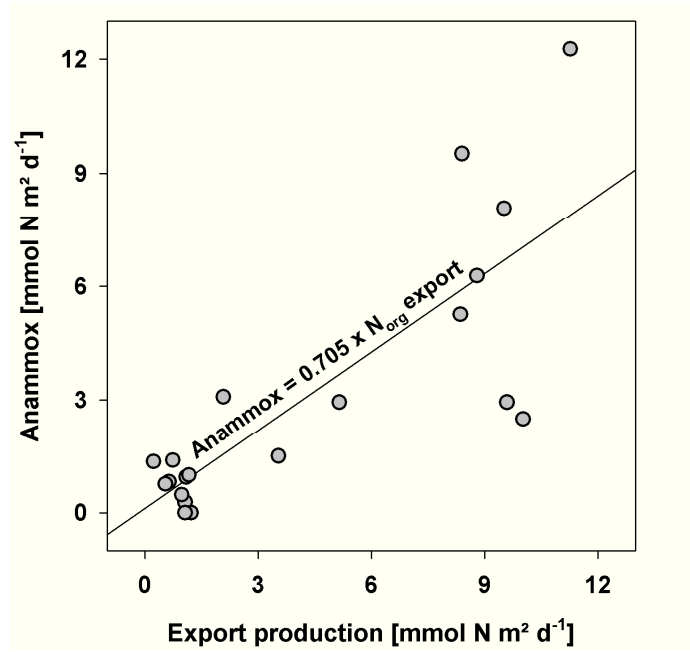


Figure S4 | Anammox as a function of export of organic nitrogen (N_{org}).

Table S1 | Sampling locations and corresponding depth-integrated nutrient concentrations as well as N-fluxes.

Station §	Lat / °N	Lon / °E	Bottom depth / m	Extent of OMZ † / m	NH ₄ ⁺ / mol m ⁻²	NO ₂ ⁻ / mol m ⁻²	N* / mol m ⁻²	H ₂ S / mol m ⁻²	NH ₃ oxidation to NO ₂ ⁻ *	NO ₂ ⁻ oxidation to NO ₃ ⁻ *	NO ₃ ⁻ reduction to NO ₂ ⁻ *	Anammox *	Denitrification with H ₂ S *	N _{org} export to OMZ *
119 (3)	-3.58	-81.34	1216	331	0.00	0.00	-1.71	0.00	0.45 (±0.02)	4.03 (±0.33)	5.08 (±0.34)	0.82 (±0.22)		0.64
109 (4)	-3.58	-85.83	3266	433	0.00	0.00	-2.08	0.00	n.d.	0.67 (±0.19)	3.91 (±0.61)	0.28 (±0.05)		1.06
805 (4)	-6.00	-81.36	999	558	0.15	0.11	-4.87	0.00	4.72 (±0.29)	11.5 (±1.53)	9.61 (±1.34)	2.49 (±0.33)		10.0
123 (4)	-6.00	-81.50	2426	549	0.03	0.15	-3.03	0.00	0.54 (±0.00)	5.24 (±1.58)	13.1 (±2.45)	2.93 (±0.29)		9.59
134 (5)	-6.00	-85.83	4110	472	0.00	0.11	-2.77	0.00	n.d.	2.38 (±0.43)	5.45 (±0.45)	0.47 (±0.12)		0.97
807 (6)	-10.00	-78.38	115	105	0.01	0.82	-1.31	0.00	0.97 (±0.10)	16.1 (±4.30)	76.7 (±20.0)	12.3 (±1.42)		11.3
811 (6)	-10.00	-78.97	145	91	0.01	0.10	-0.19	0.00	1.00 (±0.11)	6.40 (±0.51)	6.00 (±1.07)	1.50 (±0.25)		3.54
3 (6)	-10.00	-81.50	4697	673	0.40	0.46	-3.02	0.00	1.17 (±0.10)	2.63 (±0.30)	2.62 (±0.89)	1.39 (±0.29)		0.74
5 (6)	-10.00	-84.00	4525	657	0.14	0.16	-3.70	0.00	0.23 (±0.03)	8.46 (±1.27)	3.89 (±0.69)	0.94 (±0.05)		1.09
101 (5)	-10.00	-85.83	4407	589	0.07	0.04	-3.24	0.00	n.d.	1.96 (±0.07)	3.80 (±0.39)	n.d.		1.23
13 (6)	-12.03	-77.79	356	328	0.02	0.83	-2.30	0.00	0.62 (±0.06)	5.11 (±1.25)	18.2 (±1.39)	5.26 (±0.73)		8.36
8 (6)	-12.03	-80.95	4678	487	0.07	0.55	-3.80	0.00	0.32 (±0.01)	2.36 (±0.27)	5.79 (±0.73)	1.00 (±0.22)		1.17
19 (4)	-12.36	-77.00	105	93	0.26	0.05	-4.12	0.11					69.7 (±9.62)	
65 (4)	-13.35	-76.50	109	93	0.33	0.04	-4.45	0.31					50.9 (±15.9)	
62 (6)	-13.35	-76.75	160	136	0.04	0.53	-3.27	0.00	0.69 (±0.08)	24.1 (±1.27)	15.8 (±1.15)	8.08 (±0.73)		9.52
54 (6)	-13.75	-77.03	1893	636	0.14	0.96	-3.06	0.00	0.95 (±0.05)	11.0 (±1.72)	22.4 (±0.87)	6.28 (±1.04)		8.80
84 (5)	-14.00	-81.00	4813	423	0.01	0.56	-4.75	0.00	0.08 (±0.00)	2.92 (±0.35)	8.22 (±1.50)	0.76 (±0.12)		0.55
93 (5)	-14.00	-85.83	4573	352	0.01	0.11	-3.81	0.00	n.d.	n.d.	3.88 (±0.37)	n.d.		1.07
38 (3)	-16.00	-74.25	258	213	0.01	0.80	-5.91	0.00	0.92 (±0.18)	72.4 (±9.12)	20.0 (±1.64)	9.52 (±0.69)		8.40
36 (6)	-16.00	-75.00	2845	453	0.42	1.23	-5.98	0.00	1.79 (±0.16)	22.1 (±1.70)	9.99 (±1.60)	2.94 (±0.83)		5.15
28 (6)	-16.00	-77.00	2352	450	0.12	1.83	-5.43	0.00	0.61 (±0.06)	2.85 (±0.57)	no data	1.35 (±0.36)		0.23
44 (6)	-17.34	-71.94	281	220	0.03	1.13	-3.48	0.00	0.87 (±0.14)	8.86 (±0.91)	20.0 (±3.10)	3.08 (±0.35)		2.08

§ In parenthesis: depths sampled for ¹⁵N-labeling experiments.

† Vertical extent for O₂ ≤ 15 μmol L⁻¹.

* In mmol N m⁻² d⁻¹.

n.d.: non-detectable.

Grey shading: sulfidic stations.

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Microaerobic Respiration in Oxygen Minimum Zones

Kalvelage, Tim^{1*}, Gaute Lavik¹, Marlene M. Jensen^{1,4}, Sergio Contreras^{1,5}, Niels Peter Revsbech², Carolin Löscher³, Aurélien Paulmier^{1,7}, Harald Schunck⁴ and Marcel M.M. Kuypers¹

¹Max Planck Institute for Marine Microbiology, Bremen, Germany

²Department of Biological Sciences, University of Aarhus, Denmark

³Institute for General Microbiology, Kiel, Germany

⁴GEOMAR | Helmholtz Centre for Ocean Research Kiel, Germany

⁵Current address: Technical University of Denmark, Kongens Lyngby, Denmark

⁶Current address: Large Lakes Observatory, University of Minnesota Duluth, USA

⁷Current address: Laboratoire d'Etudes en Géophysique et Océanographie Spatiales, Toulouse, France

*Corresponding author. E-mail: tkalvela@mpi-bremen.de

Keywords: oxygen minimum zone, organic matter remineralization, ammonia supply, microaerobic respiration, terminal respiratory oxidase.

Acknowledgements

We wish to thank the governments of Namibia and Peru for access to their territorial waters. We sincerely thank the cruise leaders Kay Emeis (M76-2) and Martin Frank (M77-3) as well as the crews of the cruises onboard R/V Meteor for their support at sea. We are grateful for the technical and analytical assistance of Gabriele Klockgether, Daniela Franzke, Inka Boosmann, Violeta Leon, Linda Sin, Andreas Ellrott, Volker Meyer and Michael Jünemann. We thank Jasmin Franz for providing POC data. This study was supported by the DFG-funded Sonderforschungsbereich 754 “Climate-Biogeochemistry Interactions in the Tropical Ocean” and the Max Planck Society. Additional funding came from the European Research Council, grant 267233, and the Danish Council for Independent Research: Natural Sciences, grant 10-083140.

Abstract

In the oxygen minimum zones (OMZs) of the tropical oceans, strong microbial respiration of sinking organic matter results in the depletion of oxygen. When oxygen concentrations drop below $\sim 5 \mu\text{mol L}^{-1}$, organic matter is generally assumed to be respired with nitrate. However, the commonly observed activity of nitrifiers at non-detectable oxygen concentrations ($< 1 \mu\text{mol L}^{-1}$) suggests aerobic micro-organisms to be well adapted to near-anoxic conditions. Hence, a large fraction of sinking organic matter in OMZs is likely remineralized microaerobically. So far, microaerobic respiration as a potentially important remineralization pathway and ammonium source in OMZs has rarely been investigated.

