Extreme N$_2$O accumulation in the coastal oxygen minimum zone off Peru

Annette Kock$^1$, Damian L. Arévalo-Martínez$^1$, Carolin R. Löscher$^2$, Hermann W. Bange$^1$

$^1$GEOMAR Helmholtz Centre for Ocean Research Kiel, Duesternbrooker Weg 20, 24105 Kiel, Germany

$^2$Institute of General Microbiology, Christian-Albrechts University Kiel, Am Botanischen Garten 1-9, 24118 Kiel, Germany

Correspondence to: Annette Kock, akock@geomar.de
Abstract

Depth profiles of nitrous oxide (N\textsubscript{2}O) were measured during six cruises to the upwelling area and oxygen minimum zone (OMZ) off Peru in 2009 and 2012/2013, covering both the coastal shelf region and the adjacent open ocean. N\textsubscript{2}O profiles displayed a strong sensitivity towards oxygen concentrations. Open ocean profiles showed a transition from a broad maximum to a double-peak structure towards the centre of the OMZ where the oxygen minimum was more pronounced. Maximum N\textsubscript{2}O concentrations in the open ocean were about 80 nM. A linear relationship between ΔN\textsubscript{2}O and apparent oxygen utilization (AOU) could be found for all measurements within the upper oxycline, with a slope similar to studies in other oceanic regions. N\textsubscript{2}O profiles close to the shelf revealed a much higher variability, with N\textsubscript{2}O concentrations in the upper oxycline reaching up to several hundred nanomoles per liter at selected stations. Due to the extremely sharp oxygen gradients at the shelf, these maxima occurred in very shallow water depths of less than 50 m. In the coastal area, a linear relationship between ΔN\textsubscript{2}O and AOU could not be observed as extremely high ΔN\textsubscript{2}O values were scattered over the full range of oxygen concentrations. Our results indicate that the coastal upwelling off Peru at the shelf causes conditions that lead to extreme N\textsubscript{2}O accumulation, most likely due to the interplay of high rates of nitrogen cycling and a rapid switching of the OMZ waters from anoxic to oxic conditions as a result from coastal upwelling and subsequent strong remineralization in the water column along the Peruvian coast.
Nitrous oxide ($N_2O$) acts as a strong atmospheric greenhouse gas and contributes substantially to the stratospheric ozone depletion (IPCC, 2013; WMO, 2011). The ocean is a major source for $N_2O$ as it is naturally produced in the water column (Ciais et al., 2013; Bange, 2008). While in large parts of the surface ocean $N_2O$ concentrations are close to saturation, high emissions of $N_2O$ have been observed in upwelling areas where subsurface waters enriched in $N_2O$ are transported to the surface (e.g. Nevison et al. (2004)). The global distribution of $N_2O$ in the ocean is closely linked to the oceanic oxygen distribution, and particularly high supersaturations are found in upwelling areas which overlay pronounced oxygen minimum zones (OMZ), e.g. in the Arabian Sea (Bange, 2004) or in the eastern South Pacific Ocean (Charpentier et al., 2010).

These OMZs are key regions for the marine nitrogen (N) cycling where active N loss via canonical denitrification and anaerobic ammonium oxidation (anammox) takes place. Recent studies furthermore indicate that they are also zones of intense nitrogen fixation (Deutsch et al., 2007; Loescher et al., 2014; Fernandez et al., 2011), and in areas where the OMZ is fuelled by high export production, high rates of other N transformation processes, such as nitrification, have been observed (Hu et al., 2015; Kalvelage et al., 2013).

Within the nitrogen cycle, $N_2O$ evolves during nitrification and denitrification (Bange, 2008). Both processes strongly depend on the oxygen availability in the water column, with different responses to the oxygen concentration. Under oxic conditions the first step of nitrification, ammonium-oxidation to nitrite, is known to be the main production pathway for $N_2O$, with an increasing $N_2O$ yield at decreasing oxygen concentrations (Goreau et al., 1980; Löscher et al., 2012; Frame and Casciotti, 2010). During bacterial ammonium-oxidation, $N_2O$ can either be produced as a side product during the oxidation of ammonia to nitrite or through the reduction of nitrite to $N_2O$ (nitrifier-denitrification) (Stein, 2011). Nitrifier-denitrification has been identified as an important production pathway of $N_2O$ at low oxygen concentrations and may thus be responsible for the increased $N_2O$ production under these conditions (Ni et al., 2014).
While the N\(_2\)O production pathways during bacterial nitrification have been studied for several decades, archaeal ammonium oxidation has only recently come into focus as a main production pathway for N\(_2\)O. The exact mechanism and the extent to which ammonium oxidation or a nitrifier-denitrification pathway are responsible for archaeal N\(_2\)O production as well as the effect of environmental controls on archaeal N\(_2\)O production are subject to ongoing research (Stieglmeier et al., 2014; Löscher et al., 2012; Santoro et al., 2011).