Here, we report extensive measurements of oxygen consumption in the Namibian and Peruvian OMZs. Short-term incubation experiments with labelled oxygen ($^{18}\text{O}_2$) revealed persistent aerobic microbial activity at non-detectable concentrations of O_2 ($\leq 50 \text{ nmol L}^{-1}$). Accordingly, abundant genes encoding for terminal respiratory oxidases with high O_2 affinities were detected by metagenomic analyses. In the upper OMZs ($\leq 20 \mu\text{mol L}^{-1}$ of O_2), microaerobic respiration was the dominant pathway of organic matter remineralization and provided sufficient ammonium to sustain previously determined rates of ammonia oxidation and anammox.

Introduction

Most of the organic matter in the world's oceans is remineralized via aerobic microbial respiration. Only when oxygen (O_2) becomes scarce, thermodynamically less favourable electron acceptors are used by micro-organisms for the oxidation of organic matter (Falkowski 2008).

Large, permanently O_2 -depleted water masses, the so-called oxygen minimum zones (OMZs), are found at depths of 100-1,000 m in the tropical oceans (Kamykowski and Zentara 1990; Helly and Levin 2004; Karstensen et al. 2008). Here, coastal upwelling of nutrient-rich deep waters fuels high surface productivity and thus strong O_2 consumption via degradation of organic matter in the subsurface. When O_2 concentrations drop below $\sim 5 \mu\text{mol L}^{-1}$ in OMZs, nitrate (NO_3^-) is generally assumed to replace O_2 as the major electron acceptor in organic matter respiration (Devol 1978; Codispoti 2001; Paulmier et al. 2009).

However, O_2 -dependent nitrification has been measured in samples from virtually anoxic depths in OMZs, while NO_3^- reduction and anaerobic ammonia oxidation (anammox) have been found active in the upper OMZs, where O_2 concentrations often exceed $5 \mu\text{mol L}^{-1}$ (Ward et al. 1989; Lipschultz et al. 1990; Kuypers et al. 2005; Hamersley et al. 2007; Lam et al. 2009; Lam et al. 2011). In accordance, O_2 -sensitivity assays recently revealed a broad range of O_2 concentrations for the co-occurrence of aerobic and anaerobic processes in OMZs. In the OMZs off Namibia and Peru, the activities of aerobic ammonia (NH_3) and nitrite (NO_2^-) oxidation were little affected by a decrease in O_2 to non-detectable levels ($< 1 \mu\text{mol L}^{-1}$). At the same time, NO_3^- reduction and anammox proceeded until $\sim 20 \mu\text{mol L}^{-1}$ of O_2 (Füssel et al. 2011; Kalvelage et al. 2011; Chapter 3).

Pronounced activities of anammox and aerobic NH_3 oxidation have often been measured in the upper OMZs. In the upper Peruvian OMZ, remineralization of organic matter via NO_3^- respiration was recently shown to supply $\sim 15\text{-}50\%$ of the NH_4^+ required for

anammox and aerobic NH_3 oxidation (Lam et al. 2009; Chapter 3). Microaerobic organic matter respiration has been suggested as the major source of NH_4^+ near the upper OMZ boundary. Here regular intrusions of oxygenated surface waters or mixing events, such as related to eddy activity, could sustain aerobic microbial activity (Bertrand et al. 2010).

However, direct measurements of O_2 consumption in OMZs are virtually non-existent, mainly due to a lack of available methods for precise measurements at low O_2 concentrations. A single measurement of microaerobic respiration has been carried out in the upper Peruvian OMZ. Revsbech et al. (2009) could determine an O_2 consumption rate of $\sim 2 \mu\text{mol L}^{-1} \text{d}^{-1}$, which exceeds O_2 consumption due to nitrification in this area by about one order of magnitude and thus can mainly be attributed to heterotrophic activity.

Here, we report extensive measurements of O_2 consumption throughout the OMZs off Namibia and Peru. Rates of aerobic respiration were determined in short-term incubation experiments with labeled O_2 ($^{18}\text{O}_2$). Supported by metagenomic analysis, we show that the microbial community inhabiting the OMZ waters has the capacity to exploit sub-micromolar levels of O_2 . Our results further indicate that in the upper Namibian and Peruvian OMZ, microaerobic organic matter remineralization is sufficient to sustain high rates of the NH_4^+ -dependent processes of anammox and NH_3 oxidation.

Materials & Methods

Water sampling – Samples were taken on two cruises onboard R/V Meteor: in May/June 2008 over the Namibian shelf (M76-2) and in December/January 2008/2009 in the eastern tropical South Pacific (ETSP) OMZ off Peru (M77-3). On both cruises, a pump-CTD system was used to collect water samples throughout the OMZ (down to ~ 375 m depth). The pump CTD system was equipped with a conventional amperometric O_2 micro-sensor for dissolved O_2

profiling and a highly sensitive STOX (Switchable Trace amount OXYgen) sensor for high-accuracy O₂ measurements (detection limit: 50-100 nmol L⁻¹ during our deployments) at selected depths (Revsbech et al. 2009). In parallel, a CTD-rosette system (24 x 10-L Niskin bottles) equipped with a fluorescence sensor (Dr. Haardt; calibrated against chlorophyll-a measurements) was deployed in the Peruvian OMZ. Over the Namibian shelf, additional samples were collected in the benthic boundary layer (BBL), at about 30 cm to 2 m above the seafloor, with a bottom water sampler (Holtappels et al. 2011).

Ammonia, chlorophyll-a and particulate organic matter analyses – Samples for NH₄⁺ analyses were taken with a depth resolution of 1-2 m and measured fluorometrically (Holmes et al. 1999) (detection limit: 10 nmol L⁻¹). Concentrations of chlorophyll-a were determined with a fluorometer (Turner Designs) after filtration of 0.5-2 L of seawater onto 25 mm Whatman GF/F filters followed by chlorophyll-a extraction with acetone. Both NH₄⁺ and chlorophyll-a were measured directly on board. Water samples (0.5-2 L) for particulate organic carbon (POC) were filtered onto combusted (5 h at 450°C) 25 mm Whatman GF/F filters and stored frozen (-20°C). In a shore-based laboratory, samples were measured using an elemental analyzer (EURO EA and Thermo Flash EA, 1112 Series) after drying and decalcification with hydrochloric acid (37%) for ~15 h.

¹⁸O₂-labeling experiments – Microaerobic respiration was measured on the Namibian shelf as well as the coastal and offshore Peruvian OMZ (Table 1), as the consumption of ¹⁸O₂ in time-series incubations. Due to the low natural abundance of the stable oxygen isotope ¹⁸O (~0.2%) compared to the most abundant oxygen isotope ¹⁶O (~99.8%), trace amounts of double-labelled O₂ (¹⁸O₂) can be measured with high accuracy using mass spectrometry. At each station, up to six depths - from the base of the upper oxycline down to the core of the OMZ - were chosen for ¹⁸O₂-labeling experiments. Before sampling, a super-saturated (~1 mmol L⁻¹)

$^{18}\text{O}_2$ stock solution was prepared by replacing 1 ml of sterile seawater in a 12-ml Exetainer (Labco, UK) with 99% enriched $^{18}\text{O}_2$ (Sigma-Aldrich, Germany). Water samples were collected in 250-mL serum bottles and purged with helium (He) for approximately 15 min to remove any initial O_2 . During He-purging, a small sample volume was lost such that the bottles had to be refilled with a second He-purged sample from the same depth to avoid headspace. Afterwards, a defined volume of the $^{18}\text{O}_2$ stock solution was added to the serum bottles (using a gas tight syringe) in exchange for part of the de-oxygenated water. The samples were adjusted to the following $^{18}\text{O}_2$ concentrations: Namibian OMZ: $\sim 2.5 \mu\text{mol L}^{-1}$; Peruvian OMZ: $\sim 7.0 \mu\text{mol L}^{-1}$ (upper-most sampling depth), $\sim 3.5 \mu\text{mol L}^{-1}$ (upper OMZ) and $\sim 1.5 \mu\text{mol L}^{-1}$ (lower OMZ/core of OMZ). To ensure complete homogenization of the samples with respect to $^{18}\text{O}_2$, the bottles were placed on a magnetic stirrer plate (a stirring bar had been added to each bottle prior to sampling) for ~ 10 min at ~ 450 rpm. Samples were then transferred into 12-ml Exetainers using He-overpressure (see Holtappels et al. (2011) for detailed description) to avoid contamination with $^{16}\text{O}_2$ from air. One Exetainer each was sacrificed to determine initial $^{18}\text{O}_2$ concentrations with a custom-built, fast-responding O_2 micro-sensor (Clark-type, MPI Bremen; detection limit: $\sim 0.5 \mu\text{mol L}^{-1}$). The remaining Exetainers were incubated in the dark at in situ temperature (± 2.5 °C). After about 0, 6, 12, 24 and 48 h, biological activity was terminated in two replicate Exetainers by adding saturated mercuric chloride. The concentration of $^{18}\text{O}_2$ in these experiments was determined by membrane inlet mass spectrometry (MIMS; GAM200, IPI) in a shore-based laboratory. Respiration rates were calculated from the slope of linear regression of $^{18}\text{O}_2$ consumption as a function of time (Fig. 1). Only significant and linear consumption of $^{18}\text{O}_2$ was considered (t -tests, $p < 0.05$).

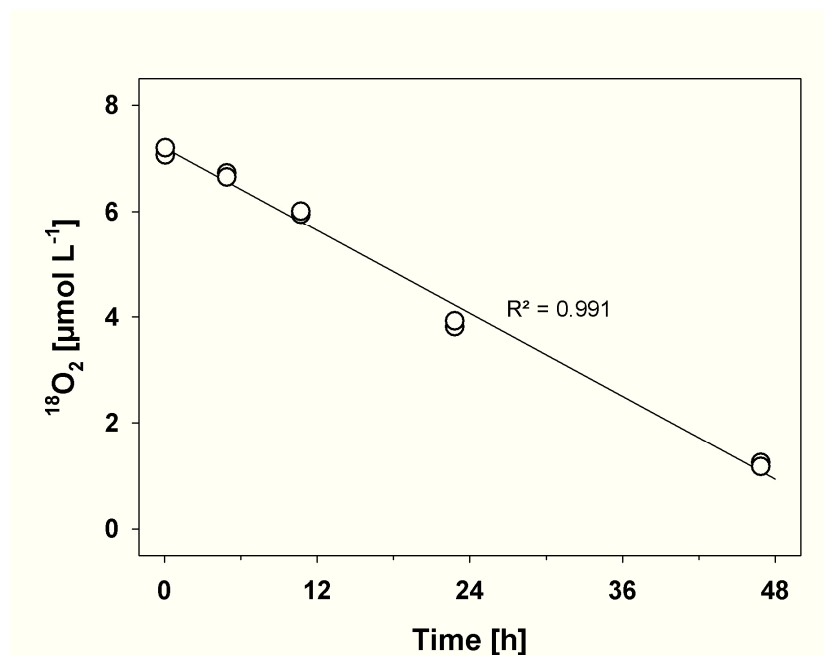


Figure 1 | Example of linear $^{18}\text{O}_2$ consumption in short-term incubation experiments.

The data shown here were obtained at St. 805 (15 m) in the Peruvian OMZ.

Metagenome sequencing – Samples for nucleic acid extraction (1.5-2 L) were prefiltered (10 μm) and collected onto 0.22- μm Durapore Membrane filters (Millipore). Filters were immediately frozen at -80°C until further analysis. Nucleic acids were extracted using a DNA/RNA-Allprep kit (Qiagen) following the manufacturers protocol with minor modifications (Schunck et al. 2012 submitted). Sequencing of 50- μl DNA samples was done on a GS-FLX 454 pyrosequencer (Roche).

Sequence analysis – A total of 1,204,437 raw reads were obtained. The raw reads were clustered using Cd-hit (Li and Godzik 2006) with a sequence identity threshold of 98% and a word length of 8. The ribosomal-gene cluster representative sequences were identified by BLASTn searches (Altschul et al. 1990) against the SILVA database (Prüsse et al. 2007) (bit score cut off: 86). Of all sequences, 0.24% was of ribosomal gene origin and subsequently separated from non-ribosomal-gene cluster representative sequences using MEGAN (Huson

et al. 2007). Those sequences were compared against the non-redundant NCBI database using BLASTx (bit score cut off: 35) and scanned with profile hidden Markov models of the ModEnzA Enzyme Commission groups (Desai et al. 2011). Of all non-ribosomal-gene sequences, 69.6% were identified as protein-coding; the remainder could not be assigned. Sequences, cluster size and cluster identification number as well as results from the BLAST searches and EC scans were added to a MySQL database for analysis (Desai et al. submitted). For the functional (type of cytochrome oxidase) and taxonomic assignment of the cluster representatives the top hit of each BLAST-search was used.

Table 1 | Sampling locations with the pump-CTD system for $^{18}\text{O}_2$ -labeling experiments during cruises M76-2 (Namibian OMZ) and M77-3 (Peruvian OMZ).

	Station	Latitude	Longitude	Depth
Namibian OMZ	225	19.02°S	12.24°E	123 m
	231	21.00°S	13.25°E	123 m
	243	22.10°S	13.87°E	103 m
	252	23.00°S	14.23°E	111 m
Peruvian OMZ	805*	6.00°S	81.36°W	999 m
	807	10.00°S	78.38°W	115 m
	811	10.00°S	78.97°W	145 m
	3	10.00°S	81.50°W	4697 m
	5	10.00°S	84.00°W	4525 m
	13	12.03°S	77.79°W	356 m
	36	16.00°S	75.00°W	2845 m

* At St. 805, samples were collected with a CTD-rosette system.

Results & Discussion

The hydrochemical setting at the time of sampling over the Namibian shelf as well as in the Peruvian OMZ has been described in detail previously (Füssel et al. 2011; Kalvelage et al. 2011; Chapter 3). Thus, we will here focus on the distribution of O₂ and the potential for oxic respiration under microaerobic conditions in both systems. We will discuss in more detail the consumption of O₂ in the Peruvian OMZ in relation to surface productivity, organic matter availability and NH₄⁺ accumulations. Furthermore, we will explore the genetic capacity for aerobic respiration of micro-organisms thriving in the OMZ waters and finally compare supply and demand of NH₄⁺ in the upper OMZs off Namibia and Peru.

Microaerobic respiration in the Namibian shelf OMZ – At four stations over the Namibian shelf, marked by high primary productivity (Füssel et al. 2011), O₂ concentrations ranged from ~150 to 250 μmol L⁻¹ in the surface waters and gradually declined to ≤15 μmol L⁻¹ at ~65-85 m depth (Fig. 2). Oxygen concentrations in the lower micromolar range were detected in the upper part of the OMZ, whereas O₂ was generally below the detection limit of the STOX sensor (~100 nmol L⁻¹ of O₂ during M76-2) in the lower OMZ (see also Kalvelage et al. 2011). Both O₂ profiles (Fig. 2) and density gradients (data not shown) indicated a weak stratification and thus enhanced vertical mixing of the OMZ waters with more oxygenated overlying waters over the shelf at the time of sampling.

Aerobic respiration was measurable at all depths investigated, from the BBL to the upper OMZ boundary, at each of the four sampling sites. Respiration rates were fairly consistent between stations, typically around 150-500 nmol L⁻¹ O₂ d⁻¹, with the exception of noticeably higher rates in the lower OMZ and the OMZ bottom waters at St. 252 (~1.5 μmol L⁻¹ O₂ d⁻¹). A trend of elevated O₂ consumption towards the sediment-water interface, increasing from ~150 to ~350 nmol L⁻¹ O₂ d⁻¹, was also observed at St. 231. The high

potential for aerobic respiration in the bottom waters at St. 231 and 252 might owe to the accumulation of sinking and/or resuspended particulate organic matter. In the Namibian OMZ, particles have been shown to be colonized by both auto- and heterotrophic micro-organisms which use O_2 as the terminal electron acceptor (Woebken et al. 2007). In contrast, respiration remained rather constant throughout the OMZ at St. 225 and 243. Overall, there was no apparent correlation between *in-situ* concentrations of O_2 and O_2 consumption rates in the incubation experiments.

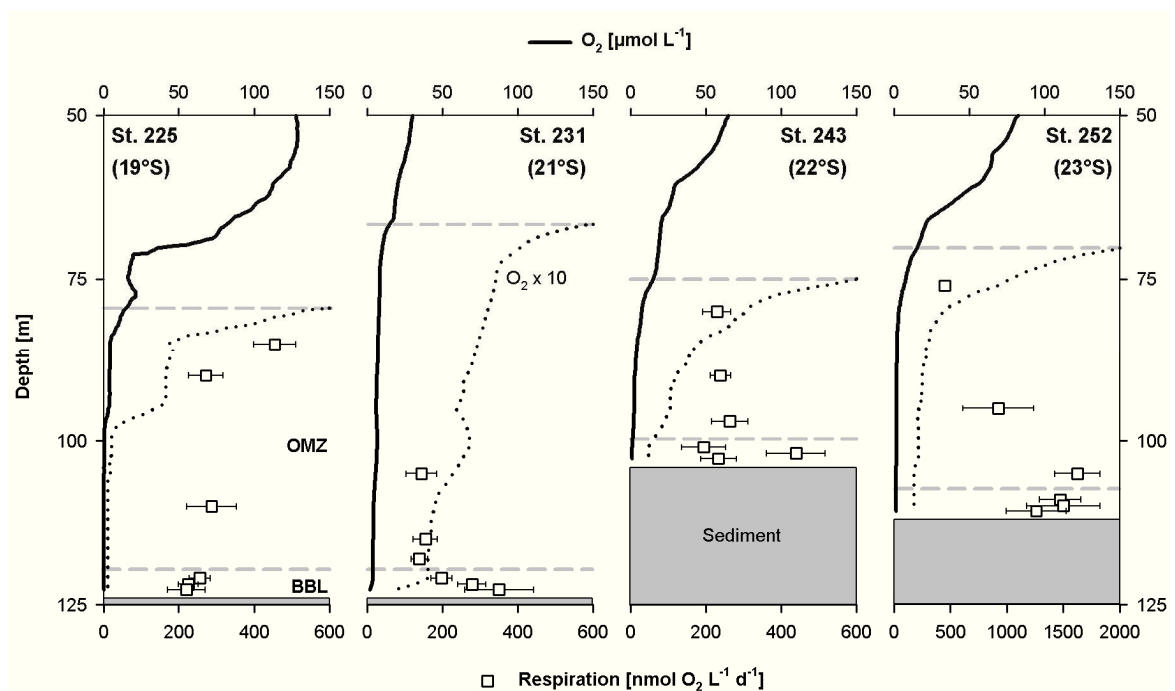


Figure 2 | Vertical distribution of O_2 (and 10 x its concentration, dotted lines) and rates of aerobic respiration in the OMZ and BBL at four stations over the Namibian shelf.