During denitrification, the canonical reduction of nitrate to molecular nitrogen, N\(_2\)O evolves as an intermediate product. Denitrifying genes are widespread among different groups of microorganisms, but active denitrification is restricted to suboxic to anoxic conditions (e.g. Firestone et al., 1980; Dalsgaard et al., 2014). Denitrification is a heterotrophic process that is stimulated by the supply of organic carbon or hydrogen sulfide (Chang et al., 2014; Dalsgaard et al., 2014; Galan et al., 2014). Depending on the environmental conditions, N\(_2\)O production or consumption due to denitrification can be observed in environmental samples. There has been evidence that N\(_2\)O consumption is more sensitive to trace amounts of oxygen than N\(_2\)O production. This could lead to N\(_2\)O accumulation when oxygen is present in low concentrations (Tiedje, 1988). Exceptionally high N\(_2\)O concentrations have been measured off the West Indian Coast where anoxic waters from the Arabian Sea frequently extend over the shelf (Naqvi et al., 2000). These are the highest marine N\(_2\)O concentrations reported so far and were associated with an increased N\(_2\)O production from denitrification during transient oxygen concentrations. In a recent study it was furthermore shown that N\(_2\)O production from denitrification could be stimulated by H\(_2\)S addition (Dalsgaard et al., 2014) which could indicate a coupling between N\(_2\)O production and sulfur cycling.

Measurements of denitrification and anammox rates in different oceanic OMZs have raised the question whether denitrification or anammox is the main pathway for nitrogen loss in the water column (Hamersley et al., 2007; Ward et al., 2009; Voss and Montoya, 2009). In the ETSP, anammox has been found to play the major role in N loss, whereas denitrification was only rarely detectable (Kalvelage et al., 2013; Hamersley et al., 2007) (Thamdrup et al., 2006). As N\(_2\)O is not supposed to be involved in the anammox process (Kartal et al., 2011),
anammox does not influence the N$_2$O distribution and only denitrification is thought to be responsible for N$_2$O consumption at suboxic to anoxic conditions (Bange, 2008). The widespread N$_2$O consumption in the OMZ core is thus an indicator for denitrification taking place in the ETSP (Farias et al., 2007). One explanation for these contradicting findings is that denitrification strongly depends on the supply of organic carbon or hydrogen sulfide (Chang et al., 2014; Dalsgaard et al., 2014; Galan et al., 2014).

As nitrification is one major process accompanying the remineralization of organic matter, a positive correlation between the excess N$_2$O ($\Delta$N$_2$O) and the apparent oxygen utilization (AOU) is often interpreted as an indication for nitrification as the main N$_2$O production pathway (e.g. Walter et al. (2006), Forster et al. (2009)). An increase in the $\Delta$N$_2$O/AOU ratio at low oxygen concentrations has been observed in several studies in different oceanic areas with reduced oxygen concentrations (Ryabenko et al., 2012; Upstill-Goddard et al., 1999; De Wilde and Helder, 1997), whereas a breakdown of this relationship due to N$_2$O consumption is observed when oxygen concentrations fall below a certain, not well defined, threshold (Zamora et al., 2012).

There is a strong indication that at low oxygen concentrations nitrification and denitrification may take place in close proximity (Kalvelage et al., 2011), and the N$_2$O production and consumption under these conditions are strongly influenced by the interaction of both processes. Stable isotope and consumption rate measurements of N$_2$O in oxygen-deficient waters indicated that N$_2$O accumulates within the oxycline as a result of the coupling between nitrification and denitrification whereas N$_2$O consumption in the OMZ core was associated with denitrification (Farias et al., 2007; Babbin et al., 2015). The exact oxygen concentration where N$_2$O consumption starts is not yet well determined, however (Cornejo and Farias, 2012; Zamora et al., 2012).