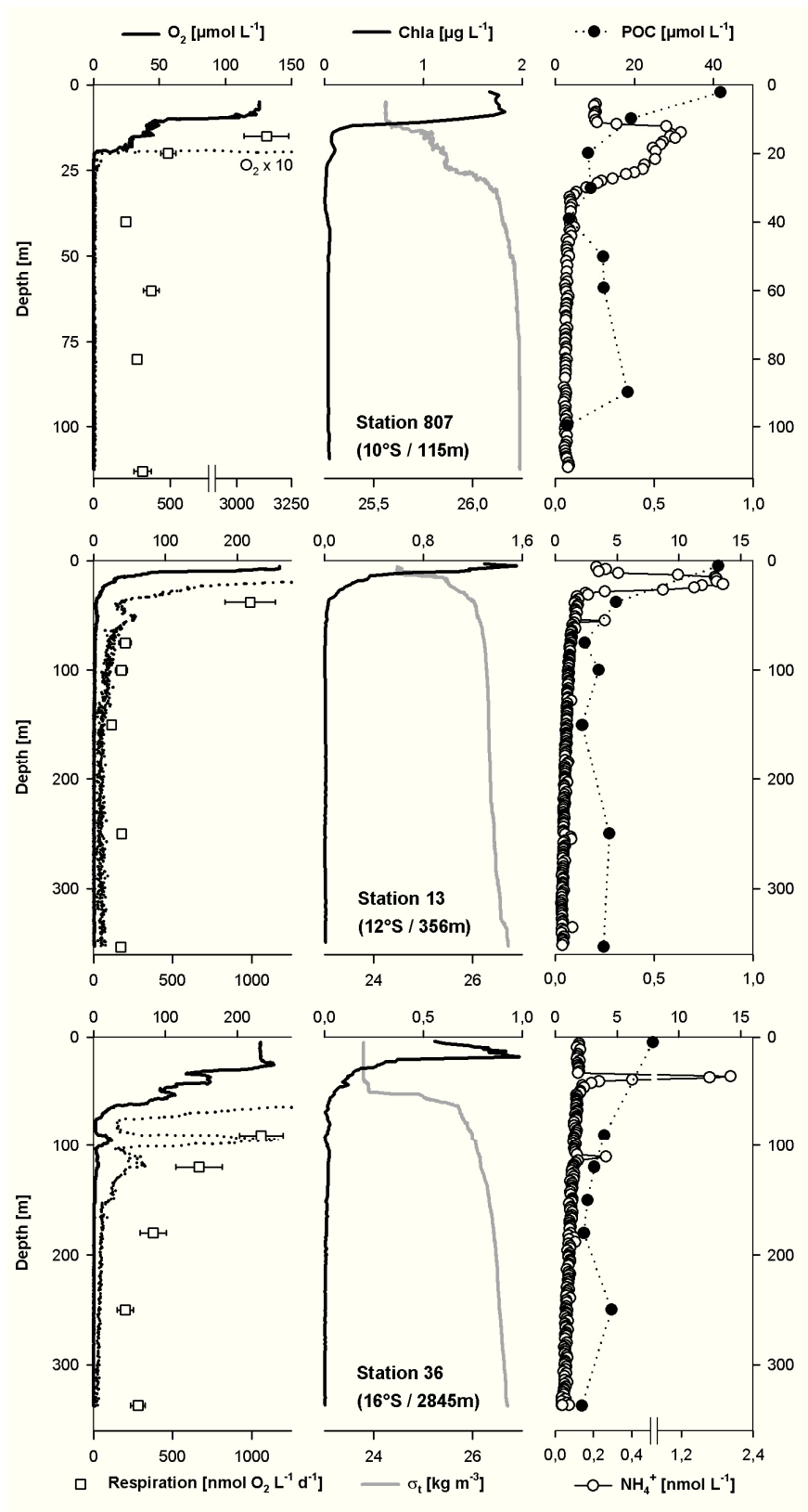


Figure 3 | Physicochemical zonation and rates of aerobic respiration at three stations in the Peruvian OMZ.

Microaerobic respiration in the Peruvian OMZ – The experimental stations off Peru covered a larger range of environmental settings, including highly productive coastal and more oligotrophic open ocean sites. Incubation experiments were conducted at seven stations, some of which will be discussed in more detail in the following.

At three stations, located on the shelf (St. 807), upper (St. 13) and lower continental slope (St. 36), we investigated the relation of surface productivity and microaerobic respiration as a potential source of NH_4^+ in the OMZ. In the shallow shelf OMZ, O_2 declined rapidly from $\sim 125 \mu\text{mol L}^{-1}$ at the surface to $\leq 15 \mu\text{mol L}^{-1}$ at ~ 20 m (Fig. 3), and remained below the detection limit of the STOX sensor (50 nmol L^{-1} of O_2 during M77-3) at depths ≥ 40 m. Similar to the Namibian shelf stations, vertical mixing of surface and OMZ waters was indicated by the erratic O_2 and density profiles between ~ 10 and 25 m at St. 807. At St. 13 and 36, surface O_2 concentrations were $\sim 250 \mu\text{mol L}^{-1}$ and decreased to $\leq 15 \mu\text{mol L}^{-1}$ at ~ 25 m and ~ 70 m, respectively. STOX measurements revealed non-measurable concentrations of O_2 at depths ≥ 100 m (St. 13) and ≥ 180 m (St. 36) (see also Kalvelage et al. 2011). Pronounced pycnoclines at St. 13 (~ 20 m) and 36 (~ 55 m) indicated a stronger vertical stratification here, compared to the Peruvian shelf waters. Lateral mixing in of more oxygenated waters into the OMZ was however clearly shown by a local O_2 maximum ($10\text{--}25 \mu\text{mol L}^{-1}$) at ~ 100 m at St. 36. The less distinct O_2 maximum at St. 13 (~ 55 m) might be a remnant of a mixing event in the past.

Oxygen consumption was highest near the upper OMZ boundary at all three stations and rapidly decreased with depth. Maximum rates were $>3 \mu\text{mol L}^{-1} \text{O}_2 \text{ d}^{-1}$ at St. 807 and $\sim 1 \mu\text{mol L}^{-1} \text{O}_2 \text{ d}^{-1}$ at St. 13 and 36. These values are in good agreement with the (to our knowledge) so far only, direct measurement of oxic microbial respiration ($\sim 2 \mu\text{mol L}^{-1} \text{O}_2 \text{ d}^{-1}$) in the upper Peruvian OMZ (Revsbech et al. 2009). Deeper in the OMZ, respiration rates showed little variability and typically ranged from 200 to $400 \text{ nmol L}^{-1} \text{O}_2 \text{ d}^{-1}$ (Fig. 3).

Release of NH_4^+ by microaerobic respiration – The shallow sampling sites near the Peruvian coast (St. 807 and 13) showed high levels of chlorophyll-a at the surface ($\sim 1.5\text{-}2 \mu\text{g L}^{-1}$), whereas further offshore (St. 36) the waters appeared less productive, as indicated by $\sim 30\text{-}50\%$ lower maximum chlorophyll-a concentrations (Fig. 3). At all three stations, peak concentrations of NH_4^+ ($\sim 0.5\text{-}2 \mu\text{mol L}^{-1}$) were observed just below the chlorophyll maximum, suggesting high rates of organic matter remineralization and subsequent NH_4^+ release near the upper OMZ boundary. In corroboration, the distributions of particulate organic carbon (POC) indicated the highest organic matter turnover in this zone. Surface POC concentrations ranged from $\sim 40 \mu\text{mol L}^{-1}$ (St. 807) to $\sim 8 \mu\text{mol L}^{-1}$ (St. 36) and quickly attenuated towards the OMZ. Throughout the OMZ, POC concentrations remained rather constant at about 10, 3 and 3 $\mu\text{mol L}^{-1}$ at St. 807, 13 and 36, respectively. Overall, the vertical distributions of POC coincided strikingly well with those of the O_2 consumption rates. Microaerobic respiration apparently was an important degradation pathway of sinking organic matter and hence an important source of NH_4^+ in the upper Peruvian OMZ.

Terminal respiratory oxidases with high O_2 affinity in the Peruvian OMZ – Though at rates up to ten times lower compared to the uppermost incubation depth (St. 807), O_2 consumption was consistently measurable in samples from apparently anoxic ($\leq 50 \text{ nmol L}^{-1}$ of O_2) depths in the Peruvian OMZ. These rates are unlikely representative of *in-situ* activity, yet they highlight the aerobic respiratory potential of micro-organisms inhabiting the deeper OMZ waters. A recent survey of the ETSP OMZ along the South American continental slope, revealed O_2 to be depleted down to at least $\leq 10 \text{ nmol L}^{-1}$ in the $\sim 200 \text{ m}$ thick core of the OMZ (Thamdrup et al. 2012). In the same study, the authors concluded that the OMZ core waters are largely functionally anoxic and possibly only contain picomolar concentrations of O_2 , due to efficient O_2 scavenging by micro-organisms. Revsbech et al. (2009) reported an apparent half-saturation coefficient (K_m) of $\leq 20 \text{ nmol L}^{-1}$ of O_2 for oxic respiration for a sample from

the Peruvian OMZ, which suggests the expression of terminal respiratory oxidases with a high O_2 affinity in these waters.