Measurements of N$_2$O consumption rates in the eastern tropical North Pacific (ETNP) Ocean furthermore provided evidence for a rapid N$_2$O cycling within the ETNP, although depth profiles of N$_2$O seemed to be relatively invariant over time (Babbin et al., 2015). These quasi-stable conditions may be disturbed by rapid changes in the environmental conditions.
Although the waters off Peru harbor one of the most prominent OMZs in the world, only a few measurements of N\textsubscript{2}O are available so far (Friederich et al., 1985; Nevison et al., 1995; Pierotti and Rasmussen, 1980). N\textsubscript{2}O measurements from the OMZ off Chile indicated the potential for high N\textsubscript{2}O production and emissions due to the proximity of the OMZ to coastal upwelling taking place in this area (Charpentier et al., 2007; Castro-Gonzalez and Farias, 2004). Here we present N\textsubscript{2}O measurements in the water column off Peru from six measurement campaigns in the ETSP. This upwelling area is one of the four major eastern boundary upwelling systems (EBUS) where alongshore trade winds induce westward transport of the surface water masses which leads to strong coastal upwelling (Chavez and Messié, 2009). The ETSP is characterized by one of the largest and most intense OMZs in the oceans, extending from the Peruvian shelf about 1000 km offshore with a maximum thickness of more than 600 m (Fuenzalida et al., 2009). It is located in the shadow zone of large ocean current systems which leads to a sluggish ventilation and long residence times of waters within the OMZ. To the North of the OMZ, equatorial current bands such as the Equatorial Undercurrent (EUC) and the Southern Subsurface Countercurrents (SSCC) supply waters to the ETSP which leads to slightly higher oxygen concentrations in the northern OMZ compared to the OMZ core (Stramma et al., 2010). The equatorial current bands also feed the poleward Peru-Chile Undercurrent (PCUC) which is the main source for waters upwelled along the coast where high primary production and high remineralization rates in the underlying waters lead to a further drawdown in oxygen concentrations (Karstensen et al., 2008). Active N loss can be observed in large parts of the OMZ which is reflected in a pronounced secondary nitrite maximum and a strong nitrogen deficit in the OMZ core (Codispoti et al., 1986). The OMZ extends over large parts of the Peruvian shelf where sulfidic conditions within the water column are frequently observed (Schunck et al., 2013). While year-round upwelling can be observed along the Peruvian coast, the region is strongly influenced by the El Niño Southern Oscillation (ENSO) leading to large interannual variability in the upwelling intensity which could lead to the interruption of the upwelling during El Niño events (Dewitte et al., 2012). While the OMZ core is largely unaffected by ENSO, a deepening of the upper oxycline and the re-oxygenation of the Peruvian shelf due to the propagation of coastal trapped waves can be observed (Gutierrez et al., 2008).
2 Methods

In total, 146 depth profiles (0-~4200 m) of N\textsubscript{2}O were measured on two cruises between December 2008 and February 2009 (M77-3 & M77-4) and four cruises between October 2012 and March 2013 (M90 - M93) to the upwelling area and the adjacent open ocean off Peru onboard the German research vessel Meteor. The Southern Oscillation Indices (http://www.ncdc.noaa.gov/teleconnections/enso/indicators/soi/) from 2008/2009 and 2012/2013 did not indicate the presence of an El Niño event during our measurement campaigns, and similar conditions between both measurement campaigns could be expected. The locations of the sampled stations are shown in Fig. 1. While the M77-4 and M90 cruises mainly covered the open ocean area, the M77-3 and M91-M93 cruises mainly took place in the Peruvian shelf area. The work was part of the German DFG collaborative research project (SFB) 754 (https://www.sfb754.de/) and the BMBF project SOPRAN (Surface Ocean PRocesses in the Antropocene, www.sopran.pangaea.de). The N\textsubscript{2}O data set described here has been archived in MEMENTO, the MarinE MethanE and NiTrous Oxide database (https://memento.geomar.de) (Kock and Bange, 2015).

Triplicate samples were taken from 10 L Niskin bottles mounted on a rosette water sampler or a pump-CTD (M77-3) in 25 ± 0.11 mL (M77-3 & M77-4) and 20 ± 0.14 mL (M90 - M93) opaque glass vials and sealed with butyl rubber stoppers and aluminum caps, thereby avoiding the inclusion of air bubbles.

Samples were treated with 0.2 mL (M77-3 & M77-4) and 0.05 mL (M90 - M93) of a saturated mercuric chloride solution directly after the sampling to inhibit microbial N\textsubscript{2}O production or consumption. The samples were either analyzed onboard (M77-3 & M77-4, M91, partly M90 & M93) within a few days or shipped to GEOMAR by air freight for later analysis (M92, partly M90 & M93). Samples that were shipped to Germany were additionally sealed with paraffin wax and stored upside down to avoid the formation of air bubbles in the samples due to temperature and pressure changes during transportation.

Samples were analyzed using a static equilibration method: 10 mL helium (99.9999% AirLiquide, Düsseldorf, Germany) was manually injected into each vial which was equipped with a second syringe to collect the
overflowing water. Vials with added headspace were vigorously shaken for about 20 s and allowed to equilibrate at ambient temperature for a minimum of two hours. A subsample of the equilibrated headspace was manually injected into a GC-ECD system (Hewlett-Packard 5890 Series II, Agilent Technologies, Santa Clara, CA, USA), equipped with a 6’ 1/8” packed column (molsieve, 5Å, W. R. Grace & Co.-Conn., Columbia, MY). The GC was operated at 190 °C, using argon/methane (95%/5%, ECD purity, AirLiquide, Düsseldorf, Germany) as carrier gas at a flow rate of 30 mL min⁻¹.

The GC was calibrated on a daily basis with a minimum of 2 (M77-3 & M77-4) or 4 (M90 - M93) different standard gas mixtures (N₂O in synthetic air, Deuste-Steininger GmbH, Mühlhausen, Germany and Westfalen AG, Münster, Germany). Standard gases were either injected as pure gas or further diluted with helium (1:3, 1:1 or 3:1) to obtain additional standard gas concentrations. Pure standard gases were calibrated against NOAA primary standards at the Max Planck Institute for Biogeochemistry in Jena, Germany, if the standard gas concentrations were within the calibration range of the NOAA gases. Gases with N₂O concentrations outside the NOAA calibration range were internally calibrated using an LGR N₂O/CO analyzer (Los Gatos Research, Mountain View, CA, USA), which was proven to have a linear response and minimal drift within the calibration range (Arevalo-Martinez et al., 2013). The N₂O concentration in the samples was calculated according to Walter et al. (2006) using the solubility function of Weiss and Price (1980). The average precision of the measurements, calculated as median standard deviation from triplicate measurements, was 0.7 nM.

ΔN₂O was calculated as the difference between the in-situ concentration [N₂O]ₜ and the equilibrium concentration [N₂O]ₜₑq:

\[ ΔN₂O = [N₂O]ₜ - [N₂O]ₜₑq \]  (1)

We used the contemporary atmospheric mixing ratio measured at Cape Grim, Tasmania (http://agage.mit.edu/data/agage-data) for the calculation of [N₂O]ₜₑq. This calculation underestimates the N₂O excess in subsurface waters which have been isolated from the surface for a long time as it does not account
for the increase in the atmospheric mixing ratio since the beginning of the industrial revolution (Freing et al., 2009). The use of the contemporary N₂O mixing ratio of 2013 would lead to a maximum ~17% overestimate of \([N_2O]_{eq}\), thus leading to only a small error compared to the maximum N₂O concentrations measured in our study, and the use of the contemporary atmospheric mixing ratio still allows a qualitative analysis of the ΔN₂O/AOU relationship in order to investigate the formation and consumption processes of N₂O.

The potential temperature of the water parcel at a certain depth was calculated using the Gibbs Seawater Oceanographic Toolbox (McDougall and Barker, 2011).

Oxygen concentrations were measured either with a Seabird (M77-3 & M77-4: SBE-5; M90-M93: SBE 43) oxygen sensor (Sea-Bird Electronics, Bellevue, WA, USA) mounted on the CTD rosette or from 100 mL discrete samples taken from the Niskin bottles and analyzed using the Winkler titration method (Grasshoff et al., 1999). The oxygen sensor was calibrated against the Winkler measurements.

Recent studies using highly sensitive STOX (Switchable Trace amount Oxygen) sensors for oxygen measurements indicate that measurements with conventional oxygen sensors that are calibrated against Winkler measurements may be biased towards higher concentrations at near-zero oxygen conditions. Thamdrup et al. (Thamdrup et al., 2012) therefore argued that anoxic conditions are prevalent in the core of the Peruvian OMZ where oxygen concentrations of several µM have been found using the conventional Winkler-calibrated measurements. As STOX sensor measurements were not available for all measurement campaigns presented here, the minimum oxygen measurements reported here from the core of the OMZ (3-5 µM) should be considered as an overestimation.

The Apparent Oxygen Utilization (AOU) was calculated from the oxygen concentrations \([O_2]_w\) using the CSIRO SeaWater library, version 3.2 (http://www.cmar.csiro.au/datacentre/ext_docs/seawater.htm) to calculate oxygen saturation \([O_2]_{eq}\):

\[
AOU = [O_2]_w - [O_2]_{eq}
\] (2)
Nutrient samples from the CTD rosette were analyzed onboard following the nutrient analysis methods according to Hansen et al. (1999). Samples taken from the pump-CTD during M77-3 were stored at -20°C and shipped to Germany for later analysis. $N'$ was calculated as a measure for the nitrogen deficit from the nitrate ($\left[\text{NO}_3^-\right]$), nitrite ($\left[\text{NO}_2^-\right]$) and phosphate ($\left[\text{PO}_4^{3-}\right]$) concentrations as follows (Altabet et al., 2012):

$$N' = \left(\left[\text{NO}_3^-\right] + \left[\text{NO}_2^-\right]\right) - 16\left[\text{PO}_4^{3-}\right]$$ (3)