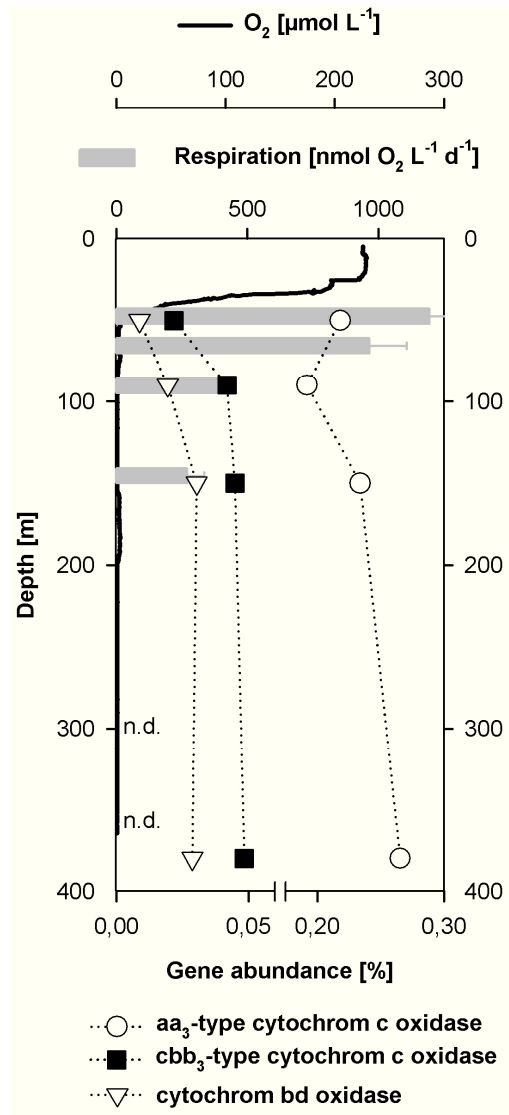


Figure 4 | Aerobic respiration and abundance of selected terminal respiratory oxidases (in % of all protein-encoding sequences) from metagenomes obtained for four depths at St. 3 in the Peruvian OMZ.

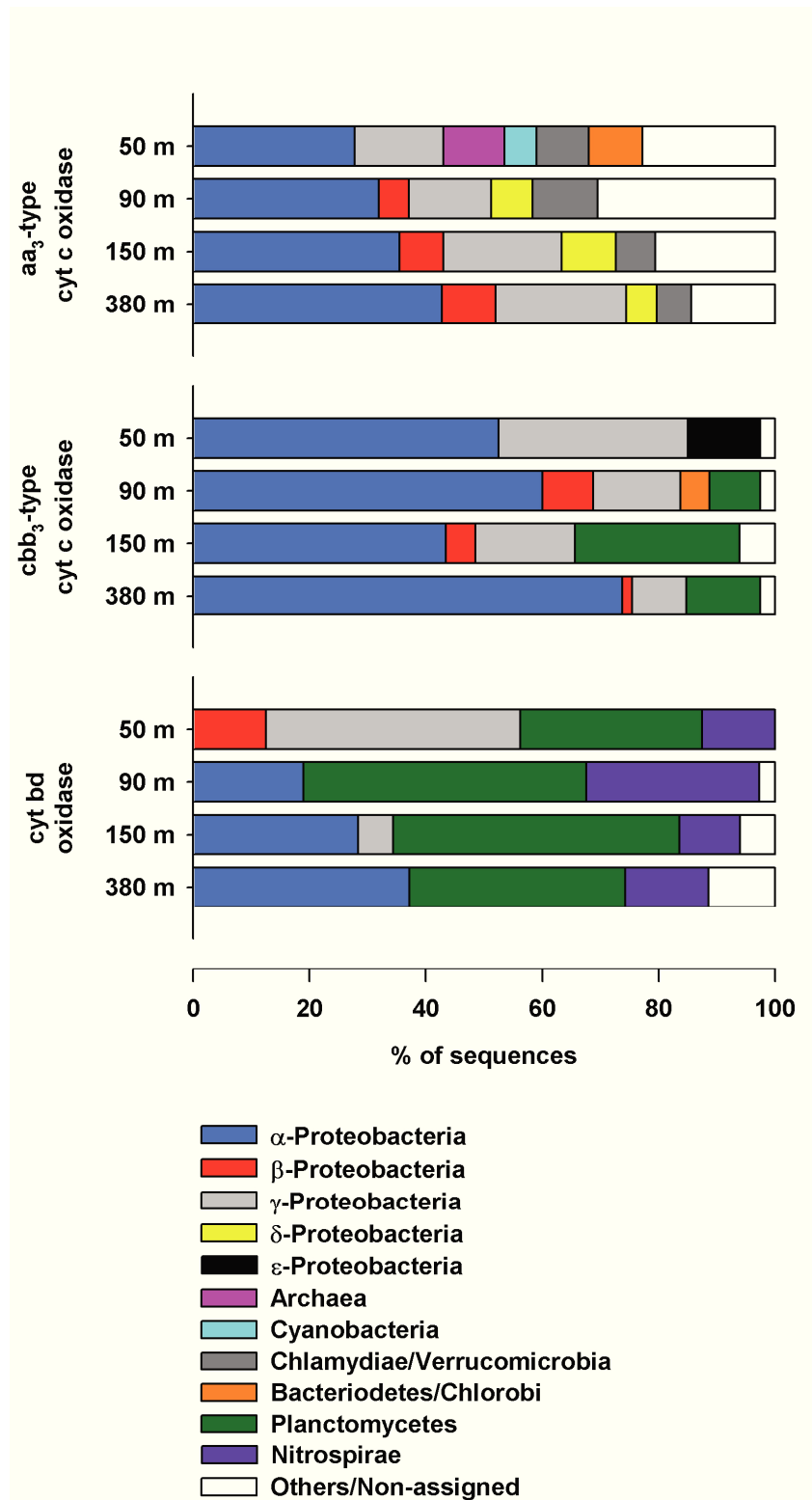


Figure 5 | Taxonomic assignments of terminal respiratory oxidases. Only phyla represented by at least 5% of the respective oxidase-encoding sequences are shown.

We obtained metagenomes for four depths at St. 3, to explore the genetic potential of micro-organisms to exploit low levels of O₂ in OMZ waters. Throughout the OMZ, 0.19-0.26% of all protein-encoding sequences could be assigned to common aa₃-type cytochrom c oxidases. Among all sequences related to known marine prokaryotes, those similar to the aa₃-type cytochrom c (cyt c) oxidase found in the marine archaeal NH₃ oxidizer *Nitrosopumilus maritimus* (Walker et al. 2010) were amongst the most abundant ones (~10%) at the base of the oxycline (50 m) (Fig. 5). In accordance, high NH₃-oxidizing activity and strong expression of the archaeal ammonia monooxygenases subunit A was observed in previous studies in the upper Peruvian OMZ (Ward et al. 1989; Lipschultz et al. 1990; Lam et al. 2009). Furthermore, high archaeal metabolic activity in this zone was shown recently in a metatranscriptomic study in the OMZ off northern Chile (Stewart et al. 2011). Throughout the OMZ, we found a number of aa₃-type cyt c oxidase sequences related to marine bacterial NH₃⁻ oxidizers (*Nitrosococcus halophilus*, *oceanus* and *watsoni*) as well as NO₂⁻ oxidizers (*Nitrococcus mobilis*). In addition, sequences similar to the aa₃-type cyt c oxidase found in the genome of Candidatus *Pelagibacter ubique*, a heterotrophic α -proteobacterium which is highly abundant and active in OMZs (Stewart et al. 2011), were detected at 50 and 90 m.

A variety of micro-organisms has adapted to variable and extremely low O₂ concentrations by evolving terminal respiratory oxidases with very high O₂ affinities. For example, the expression of bacterial cyt bd oxidases is maximal under microaerobic to anoxic conditions (Fu et al. 1991; Tseng et al. 1996) and apparent K_m values as low as 3-8 nmol L⁻¹ of O₂ were measured for the cyt bd oxidase of *Escherichia coli* (D'mello et al. 1996). In a recent experiment, Stolper et al. (2010) demonstrated that *Escherichia coli* could grow aerobically at O₂ concentrations down to at least 3 nmol L⁻¹. Pathogens and other host-associated micro-organisms (e.g. Rhizobia in soybean nodules) possess another type of oxidases with a high O₂ affinity, the cbb₃-type cyt c oxidase, for which a K_m value as low as 7 nmol L⁻¹ of O₂ was reported (Preisig 1993; Preisig et al. 1996; Baar et al. 2003; Pitcher and

Watmough 2004). At St. 3, sequences encoding for both cyt bd oxidases and cbb3-type cyt c oxidases were detected throughout the OMZ with a large fraction (~30-50%) of both oxidase types closely resembling cyt oxidases present in the anammox bacterium *Candidatus Kuenenia stuttgartiensis* (phylum planctomycetales; Fig. 5). The O₂-sensitive anammox bacteria likely synthesize these cyt oxidases to detoxify O₂. Of the cyt bd oxidase-encoding sequences, ~10-30% matched the cyt bd-like oxidase recently found in the genome of the NO₂⁻ oxidizer *Candidatus Nitrospira defluvii* (Lücker et al. 2010) (Fig. 5). This agrees well with the oxidation of NO₂⁻ persistently measured in samples from the base of the oxycline down to the core of the Peruvian OMZ (Ward, et al. 1989; Lipschultz et al. 1990; Chapter 3). Further, at all depths sampled cyt bd oxidase and cbb3-type cyt c oxidases encoding-sequences could be assigned to a number of heterotrophic Alpha- (mostly Rhodobacteraceae) and Gammaproteobacteria, respectively, including known marine representatives of both phyla.