To distinguish between coastal and open ocean stations we calculated the distance of each station from the continental slope (2000 m isobath) and used the first baroclinic Rossby radius of deformation as described by Chelton et al. (Chelton et al., 1998) as threshold distance for stations that were influenced by coastal upwelling.
Results and Discussion

The oxygen profiles revealed an intense oxygen minimum zone throughout the studied area, with a vertical thickness of several hundreds of meters. In the open ocean, the oxygen concentrations in the core of the OMZ increased towards the north from below 3 µM south of 5 °S to ~10 µM at the equator. South of 13°S the mixed layer depth significantly increased from ~50 m to ~100 m, which is reflected in the oxygen and N₂O distributions (Fig. 2). Due to the coastal upwelling, the depth of the upper OMZ boundary significantly decreased towards the coast, with a well oxygenated mixed layer of ~50 m in the open ocean and a mixed layer depth of less than 5 m on the shelf. Oxygen was strongly undersaturated in the surface waters on the shelf as a result of upwelling of waters from the underlying OMZ. Elevated phosphate concentrations in the surface waters at the coast also reflected the upwelling on the shelf (Fig. 3).

The vertical profiles showed characteristic nutrient distributions that marked the zones of nitrogen depletion: accumulation of nitrite was observed in the core of the OMZ where oxygen concentrations fell below ~5 µM and low N' values coincided with the nitrite maxima in the OMZ. The maximum nitrite concentration reached ~13 µM, with a more pronounced maximum at the shelf than in open ocean waters. Additionally, many profiles showed an additional, less pronounced primary nitrite maximum within the upper oxycline that is associated with nitrification (Codispoti and Christensen, 1985) (Fig. 4). At the shelf, strong signals of N loss throughout the water column are reflected in more negative N' and low nitrate values at the coast (Fig. 3).

The water mass distribution in our dataset agrees well with the data presented by Pietri et al. (2014) (Fig. 5). Due to the larger area covered by our measurements our data showed a broader scattering, but we could identify the same water masses in our data: below 500 m, both the coastal and the offshore profiles carry fresh (S~34.8) and cool (T_{pot}~5°C) Antarctic Intermediate Water (AAIW) (Pietri et al., 2014), while shallower subthermocline waters are covered by the Equatorial Subsurface Water (ESSW). Waters with low salinities (~34.6) and potential temperatures between 10°C and 15°C in the offshore waters can be traced back to Eastern South Pacific Intermediate Water (ESPIW) (Schneider et al., 2003). This water mass can hardly be
identified in the coastal data. Cold Coastal Water (CCW) with $S \sim 15$ and $T_{\text{pot}} \sim 17^\circ\text{C}$ could only be identified in the coastal data as it is directly related to the coastal upwelling, whereas the offshore surface data were associated with Subtropical Surface Water (STSW) (Pietri et al., 2013). The $\text{N}_2\text{O}$ depth distribution showed a strong sensitivity to oxygen concentrations throughout the study area, and strong differences between coastal and offshore stations could be observed. In the offshore waters, surface $\text{N}_2\text{O}$ concentrations were close to saturation, with a strong increase below the mixed layer (Fig. 2). Two types of depth profiles could be identified, reflecting marginal differences in the minimum oxygen concentrations observed in the OMZ: a broad $\text{N}_2\text{O}$ maximum at the depth of the oxygen minimum was found at the northern and southern periphery of the oxygen minimum zone where the minimum oxygen concentrations did not fall below 5 µM. In contrast, $\text{N}_2\text{O}$ depletion was found in the core of the OMZ, where oxygen concentrations below 5 µM were observed over a wide depth range. The $\text{N}_2\text{O}$ depth profiles in the central OMZ thus revealed a double-peak structure with narrow $\text{N}_2\text{O}$ maxima in the upper and lower oxycline (Fig. 4). This depth profile structure has been frequently observed in other oceanic areas with highly depleted oxygen concentrations (e.g. (Bange et al., 2010)). $\text{N}_2\text{O}$ depletion coincided with nitrite accumulation in the OMZ core and high nitrate to phosphate ratios. In all offshore profiles $\text{N}_2\text{O}$ concentrations did not exceed 80 nM.