Overall, both type of oxidases with high O₂ affinities revealed a clear trend of increasing relative abundance from the upper OMZ towards the core of the OMZ (cyt bd: +330%; cbb₃-type cyt c: +222%), which indicates an adaptation of the aerobic microbial community, at least on a gene level, to trace concentrations of O₂ deeper in the OMZ. However, although the genetic potential appeared to be present, O₂ consumption rates were non-detectable (detection limit: ~50 nmol O₂ L⁻¹ d⁻¹) at 300 and 365 m at St. 3. Similarly, rates were below detection at the core of the OMZ at St. 8 (275 and 360 m; data not shown). Both stations were located more than 250 km off the Peruvian coast. In comparison, microaerobic respiration remained detectable at equivalent depths at the (near) coastal stations (e.g. 13 and 36). Near the Peruvian coast, O₂ supply, e.g. by eddy activity, might be sufficient for certain micro-organisms to keep their aerobic lifestyle deeper in the OMZ (Chaigneau et al. 2012).

Sources and sinks of NH₄⁺ in the upper Namibian and Peruvian OMZ – Near the upper OMZ boundary, activity of both aerobic and anaerobic N-cycle processes is often enhanced, which has been interpreted as high organic matter remineralization and subsequent NH₄⁺ release in this zone (Thamdrup et al. 2006; Hamersley et al. 2007; Lam et al. 2009; Jensen et al. 2011; Kalvelage et al. 2011). Recent studies investigating both sinks and sources of NH₄⁺ in the Peruvian OMZ, revealed noticeably higher NH₄⁺ demands of NH₃ oxidation and anammox in the upper OMZ than could be explained (~15-50%) by the NH₄⁺ supply from NO₃⁻ reduction and DNRA (Lam et al. 2009; Chapter 3). Thus, it has been suggested that microaerobic respiration of organic matter might be responsible for a large fraction of the NH₄⁺ needed to fuel NH₃ oxidation and anammox at the base of the oxycline. The activities of N-cycle processes that either consume or produce NH₄⁺, have been reported previously for the period of our sampling in the OMZs off Namibia and Peru (M76-2 and M77-3, respectively), (Füssel et al. 2011; Kalvelage et al. 2011; Chapter 3). By combining these with the O₂ consumption rates presented here, we evaluated whether heterotrophic microaerobic respiration could close the gap between so-far identified NH₄⁺ sinks and sources in the upper Namibian and Peruvian OMZ.

Oxygen is respired by both auto- and heterotrophic micro-organisms. In the first step of the autotrophic nitrification, NH₃ oxidizers consume 1.5 moles of O₂ per mole of NH₃:

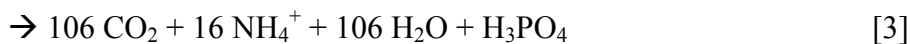


Subsequently, NO₂⁻ oxidizers further oxidize each mole of NO₂⁻ with 0.5 mole of O₂:

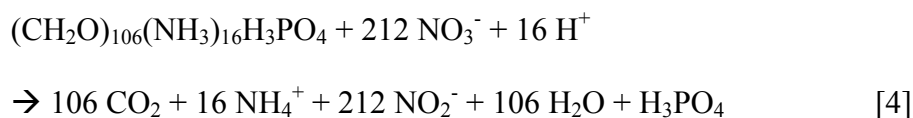


If we subtract the O₂ requirements of nitrification, using the stoichiometry given in [1] and [2], from the total O₂ consumption, we get an estimate of heterotrophic oxic respiration in the upper OMZ waters (Table 2). The aerobic degradation of 1 mole of Redfield organic matter requires 106 moles of O₂, whereby 16 moles of NH₄⁺ are released:

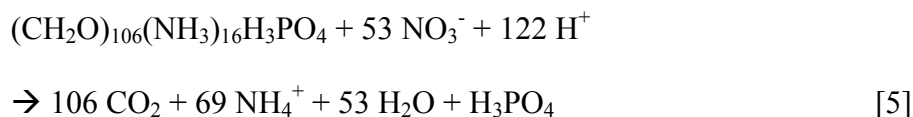




Under O₂-depleted conditions, NO₃⁻ is used as electron acceptor for the degradation of organic matter. In both the Namibian and Peruvian OMZ, NO₃⁻ was found to be mainly reduced to NO₂⁻ (Füssel et al. 2011; Chapter 3). Nitrate reduction to NO₂⁻ releases 16 moles of NH₄⁺ per mole of organic matter:



Alternatively, dissimilatory NO₃⁻ reduction to NH₄⁺ (DNRA) oxidizes 1 mole of organic matter with 53 moles of NO₃⁻ to produce 69 moles of NH₄⁺:



In the upper Namibian and Peruvian OMZ, heterotrophic oxic respiration appeared to be the dominant and often even only source of NH₄⁺ among those processes (Table 2).

Aside from aerobic NH₃ oxidation, anammox, the anaerobic oxidation of NH₄⁺ with NO₂⁻ is the major sink for NH₄⁺ in OMZs:



The combined NH₄⁺ demands of NH₃ oxidation and anammox near the upper OMZ boundary off Peru, were generally well below the estimated supply of NH₄⁺, resulting in net production rates of 66-365 nmol NH₄⁺ L⁻¹ d⁻¹ (Table 2). The highest net rates (St. 805 and 13) coincided with pronounced NH₄⁺ maxima (~0.6 and 0.8 μmol L⁻¹, respectively) below the chlorophyll maximum (Fig. 3). For St. 805 off Peru as well as St. 243 and 252 on the Namibian shelf, we estimated slightly negative NH₄⁺ balances, which were however close to the margin of error. Ammonia production rates are somewhat lower if we consider assimilation as an additional sink for NH₄⁺. Ammonia assimilation rates have been reported on the order of ~50 nmol NH₄⁺ L⁻¹ d⁻¹ for the upper Peruvian OMZ (Lipschultz et al 1990). Nevertheless, this would still leave sufficient NH₄⁺ to fuel NH₃ oxidation and anammox at the reported rates.

Table 2 | Ammonia production and consumption near the upper oxycline in the OMZs off Namibia and Peru. For the sake of clarity, standard errors for the individual processes determined at each station are not listed here. Typically, the standard error was ~10% of the measured rate. Directly measured rates are underlined, the remainder have been inferred from idealized stoichiometries.

Station	Namibian OMZ		Peruvian OMZ						
	243	252	805	807	811	3	5	13	36
O ₂ [$\mu\text{mol L}^{-1}$]	7.59	1.11	7.46	~20	4.16	9.09	0.86	3.40	1.49
NH ₄ ⁺ [$\mu\text{mol L}^{-1}$]	0.00	0.12	0.27	0.58	0.05	1.27	0.07	0.10	0.05
Aerobic									
<u>NH₃ oxidation</u> ^{1,6}	21	93	89	49	13	60	5.8	14	35
<u>NO₂⁻ oxidation</u> ^{1,6}	74	112	38	928	70	35	32	29	186
<u>Total oxic respiration</u> ²	230	450	541	3136	605	1195	730	990	1060
Heterotrophic oxic respiration ^{2,4}	161	254	389	2599	552	1087	706	954	915
Anaerobic									
<u>NO₃⁻ reduction</u> ^{1,6}	17	370	18	1010	0.0	40	0.0	0.0	42
<u>DNRA</u> ^{1,6}	0.0	0.0	0.3	1.1	0.0	0.0	0.0	0.0	0.8
<u>Anammox</u> ^{1,6}	25	42	0.0	112	9.6	1.6	4.0	3.6	2.3
NH₄⁺ sinks									
<u>NH₃ oxidation</u> ³	-21	-93	-89	-49	-13	-60	-5.8	-14	-35
<u>Anammox</u> ³	-13	-21	0.0	-56	-4.8	-0.8	-2.0	-1.8	-1.2
NH₄⁺ sources									
Heterotrophic oxic respiration ^{3,5}	24	38	59	392	83	164	106	144	138
NO ₃ ⁻ reduction ^{3,5}	1.3	28	1.3	76	0.0	3.0	0.0	0.0	3.2
DNRA ^{3,5}	0.0	0.0	0.3	1.1	0.0	0.0	0.0	0.0	0.8
Net NH₄⁺ production ³	-7.9 (± 12)	-47 (± 30)	-29 (± 21)	365 (± 86)	66 (± 25)	106 (± 23)	99 (± 12)	128 (± 26)	106 (± 25)

¹ In nM N d⁻¹.

² In nM O₂ d⁻¹.

³ In nM NH₄⁺ d⁻¹.