A bilinear $\Delta\text{N}_2\text{O}/\text{AOU}$ relationship has been identified in the upper oxycline for waters with oxygen concentrations higher than 5 µM during the M77-4 cruise that took place in the offshore waters of the OMZ (Ryabenko et al., 2012). We found a very similar relationship for all offshore data with no systematic difference between the data from the M77-4 (January/February 2009) cruise and the M90 (November 2012) cruise (Figures 2, 6a). This indicates a comparable setting of the open ocean OMZ waters during both cruises. We furthermore found no difference in the $\Delta\text{N}_2\text{O}/\text{AOU}$ relationship between stations with a broad $\text{N}_2\text{O}$ maximum and a double-peak structure. These results are similar to previously reported $\Delta\text{N}_2\text{O}/\text{AOU}$ relationships from other oceanic OMZs (Upstill-Goddard et al., 1999; Cohen and Gordon, 1978; De Wilde and Helder, 1997). The $\Delta\text{N}_2\text{O}$ distribution in the TS-diagram (Fig. 5) showed that elevated $\Delta\text{N}_2\text{O}$ values were mainly associated with
AAIW and ESSW, while surface waters were close to saturation. Compared to the offshore waters, the $N_2O$ distribution on the shelf and in the adjacent deep waters showed a much larger variability. $N_2O$ depletion was in fact observed at oxygen concentrations below 5 µM, too. While several $N_2O$ profiles revealed a shape similar to the offshore profiles, an overall characteristic shape of the profiles could not be identified, however: profiles with a subsurface $N_2O$ maximum in the oxycline were observed as well as profiles with multiple maxima or a surface $N_2O$ maximum (Fig. 4). $N_2O$ accumulation with concentrations which strongly exceeded the maximum offshore $N_2O$ concentrations of 80 nM was frequently observed. Several profiles showed an extreme $N_2O$ accumulation with concentrations up to ~850 nM (Fig. 4). The location and shape of the $N_2O$ maxima in the different profiles was highly variable, which resulted in a very patchy distribution of $N_2O$ in the water column over the shelf and in the adjacent waters (Fig. 3). This is also reflected in the TS-diagram of the coastal data (Fig. 5), which showed that elevated $\Delta N_2O$ values were associated with CCW, but displayed a large variability within this water mass. High resolution measurements of surface $N_2O$ during M90, M91 and M93 also revealed a very heterogeneous surface $N_2O$ distribution with remarkably high concentrations of $N_2O$ in vicinity of the main upwelling cells off Peru (Arevalo-Martinez et al., 2015).

In contrast to the open ocean waters, a correlation between $\Delta N_2O$ and AOU was not observed for the coastal data (Fig. 6b). The $\Delta N_2O$/AOU ratio from the offshore waters serves as a lower limit for the coastal stations, where numerous values with much higher $\Delta N_2O$/AOU ratios were observed. These data were highly scattered over the full range of oxygen concentrations, indicating that for the coastal stations the $N_2O$ maxima were not associated with suboxic conditions as observed offshore. The $\Delta N_2O$ values that showed the strongest deviation from the offshore $\Delta N_2O$/AOU ratio were associated with highly negative $N'$ values as a signal for a large nitrogen deficit (Fig. 6b). This indicates that these waters with extreme $N_2O$ accumulation had been subject to extensive N loss.
Extreme accumulation of N$_2$O with concentrations up to 765 nM in the oceanic water column has also been found in the Arabian Sea (Naqvi et al., 2010; Naqvi et al., 2006) and at a time series station off Chile (Farias et al., 2015), where maximum concentrations of ~500 nM were found. Naqvi et al. (2000) explained the extreme N$_2$O accumulation over the Indian shelf with the response of denitrifying enzymes to transient oxygen depletion. N$_2$O thus accumulated when waters reached suboxic conditions. N$_2$O accumulation coincided with the accumulation of nitrite and consumption of N$_2$O started when these waters became sulfidic (Naqvi et al., 2010). Farias et al. (2015) measured N$_2$O accumulation during the transition from oxic to anoxic conditions, too, but at variable oxygen concentrations whereas N$_2$O depletion became dominant under suboxic conditions. In contrast to the results from the Indian Ocean, they identified enhanced remineralization due to short-term variability in coastal upwelling as the main driver for N$_2$O accumulation.

In our study, N$_2$O accumulation did not coincide with the accumulation of nitrite. N$_2$O was generally depleted in samples that showed marked nitrite accumulation (Fig. 4) and similar to the measurements off Chile, we found strongly elevated N$_2$O concentrations (>100 nM) over the full range of oxygen concentrations (Fig. 5), whereas N$_2$O accumulation on the Indian shelf was restricted to suboxic conditions.

The high oxygen concentrations found in the majority of our samples with extreme N$_2$O accumulation excludes in-situ denitrification or anammox (see e.g. Babbin et al. (2014), Dalsgaard et al. (2014)). The extraordinarily high N$_2$O concentrations as well as the low N' values thus have to be old signals of processes taking place under anoxic to suboxic conditions. There is no known consumption process for N$_2$O in oxygenated waters (Bange, 2008), and the strong signals of N loss that are produced under anoxic conditions are unlikely to be rapidly compensated by N fixation upon oxygenation. Both signals thus are likely to have remained preserved when oxygen concentrations increased due to (diapycnal or isopycnal) mixing with waters of higher oxygen concentration or due to direct contact with the atmosphere as a result of upwelling. Our observations of high N$_2$O concentrations in oxygenated waters furthermore indicate that this accumulation could have taken place during re-oxygenation rather than during decreasing oxygen concentrations. An increase in oxygen
concentrations would lead to the preservation of the high N\(_2\)O signals in the water column whereas further decreasing oxygen concentrations would only lead to a temporal N\(_2\)O accumulation and would eventually stimulate N\(_2\)O consumption.