⁴ Heterotrophic oxic respiration = Total oxic respiration – 1.5 x NH₃ oxidation – 0.5 x NO₂⁻ oxidation.

⁵ Heterotrophic oxic respiration: O₂/NH₄⁺ = 106/16; NO₃⁻ reduction: NO₃⁻/NH₄⁺ = 212/16; DNRA: NO₃⁻/NH₄⁺ = 53/59.

⁶ From Füssel et al. 2011, Kalvelage et al. 2011 and Chapter 3.

In summary, we show that microaerobic respiration is a widespread feature of the Namibian and Peruvian OMZ waters. The rapid consumption of O_2 in samples from the lower OMZ and the virtually anoxic OMZ core indicates that the microbial community inhabiting these zones is well adapted to exploit even traces of O_2 , which might diffuse or be mixed in from more oxygenated waters. Additional evidence is provided by an increasing relative abundance of cyt oxidases with a high O_2 affinity with depth in the Peruvian OMZ. At the base of the oxycline, where measurable concentrations of O_2 persist, aerobic respiration was several-fold higher. Here, most of the O_2 consumption could be attributed to heterotrophic microbial activity whereas the contribution of nitrification is presumably minor. Our estimates of aerobic organic matter degradation and thus NH_4^+ release in the upper OMZs reconcile measured rates of NH_3 oxidation and anammox with so far identified but insufficient anaerobic sources of NH_4^+ .

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Conclusions and outlook

Tropical OMZs amount to less than 1% of the ocean volume, but have a profound impact on the ocean's nutrient inventory as they account for ~30-50% of oceanic N-loss. The predicted expansion of OMZs as a result of global change may therefore significantly increase N-loss from the ocean. Furthermore, an enlargement of N-deficient waters would exacerbate N-limitation of phytoplankton and reduce biological C-sequestration in large parts of the tropical oceans.

During the last decade, anammox has been recognized as the major pathway for water column N-loss in OMZs. Anammox requires NH_4^+ and NO_2^- and is thus tightly linked to sinks and sources of both compounds. The regulatory mechanisms of their concurrent activities were so far poorly understood, impeding realistic model-based assessments of future ocean de-oxygenation.

The effects of O_2 on aerobic NH_3 oxidation, NO_3^- reduction to NO_2^- and anammox were explored in the Namibian and Peruvian OMZ. ^{15}N -labeling experiments targeting these processes were carried out at O_2 concentrations ranging from ~0 to $25 \mu\text{mol L}^{-1}$. Ammonia oxidation showed little to no response to decreasing O_2 concentrations while heterotrophic NO_3^- reduction to NO_2^- remained active up to at least $25 \mu\text{mol L}^{-1}$ of O_2 . In contrast, anammox activity was clearly reduced at higher O_2 levels and fully inhibited at $\sim 20 \mu\text{mol L}^{-1}$ of O_2 . Hence, N-loss appears to be directly regulated by the O_2 sensitivity of anammox itself and not indirectly by any O_2 effects on sources of NH_4^+ and NO_2^- . Current biogeochemical models allow for simultaneous aerobic and anaerobic activity in a narrow O_2 range ($\sim 4\text{-}8 \mu\text{mol L}^{-1}$). This study revealed a much broader O_2 window ($>0\text{-}20 \mu\text{mol L}^{-1}$) for concurrent aerobic and anaerobic N-cycling processes in OMZs.

Besides O_2 , export production was hypothesized to be a key-regulatory factor of NH_4^+ and NO_2^- sources and sinks, including N-loss via anammox. During a cruise to the ETSP, N-

cycling rates were determined in ^{15}N -labeling experiments in the eutrophic coastal and oligotrophic offshore OMZ waters between $\sim 3^\circ\text{S}$ and 18°S . Pronounced N-loss and offshore secondary NO_2^- maxima did not coincide. In contrast, anammox showed a strong correlation with modeled export production. High rates of organic matter remineralization and benthic NH_4^+ release fueled high anammox activity in the coastal OMZ. Advection of N-deficient shelf waters and sluggish ventilation are likely responsible for the large N-deficits offshore. While secondary NO_2^- maxima and N-deficits were poor indicators of active N-loss, export production was identified as a reliable predictor of N-loss in OMZs. Moreover, coastal waters and sediments, both of which are largely ignored in current global models and N-budget estimates, were shown to be crucial in OMZ C- and N-cycling.

In the ETSA and ETSP, the high NH_4^+ requirements of anammox and aerobic NH_3 oxidation near the upper OMZ boundary can not be supported by anaerobic organic matter remineralization alone. Regarding the persistence of aerobic nitrification in OMZs, microaerobic organic matter respiration was suggested as an important source of NH_4^+ . ^{18}O -labeling experiments revealed high O_2 consumption rates throughout the Namibian and Peruvian OMZ and could mainly be attributed to heterotrophic activity. Additional metagenomic analysis revealed abundant genes encoding for terminal respiratory oxidases with high O_2 affinities that could be assigned to marine nitrifiers as well as aerobic heterotrophs. Oxidic respiration in OMZs is likely sustained by regular intrusions of more oxygenated waters. In the upper OMZs, microaerobic respiration was the dominant remineralization pathway and sufficient to balance NH_4^+ consumption and production. Biogeochemical models, which exclusively couple organic matter remineralization to NO_3^- respiration below $\sim 4 \mu\text{mol L}^{-1}$ of O_2 , should take microaerobic respiration of organic matter into account.

In summary, this thesis provides detailed insights into the regulation of complex N-cycling in OMZs. The obtained results may help to develop reliable future OMZ scenarios.

Despite recent progress in understanding OMZ systems, significant gaps remain to be filled. Contrasting distributions of N-loss activity and N-deficits in the ETSP imply significant, but so far little investigated, offshore advection of shelf OMZ waters. N-deficits are commonly used as proxies to estimate global pelagic N-loss, but coastal OMZs are under-represented in global nutrient data sets (Gruber and Sarmiento 1997; Deutsch et al. 2001). In addition, N-deficit-based calculations of N-loss are critically dependent on nutrient residence times. However, water mass transport associated with major OMZs is not well characterized (Kessler 2006; Peña et al. 2010). With an improved understanding of OMZ circulation, pelagic N-loss estimates are expected to be revised. Further important considerations are coastal sedimentary N-loss and potentially N_2 fixation in OMZ waters, which leave negative and positive imprints, respectively, on OMZ N:P ratios (Fernandez et al. 2011; Bianchi et al. 2012).

Due to the tight coupling of anammox and export production, N-loss presumably not only depends on the quantity but also quality of organic matter. The composition of exported organic matter is largely determined by the phytoplankton community composition. While the N:P ratio of bloom-forming diatoms is well below Redfield (<10:1), many N_2 -fixing microorganisms exhibit particularly high N:P ratios (>30:1) (Arrigo 2005). Hence, the spatial dominance of either of these groups as well as temporal variability (seasonal and inter-annual) of the phytoplankton community might significantly change organic N and therefore NH_4^+ availability in OMZs. Ambient O_2 concentrations may further affect the availability of remineralized NH_4^+ , as preferential N-degradation of organic matter has been observed at O_2 concentrations below $\sim 5 \mu\text{mol L}^{-1}$ (Van Mooy et al. 2002). In-depth analyses of organic matter composition and remineralization at varying concentrations of O_2 in prospective OMZ studies would certainly provide additional insights into the regulation of oceanic N-loss.

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**Carbon, nitrogen and O₂ fluxes associated with the cyanobacterium
Nodularia spumigena in the Baltic Sea**

Ploug, Helle^{1,3*}, Birgit Adam², Niculina Musat², **Tim Kalvelage**², Gaute Lavik², Dieter Wolf-Gladrow³ and Marcel M.M. Kuypers²

¹Stockholm University, Stockholm, Sweden

²Max Planck Institute for Marine Microbiology, Bremen, Germany

³Alfred Wegener Institute for Marine and Polar Research, Bremerhaven, Germany

*Corresponding author. E-mail: helle.ploug@ecology.su.se

Published in: *The ISME Journal* (2011), 5: 1549-1558.

Keywords: stable isotopes, microsensors, N₂ fixation, photosynthesis, respiration, ammonium release.

Contribution: TK measured rates of ¹⁵NH₄⁺ release in ¹⁵N₂-labeling experiments.

Abstract

Photosynthesis, respiration, N₂ fixation and ammonium release were studied directly in *Nodularia spumigena* during a bloom in the Baltic Sea using a combination of microsensors, stable isotope tracer experiments combined with nanoscale secondary ion mass spectrometry (nanoSIMS) and fluorometry. Cell-specific net C- and N₂-fixation rates by *N. spumigena* were 81.6±6.7 and 11.4±0.9 fmol N per cell per h, respectively. During light, the net C:N fixation ratio was 8.0±0.8. During darkness, carbon fixation was not detectable, but N₂ fixation was 5.4±0.4 fmol N per cell per h. Net photosynthesis varied between 0.34 and 250 nmol O₂ h⁻¹ in colonies with diameters ranging between 0.13 and 5.0mm, and it reached the theoretical upper limit set by diffusion of dissolved inorganic carbon to colonies (41 mm). Dark respiration of the same colonies varied between 0.038 and 87 nmol O₂ h⁻¹, and it reached the limit set by O₂ diffusion from the surrounding water to colonies (41 mm). N₂ fixation associated with *N. spumigena* colonies (41mm) comprised on average 18% of the total N₂ fixation in the bulk water. Net NH₄⁺ release in colonies equaled 8–33% of the estimated gross N₂ fixation during photosynthesis. NH₄⁺ concentrations within light-exposed colonies, modeled from measured net NH₄⁺ release rates, were 60-fold higher than that of the bulk. Hence, *N. spumigena* colonies comprise highly productive microenvironments and an attractive NH₄⁺ microenvironment to be utilized by other (micro) organisms in the Baltic Sea where dissolved inorganic nitrogen is limiting growth.

Giant hydrogen sulfide plume in the oxygen minimum zone off Peru stimulates high chemoautotrophic carbon dioxide fixation

Schunck, Harald^{1§}, Gaute Lavik^{2§}, Dhvani K. Desai¹, Tobias Großkopf¹, **Tim Kalvelage**², Carolin Loescher³, Aurélien Paulmier^{2,4}, Marc Mußmann², Moritz Holtappels², Sergio Contreras², Herbert Siegel⁵, Philip Rosenstiel⁶, Markus B. Schilhabel⁶, Michelle Graco⁷, Ruth A. Schmitz³, Marcel M. M. Kuypers² and Julie LaRoche^{1,8*}

¹GEOMAR | Helmholtz Centre for Ocean Research Kiel, Germany

²Max Planck Institute for Marine Microbiology, Bremen, Germany

³Institute for General Microbiology, Kiel, Germany

⁴Laboratoire d'Etudes en Géophysique et Océanographie Spatiales, Toulouse, France

⁵Leibniz Institute for Baltic Sea Research Warnemünde, Rostock, Germany

⁶Institute of Clinical Molecular Biology, Kiel, Germany

⁷Instituto del Mar Perú, Callao, Peru

⁸Dalhousie University, Halifax, Canada

§these authors contributed equally to the work

*Corresponding author. E-mail: jlaroche@geomar.de

Submitted to: *PLoS Biology*

Keywords: oxygen minimum zone, oxygen-depletion, upwelling, eastern boundary upwelling system, Peru, hydrogen sulfide, sulfidic, sulfur oxidation, sulfate reduction, metagenomic, metatranscriptomic, fluorescence in situ hybridization, chemolithoautotroph, chemoautotroph, sulfur-driven autotrophic denitrification, carbon fixation, nitrogen-loss, anammox, denitrification, SUP05.

Contribution: TK aided in nutrient sampling and carrying out ¹⁵N-labeling experiments on board R/V Meteor, measured production rates of various ¹⁵N-species and provided a figure.

Abstract

In Eastern Boundary Upwelling Systems nutrient-rich waters are transported to the ocean surface, fuelling high photoautotrophic primary production. The subsequent heterotrophic decomposition of the produced biomass leads to oxygen-depletion at intermediate water depths, resulting in the formation of oxygen minimum zones (OMZs). These OMZs can sporadically accumulate substantial amounts of hydrogen sulfide, which is toxic to multicellular organisms and have been evoked for massive fish kills.

During a cruise to the OMZ off Peru we found a >8000 square kilometer covering sulfidic plume in shelf waters, which contained $\sim 3.5 \times 10^4$ tons of hydrogen sulfide. To our knowledge, this is the first time that hydrogen sulfide was measured in the Peruvian OMZ and the largest plume ever reported for ocean waters. To assess the phylogenetic and functional diversity of the inhabiting microbial community, we applied high-throughput sequencing of community DNA and RNA, and analyzed the sequence information in the context of group specific microbial cell counts, as well as of rate measurements of carbon dioxide fixation and nitrogen transformation processes. Some of the micro-organisms previously detected in high abundances in oxygen minimum zone waters were very scarce. Instead, the waters were dominated by several distinct α -, γ -, δ - and ϵ -proteobacterial taxa associated with either sulfur oxidation or sulfate reduction. Our combined results indicated these chemolithoautotrophic bacteria utilized several oxidants (oxygen, nitrate, nitrite, nitrous oxide and nitric oxide) to detoxify the waters well below the oxic surface. The chemolithoautotrophic activity led to high dark inorganic carbon fixation, representing $\sim 30\%$ of the photoautotrophic carbon fixation.

Postulated changes such as eutrophication and global warming, which may lead to an expansion and intensification of oxygen-depletion, might also increase the frequency of

sulfidic waters. The chemoautotrophically fixed carbon could fuel further sulfate reduction and thus potentially stabilize the sulfidic OMZ waters.

Nitrogen isotope effects by anammox (*K. stuttgartiensis*)

Brunner, Benjamin^{1*}, Moritz F. Lehmann², Sergio Contreras^{1,§}, Olga Matantseva¹, Mark Rollog², Tim Kalvelage¹, Gabi Klockgether¹, Gaute Lavik¹, Mike S.M. Jetten³, Boran Kartal³ and Marcel M.M. Kuypers¹

¹Max Planck Institute for Marine Microbiology, Bremen, Germany

²Institute for Environmental Geoscience, Basel, Switzerland

³Institute of Water and Wetland Research, Nijmegen, The Netherlands

[§]Current address: Large Lakes Observatory, University of Minnesota Duluth, USA

*Corresponding author. E-mail: bbrunner@mpi-bremen.de

In preparation for: *PNAS*

Keywords: anammox, *Kuenenia stuttgartiensis*, isotope fractionation.

Contribution: TK together with SC carried out culture experiments to determine natural abundance N isotope effects of anammox bacteria.

Abstract

Anaerobic ammonium oxidation (anammox) converts ammonium and nitrite into dinitrogen gas (N_2) and nitrate. This process has been identified as important fixed nitrogen (N) sink in the world's oceans. Here, we studied for the first time the N isotope signature of anammox in batch culture experiments. Anammox preferentially removes light N from the ammonium pool ($\epsilon_{NH_4 \rightarrow N_2} \sim +30\text{‰}$). For the conversion of nitrite to N_2 and nitrate, the isotope fractionation is more intricate. Anammox activity can induce N isotope exchange between nitrate and nitrite, leading to the superposition of isotope exchange effects upon kinetic N isotope fractionation. In our experiments, N isotope effects result from: i) inverse kinetic N isotope fractionation associated with the conversion of nitrite to nitrate ($\epsilon_{NO_2 \rightarrow NO_3} \sim -30\text{‰}$), ii) normal kinetic N isotope fractionation during the conversion of nitrite to N_2 ($\epsilon_{NO_2 \rightarrow N_2} \sim +15\text{‰}$), and iii) from an equilibrium isotope effect between nitrate and nitrite ($\epsilon_{NO_2 \leftrightarrow NO_3} \sim -60\text{‰}$). Our findings imply that in the ocean, anammox may be responsible for thus far unresolved large N isotopic offsets between nitrate and nitrite in oxygen minimum zones (OMZs). Irrespective of the extent of N isotope exchange between nitrate and nitrite, N removed from the combined nitrite and nitrate (NO_x) pool is depleted in the heavy isotope ^{15}N relative to NO_x , as is the case for canonical denitrification. The isotope effect for loss of fixed N (NO_x and ammonium) from OMZs is likely smaller than $+25\text{‰}$ commonly attributed to water column denitrification.



Acknowledgements

This thesis would not have been realized without support and encouragement of a great number of colleagues, friends and of course my family.

Marcel, I would like to thank you, for convincing me to work on this exciting project, for giving me a lot of freedom throughout my PhD, yet enough guidance and critical feedback. I admire your seemingly unshakable scientific enthusiasm, which helped me to keep going at times when I had enough of all those Exetainers.

Gaute, in the end you were right, it was worth it to fill them by the thousands. I am grateful for your introduction to the secret world of labeling experiments, for having been on an unparalleled cruise with you, for your ever-open office door, your open-mindedness and for good times outside office hours.

Inka, I am deeply indebted to you. Without you I would still be sitting in front of that machine. The success of this thesis is to a great part based on your excellent job. Good luck on your own scientific path!

Sergio, you not only killed most of the bugs for me but you have enriched my time at the MPI in so many ways. Thanks, for being a good friend and my companion on numerous trips.

Gabi, thanks for all the technical support and our afternoon chats. I hope we will have a chance to go at sea again together next year.

Phyllis, you are definitely an admirable scientist and I am grateful for all your efforts on helping me to improve my manuscripts.

Gail, Rachel and Tim, I would also like to thank you for valuable feedback.

Daniela, Sandra, Ulrike, Andreas, Bernd, Michael, Thomas und Volker, without your administrative, logistical and technical support all my expeditions and international travels would not have been possible.

Hermann, I am grateful that you offered to review my thesis on very short notice. Particularly, since you have been chief scientist on my first research cruise, after which I was determined to pursue a marine scientific career.

Julie, since my early days at the GEOMAR you have constantly accompanied my scientific development. Thanks for your advice and support throughout the years.

Jassi, Paddy, Leni, Caro and Harald, thanks for fruitful collaborations and a lot of fun during our cruises and all those SFB meetings.

MarMic C11, I am glad to have met each of you and I very much enjoyed the time we spent together during the last years.

Hannah, Morten and Renzo, the same is true for you.

Shalin and Petra, thanks for having provided a second home for me.

Jessika, your unconditional support, especially during the final weeks of this thesis, means a lot to me. Without you I would not have been where I am now.

Finally, I would like to thank my family for being there any time.