Enhanced production of N\(_2\)O after transition from anoxic to oxic conditions is a known process occurring in soils (e.g. Morley et al. (2008)) and may be explained by different sensitivity of denitrifying enzymes to trace concentrations of oxygen (Tiedje, 1988). In a recent incubation study, Dalsgaard et al. (2014) found no indication for an increased N\(_2\)O production by denitrification due to changes in the oxygen concentration at nanomolar levels, however. Instead, autotrophic denitrification and N\(_2\)O production have been shown to be stimulated by the addition of hydrogen sulfide (H\(_2\)S) (Galan et al., 2014; Dalsgaard et al., 2014). We did not find direct evidence for a coupling between N\(_2\)O production and the presence of H\(_2\)S in our measurements, as high N\(_2\)O accumulation was often found in proximity to H\(_2\)S plumes but was also detected when H\(_2\)S was absent in the water column. We cannot exclude that the high N\(_2\)O production we frequently observed at the shelf is stimulated by a coupling of denitrification with sulfur cycling, though: Canfield et al. (2010) found evidence for active sulfur cycling in the ETSP without H\(_2\)S accumulation, and a coupling between H\(_2\)S oxidation and denitrification has been shown before (Galan et al., 2014; Jensen et al., 2009). Indeed, active denitrification was found in proximity to H\(_2\)S plumes in the water column during M77-3 (Kalvelage et al., 2013; Schunck et al., 2013).

In the ocean, increased N\(_2\)O production was also associated with the onset of nitrification after re-ventilation of the water column in a seasonal study in the Baltic Sea, but with relatively low resulting N\(_2\)O concentrations (Naqvi et al., 2010). Yu et al. (2010) found strongly increased N\(_2\)O production by nitrifying bacteria that was stimulated by the availability of ammonium during recovery from anoxic conditions in a chemostat culture experiment. Their results point towards an increased N\(_2\)O production via the ammonium-oxidation pathway, while N\(_2\)O production by nitrifier-denitrification seemed not to be stimulated by the shift from anoxic to oxic conditions. We frequently measured high ammonium concentrations along the Peruvian shelf, indeed (Fig. 4),
which could have stimulated $\text{N}_2\text{O}$ production from ammonium oxidation. A direct correlation between $\text{N}_2\text{O}$ and ammonium could not be identified, however. From our concentration measurements alone we thus cannot distinguish if the observed high production of $\text{N}_2\text{O}$ is a result of denitrification or nitrification processes. Studies of the isotopic and isotopomeric $\text{N}_2\text{O}$ composition could reveal more detailed insights whether $\text{N}_2\text{O}$ is produced via the ammonium oxidation or the nitrite reduction pathway during extreme accumulation.

Together with the high $\text{N}_2\text{O}$ concentrations we found low $\text{N}'$ values that were associated with oxygenated waters only at the shelf, whereas in the open ocean $\text{N}$ depletion was restricted to the OMZ core (Fig. 6). This could indicate that the re-oxygenation of the oxygen-deficient waters mainly happens at the shelf, whereas waters in the open ocean OMZ are less affected by mixing processes. Strong diapycnal and isopycnal mixing on the shelf has indeed been reported from the Peruvian and Mauritanian upwelling region (Schafstall et al., 2010; Thomsen et al., JGR, submitted, 2015, (Pietri et al., 2014)).

The upwelling-induced high primary production in the surface ocean furthermore fuels rapid oxygen consumption in the underlying waters due to the export and remineralization of organic matter, thereby creating strong small-scale variability in oxygen concentrations. Kalvelage et al. (2013) showed that these high remineralization rates also induce strong N cycling in the subsurface layer. Turnover rates for different N species are therefore much faster on the shelf than in the open ocean OMZ (Hu et al., 2015), which is also reflected in the distribution of different functional gene abundances (Loescher et al., 2014). One factor that also contributes to the $\text{N}_2\text{O}$ accumulation on the shelf could thus be generally higher rates of nitrification and/or denitrification on the shelf than in the open ocean.
Summary and Conclusions

We observed extreme N$_2$O accumulations over the Peruvian shelf and in the adjacent waters with maximum concentrations similar to the observations made by Naqvi et al. (2000) over the Indian shelf and Farías et al. (2015) off Chile, whereas N$_2$O concentrations in the open ocean OMZ off Peru were comparably moderate.

We found strong evidence that N$_2$O accumulations are preserved when oxygen concentrations increased as a result of mixing and exchange with the overlying atmosphere in the upwelling zone. Waters with high N$_2$O concentrations can thus be directly and frequently transported to the surface ocean. This makes this region one of the most important oceanic regions for N$_2$O emissions to the atmosphere (Arevalo-Martinez et al., 2015). This direct link between unusually high N$_2$O production and emissions over the Peruvian shelf makes it necessary to understand the biogeochemical processes involved in N$_2$O production and consumption to produce reliable predictions of oceanic emissions from this area. Current approaches to model the N$_2$O distribution rely on parameterizations based on the linear $\Delta$N$_2$O/AOU relationship (Suntharalingam and Sarmiento, 2000; Nevison et al., 2003; Freing et al., 2012). These approaches could in fact reproduce the oxygen distribution in the open ocean OMZ off Peru reasonably well, but they fail to account for the extreme N$_2$O accumulation and its high spatial and temporal variability over the shelf area. They thus significantly underestimate the emissions from the Peruvian upwelling and potentially other upwelling areas with similar conditions, too.
Acknowledgements

We would like to thank the captains and crew of the R/V Meteor for their professional support and the chief scientists of M77-3 & M90-M93, Martin Frank, Lothar Stramma, Stefan Sommer and Gaute Lavik for the opportunity to collect samples during their cruises. We would also like to thank Annie Bourbonnais and Johanna Maltby for the collection of N$_2$O samples during M92, and Gesa Eirund, Joel Craig, Georgina Flores, Jennifer Zur, Moritz Baumann, Tina Baustian and Dörte Nitschkowski for their help in analyzing the samples.

We would like to thank Frank Malien, Mirja Dunker, Violeta Leon, Peter Fritsche, Tina Baustian, Kerstin Nachtigall, Martina Lohmann, Gabriele Klockgether and Tim Kalvelage for the sampling and analysis of oxygen and nutrient samples during M77-3 & M77-4 and M90-M93. The work presented here was made possible by the DFG-supported projects SFB754 Phase I and II (http://www.sfb754.de) and the BMBF joint projects SOPRAN II and III (FKZ 03F0611A and FKZ 03F662A).

References


Figures:

Figure 1: Station maps of the sampled N₂O stations from cruises A) M77-3, December 2008 – January 2009 (●) and M77-4, January – February 2009 (◯), B) M90, November 2012 (●) and M91, December 2012 (◯), C) M92, January 2013 (●) and M93, February – March 2013 (◯). Section annotations in A) and B) correspond to the vertical sections shown in Fig. 2 and 3.

Figure 2: Spatial distributions of oxygen (A, B), nitrite (C, D) and N₂O (E, F) along 86°W during M77-4 (2009, A, C, E) and M90 (2012, B, D, F). Small dots indicate location and depth of the discrete samples. Data gridding: ODV/DIVA.

Figure 3: Cross-shelf distribution of A) Oxygen, B) Phosphate, C) Nitrate, D) N’, e) Nitrite and f) N₂O during M91 (Section F).

Figure 4: Selected depth profiles of oxygen (black dots, dotted line), sigma-theta (grey line) and N₂O (red line, open circles) (left panel) and nitrate (grey line), nitrite (black circles, dotted line), ammonium (blue diamonds, straight line) and N’ (red line, small dots) (right panel) from selected open ocean and shelf stations during M90-93. Depth profiles of oxygen and sigma-theta were taken from the CTD sensors, the other parameters are taken from discrete samples. The locations of the respective stations are shown in the map. Red signals denote stations classified as “coastal” stations whereas blue signals denote “offshore” stations. Please note the changes in the scales for N₂O, sigma-theta, nitrite and ammonium.

Figure 5: Temperature-Salinity diagrams with ΔN₂O color coded for a) the offshore stations and b) the onshore stations. Different symbols denote different cruises: ☆ M77-3; ◯ M77-4; ○ M92; △ M90; ◊ M91; ★ M93.

Figure 6: ΔN₂O/AOU relationship from a) offshore stations and b) coastal stations. Samples from the upper OMZ and oxycline (sample depth < 350 m) are color coded with N’. Different symbols for different cruises are denoted the same as in Figure 5. The black line denotes the ΔN₂O/AOU relationship from the offshore
data for samples with O2>50 µM and depth < 350 m (y=0.13x+3.73; r²=0.83). Please note the change in the scaling for ΔN₂O values of 0 - 100 nM and 100 - 1000 nM (dotted line).

Figure 1:
Figure 2:
Figure 3:
Figure 4:
Figure 5: