Intact polar lipids of Thaumarchaeota and anammox bacteria as indicators of N-cycling in the Eastern Tropical North Pacific oxygen deficient zone

Martina SOLLAI[1*], Ellen C. HOPMANS[1], Stefan SCHOUTEN[1,3], Richard G. KEIL[2] and Jaap S. SINNINGHE DAMSTÉ[1,3]

[1] *NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Organic Biogeochemistry, P.O. Box 59, 179AB Den Burg, the Netherlands.*

[2] *School of Oceanography, University of Washington, Seattle, WA 98195, USA*

[3] *University of Utrecht, Faculty of Geosciences, P.O. Box 80.021, 3508 TA Utrecht, the Netherlands*.

* Corresponding author: *martina.sollai@nioz.nl*

Abstract

In the last decade our understanding of the marine nitrogen cycle has improved considerably thanks to the discovery of two novel groups of microorganisms: ammonia-oxidizing archaea (AOA) and anaerobic ammonia-oxidizing (anammox) bacteria. Both groups are important in oxygen deficient zones (ODZs), where they substantially affect the marine N-budget. These two groups of microbes are also well known for producing specific membrane lipids, which can be used as biomarkers to trace their presence in the environment. We investigated the occurrence and distribution of AOA and anammox bacteria in the water column of the Eastern Tropical North Pacific (ETNP) ODZ, one of the most prominent ODZs worldwide. Suspended particulate matter (SPM) was collected at different depths of the water column in high resolution, at both a coastal and an open ocean setting. The SPM was analyzed for AOA- and anammox bacteria-specific intact polar lipids (IPLs), i.e. hexose-phosphohexose (HPH)-crenarchaeol and phosphatidylcholine (PC)-monoether ladderane. Comparison with oxygen profiles reveals that both the microbial groups are able to thrive at low (<1 μM) concentrations of oxygen. Our results indicate a clear niche segregation of AOA and anammox bacteria in the coastal waters of the ETNP, but a partial overlap of the two niches of these microbial species in the open water setting. The latter distribution suggests the potential for an interaction between the two microbial groups at the open ocean site, although the nature of this hypothetical interaction (i.e. either competition or cooperation) remains unclear.

1. Introduction

The marine nitrogen cycle has been widely investigated, as nitrogen is one of the main limiting factors of primary production in the upper sunlit layers of the oceans (Arrigo, 2005; Codispoti L.A., 1997) and the ocean accounts for about half of the global net primary production (Field et al., 1998; Gruber and Galloway, 2008). In the traditional view, the marine nitrogen cycle includes nitrogen fixation as the main input of nitrogen in the ocean and dinitrogen gas formed by denitrification as the main output, so that these two pathways are mainly responsible for the marine nitrogen budget status (Karl et al., 1997). In 2001, Codispoti and coauthors suggested that in the present-day ocean, the nitrogen budget is not in a steady state, but rather out of balance, with denitrification fluxes being underestimated (Codispoti et al., 2001). Nitrogen fixation is mediated by few microorganisms, including cyanobacteria, while denitrification is performed by a wide range of microorganisms with different metabolic features, able to switch from aerobic to anaerobic nitrate (NO_3^-) dependent respiration modes (Lam and Kuypers, 2011). In this classical view, nitrification, representing the major oxidative part of the cycle, connecting organic nitrogen to NO_3^- (Codispoti et al., 2001; Lam and Kuypers, 2011), was seen exclusively as an aerobic process carried out by ammonia- (AOB), and nitrite-oxidizing bacteria, members of the β- and γ-proteobacteria. The nitrification reaction is divided into two steps, performed by distinct bacterial groups. In the first part ammonium (NH_4^+) is oxidized to nitrite (NO_2^-), whereas in the second NO_2^- is oxidized to nitrate (NO_3^-). In both cases oxygen serves as electron acceptor, although AOB have been reported to perform nitrification in sub-oxic conditions (Lam et al., 2007; Schmidt and Bock, 1997).

The overall understanding of the marine nitrogen cycle has substantially changed in the last decade (Fig. 1). Specific archaea were discovered to be important players in the marine nitrogen cycle (Venter et al., 2004) as some of them perform nitrification in the marine

water column and sediment (Francis et al., 2005; Könneke et al., 2005; Wuchter et al., 2006). The group of archaea capable of nitrification has recently been relocated in a separate phylum named Thaumarchaeota (Brochier-Armanet et al., 2008; Spang et al., 2010). Compared to their bacterial counterpart, ammonia oxidizing archaea (AOA) are often more abundant in the ocean (Karner et al., 2001; Lam et al., 2007; Wuchter et al., 2006), accounting for 20% of picoplankton and 40% of the estimated total number of cells (Karner et al., 2001). These microorganisms are able to cope with low oxygen conditions (Coolen et al., 2007; Lam et al., 2007; Park et al., 2010; Pitcher et al., 2011b; Sinninghe Damsté et al., 2002a), have low substrate requirements (Martens-Habbena et al., 2009) and are able to utilize a highly energy efficient CO_2- fixation pathway (Könneke et al., 2014); new coastal marine AOA isolates show obligate mixotrophy and vary in their adaptive ability to different environmental parameters (Qin et al., 2014). All these features have been suggested to provide a reason for AOA observed dominance over ammonia oxidizing bacteria (AOB) as ammonia oxidizers in the open oceans (Könneke et al., 2014; Pester et al., 2011). Moreover, a 'novel' process in the nitrogen cycle, named anammox, was discovered. Anaerobic ammonia oxidizing (anammox) bacteria, are a unique group of microorganisms member of the order of *Planctomycetales* (Strous et al., 1999). They are able to oxidize ammonium (NH_4^+) to molecular nitrogen (N_2) under anoxic conditions, using nitrite (NO_2^-) as electron acceptor (van de Graaf et al., 1995). Anammox bacterial activity has been detected in marine anoxic sediments and waters (Dalsgaard et al., 2003; Kuypers et al., 2003; Thamdrup and Dalsgaard, 2002) and has been recognized to contribute, along with denitrifying bacteria, to the loss of N_2 from the ocean (Galán et al., 2009; Hamersley et al., 2007; Kuypers et al., 2005; Lam et al., 2009; Thamdrup et al., 2006). Despite different oxygen tolerances, anammox bacteria and Thaumarchaeota have been observed to co-exist in different settings, particularly in oxygen deficient zones (ODZs) and anoxic waters (Coolen et al., 2007; Francis et al., 2005; Lam et al., 2007; Pitcher

et al., 2011b; Woebken et al., 2007). These two microbial groups can potentially benefit from each other, because the thaumarchaeotal nitrification might be coupled with the anammox process by providing the NO_2^- anammox bacteria need and, at the same time, consume oxygen to which anammox bacteria are sensitive. Alternatively, when nitrite is provided to anammox by other sources, the two groups might compete for NH_4^+ (Yan et al., 2012).

In this study we investigated the occurrence and depth distribution of Thaumarchaeota and anammox bacteria in the Eastern Tropical North Pacific (ETNP) oxygen deficient zone, one of the most extended ODZs in the contemporary ocean. The presence of AOA and anammox bacteria has been reported in the ETNP oxygen deficient zone by a few studies (Beman et al., 2008, 2012, 2013; Francis et al., 2005; Podlaska et al., 2012; Rush et al., 2012) and the significance of the two microbial groups to local marine nitrogen cycling is starting to be elucidated for other ODZs (Dalsgaard et al., 2003; Galán et al., 2009; Kalvelage et al., 2013; Kuypers et al., 2003, 2005; Lam et al., 2007, 2009; Pitcher et al., 2011b; Ward et al., 2009). However, the spatial distribution and the possible co-occurrence of the two groups in the ETNP have not been investigated in detail as well as the relative contribution of AOA and anammox to the local N-cycle and their possible interactions. To the best of our knowledge only one study so far has examined concurrently the presence of the two microbial groups in the southern part of the ETNP (Podlaska et al., 2012). Other studies on the ETNP ODZ have investigated the presence of AOA along a north-south transects following the coastal line of southern California (Beman et al., 2008, 2012, 2013) and the occurrence of anammox bacteria in the southern ETNP ODZ (Rush et al., 2012). These studies did not investigate the occurrence of AOA and anammox bacteria at true open ocean sites and a comparison of AOA and anammox bacteria dynamics between coastal and open ocean waters is still missing. To fill this gap in the current knowledge we performed high resolution water sampling, at both coastal and open ocean settings in the ETNP ODZ.

To trace the two microbial groups we applied intact polar lipids (IPLs) specific for these groups, which have proved to be good biomarkers in various settings (Bale et al., 2013; Buckles et al., 2013; Lengger et al., 2012; Pitcher et al., 2011b, 2011c). Anammox bacteria produce unique ladderane fatty acids which contain 3-5 concatenated cyclobutane moieties (Sinninghe Damsté et al., 2002c). They are attached to the glycerol backbone with polar head groups comprising phoshocholine (PC) and phosphoethanolamine (PE) (Boumann et al., 2006; Rattray et al., 2008). Thaumarchaeota produce also specific biomarker lipids, i.e. crenarchaeol, a glycerol dibiphytanyl glycerol tetraether (GDGT) lipid, containing a cyclohexane moiety beside four cyclopentane moieties (de la Torre et al., 2008; Pitcher et al., 2010; Schouten et al., 2008; Sinninghe Damsté et al., 2002b, 2002c). Attached to crenarchaeol are various polar headgroups such as monohexose (MH), dihexose (DH) and a hexose-phosphohexose (HPH) (Schouten et al., 2008) with the latter being the most suitable for tracing living active cells (Pitcher et al., 2011a). By applying these specific IPLs, i.e. HPH-crenarchaeol and PC-monoether ladderane, respectively, we investigate the depth habitat of the Thaumarchaeota and anammox bacteria in the ETNP oxygen deficient zone and the factors controlling their ecological niche.

2. Materials and methods

2.1. Environmental setting of the ETNP

The ETNP ODZ is one of the thickest in the contemporary ocean and extends to depths as deep as ~1000 m. Geographically it ranges from ~25°N (i.e. Baja California) to ~10°N (i.e. Costa Rica) and from ~160°W in the North Pacific Ocean to the coast of Mexico and Costa Rica. It is a permanent feature of the eastern tropical Pacific region (Paulmier and Ruiz-Pino, 2009). The region is important for its role in the global carbon cycle, for its involvement in El Niño Southern Oscillation, and it is economically relevant for fishery (Fiedler and Lavín, 2006). A shallow and strong thermocline causes water stratification and weak exchanges of nutrients and oxygen between surface waters and sub-thermocline layers, which are poorly ventilated (Lavín et al., 2006). This feature is further exacerbated by Ekman pumping which causes coastal and open ocean upwelling (Lavín et al., 2006). The hydrology of the eastern tropical Pacific is influenced by water circulation features and by strong winds in the part close to the American continent (Kessler, 2006). The ETNP oxygen deficient zone comprises part of the North Pacific sub-tropical gyre, specifically it is delimited southeastern by the California Current (CC), the North Equatorial Current (NEC) and the North Equatorial Countercurrent (NECC) (Karstensen et al., 2008). The boundary area where the CC, flowing along the coast of the Baja California and southern, encounters the NEC is characterized by Ekman transport westward and upwelling mainly off the Californian coast and in a weakened magnitude off the north Mexico coast (Kessler, 2006; Lavín et al., 2006). Our sampling area lies in this transition region; however the upper water circulation in this region is not fully understood yet (Kessler, 2006). Although ODZs have been now studied for almost a century, only recently it has been possible to determine in situ concentration in these areas to the accuracy of nanomolar O_2 (Revsbech et al., 2009). This has allowed to prove that ODZ regions, including the ETNP, are functionally devoid of oxygen, although respiratory rates

indicate that aerobic metabolisms successfully occur, even in such extreme conditions (Canfield et al., 2010; Jensen et al., 2011; Revsbech et al., 2009; Thamdrup et al., 2012; Tiano et al., 2014).

2.2. Sampling

Sampling was performed at twelve stations during the Eastern Tropical North Pacific (TN278) cruise (R/V Thomas G. Thompson, March-April 2012) (Fig. 2. panel A). The cruise route was split in two legs with the former one comprising six sampling stations (Fig 2. panel B), in very close proximity to each other, in coastal waters, north-west of the departure port of Manzanillo (Colima, MX), and the latter including six sampling stations, clustered closely together, in open ocean waters south-west of the departure port, around the area known as the Moctezuma Trough (Fig. 2. panel C). Suspended particulate matter (SPM) samples were collected on pre-ashed 0.7 μm pore size glass fiber (GF) filters, mounted in McLane WTS-LV in situ filtration systems. At each sampling site four McLane pumps were deployed simultaneously at different depths (Table 1). The volume of water filtered varied according to the depth and the material collected (Table 1). Upon the recovery of the pumps the GF filters were removed, split in two halves and frozen at -40°C.

Physical parameters of the water column were recorded by conductivity-temperature-density (CTD) equipment (SBE-911, Sea-Bird Electronics); dissolved-oxygen depth concentrations were measured by a SBE 43 electrochemical sensor mounted on the CTD rosette. Sensor oxygen concentrations were calibrated against on-deck Winkler titrations. The data reported here do not take into account recent evidence that these techniques (i.e. Clark electrodes, Winkler titrations) overestimate oxygen at the very lowest concentrations (Tiano et al., 2014). Water samples for inorganic nutrient profiles were collected using 24×10 L Niskin bottles mounted on a rosette to the CTD. The CTD was cast shortly before or after the

deployment of the McLane pumps. In case this was not possible, data from another station, the closest in time and space to that of the deployed in situ pumps, were used. This means that at some sites the depths sampled for nutrients data do not always directly correspond to the depths at which SPM was sampled with the in situ pumps (Table 1). The detection limits for NO_3^-, NO_2^-, and NH_4^+ were respectively 0.08 µM, 0.01 µM and 0.07 µM. The electrochemical oxygen sensor SBE 43 has a detection limit of 1-2 µM (Tiano et al., 2014).

2.3. Intact polar lipid analysis

Intact polar lipids were extracted from freeze-dried SPM filter halves using a modified Bligh-Dyer technique as described in Sturt et al. (2004) with some adjustments as described in Schouten et al. (2008). Briefly, a known volume of methanol (MeOH):dichloromethane (DCM):phosphate buffer (P-buffer) (2:1:0.8, v/v/v) was added to the filter in a glass centrifuge tube and the total lipid contents were extracted in a sonication bath for 10 min. After centrifugation for 3 min at 2000 rpm the supernatant was removed. The extraction was repeated two more times and the supernatants combined. To induce separation of the combined supernatant into two phases, additional DCM and P-buffer were added to a new volume ratio of 1:1:0.9 DCM:MeOH:P-buffer. The mixture was centrifuged for 2 min at 3000 rpm after which the DCM layer was removed. The procedure was repeated two more times and the combined DCM phases were collected in a round bottom flask, reduced under rotary vacuum and completely dried under N_2 (Schouten et al., 2008; Sturt et al., 2004).

IPLs were analyzed directly in the extract using a high performance liquid chromatography (HPLC)-electrospray ionization (ESI)/triple quadrupole MS in selected reaction monitoring (SRM) mode as described by Pitcher et al. (2010). In order to minimize possible variations in the IPLs response factors, the extract was analyzed in the same batch. Briefly, an Agilent (Palo-Alto, CA, US) 1100 series LC equipped with a thermostat-controlled

auto-injector was used coupled to a Thermo TSQ Quantum EM triple quadrupole MS equipped with an Ion Max source with ESI probe. The SRM method for the crenarchaeol IPLs was targeting specifically HPH-crenarchaeol (Schouten et al., 2008; Pitcher et al., 2010). Due to the lack of a standard, HPH-crenarchaeol was quantified as the integrated IPL area peak response units L^{-1} (i.e. r.u. L^{-1}), unveiling the relative depth distribution of the lipid biomarker in the water column, but not providing information on its absolute abundance. The anammox-specific membrane lipid C_{20}-[3]-ladderane with a phosphocholine (PC)-monoether was analyzed according to Jaeschke et al. (2009). The intact ladderane monoether lipid was quantified referring to an external calibration curve of an isolated C_{20}-[3]-ladderane (PC)-monoether standard (Jaeschke et al., 2009).

3. Results

3.1. Oxygen and nutrient profiles

During the ETNP (TN278) cruise in March-April 2012 the water column of the ETNP ODZ was sampled at high resolution for SPM, at depths from 20 to 2000 m in coastal waters and from 50 to 2500 m in open ocean waters at a number of geographically nearby stations (Fig. 2; Table 1). To compile all our data in two (coastal and open ocean) composite profiles, we report our nutrient (Figs. 3a-c; 3g-i), oxygen (Figs. 3d and 3l), and lipid SPM (Figs. 3e and 3f; 3m and 3n) data relative to the potential density anomaly, σ_θ (kg m^{-3}) of the water masses sampled at each of the coastal or open ocean stations, respectively. In the upper water column values for σ_θ differ at the two sampling sites as a consequence of differences in salinity (data not shown).

For both locations, the oxygen profiles (Figs. 3d and 3l) obtained by an oxygen electrochemical sensor mounted on the CTD of the nearby stations were virtually identical. Station 106, which is slightly further away from the other coastal stations (Fig. 2), is an exception since the upper oxycline is located at a σ_θ of 25.5, ca. 30 m deeper than at the other stations. For the other coastal stations the oxycline occurs in shallow waters at a σ_θ of 25.0, i.e. ~30 m depth, whilst for the in open ocean waters it is located at σ_θ 25.5, which corresponds to ~100 m depth. Because of the virtual identical oxygen- σ_θ profiles, it was decided that all the data for the coastal (except for station 106) and open ocean stations could be combined in presenting the nutrient concentrations and IPL data from SPM. In this way we provide an expanded view of the vertical distribution of the two microbial species along the water column.

In the upper coastal waters the oxygen concentration varies between 250 to 50 µM until σ_θ 23.5 (i.e. ~15-20 m), then drops to values below 20 µM at the upper oxycline (Fig. 3d). In this setting, the ODZ spans from σ_θ 25.0 to 27.0 (i.e. between ~35 to ~800 m), with a

minimal oxygen concentration below 1 µM in the core (Fig. 3d) (see also Tiano et al., 2014). Below 850 m σ_θ is between 27.0 and 27.5 and the oxygen concentration increases gradually again until ~75 µM, at 2000 m depth (Fig. 3d). In the open ocean waters oxygen concentration is stable at ~200 µM until σ_θ 22.5, at ~55 m depth, and rapidly decreases to values close to 1 µM at σ_θ 25.5, at 100 m depth. The ODZ extends until σ_θ 27.0, at 850 m depth. Below the lower oxycline the oxygen concentration increases again to ~120 µM at 3000 m depth, where σ_θ ranged between 27.5 and 28.0 (Fig. 3l).

Nutrient concentration data (i.e. NO_3^-, NO_2^-, NH_4^+) from different stations were combined in one coastal and one open ocean setting, as described above for the oxygen depth profiles. The resulting profiles show distinct patterns (Figs. 3a-c; 3g-i). In both coastal and open waters nitrate is the most abundant nitrogen species and show two maxima at different σ_θ (Figs. 3a and 3g). In coastal waters the first maximum of ~25 µM occurs at the upper oxycline at σ_θ 25, then the concentrations decrease to 15-20 µM until σ_θ 26.5. The second maximum of ~45 µM occurs at σ_θ 27.4, just below the lower oxycline (i.e. at 1000 m depth) (Fig. 3a). In open ocean waters the trend in nitrate appears similar to the one in the coastal waters, although some differences can be noticed (Fig. 3g). The first maximum (i.e. ~25 µM) in this case is broader and deeper, spanning from σ_θ 24.5 to 26, where the upper oxycline is located. The second deeper maximum (i.e. ~50 µM) as well occurs where the waters start to be re-oxygenated (i.e. σ_θ 27.4, depth 1100 m). Like for nitrate, the profiles of nitrite are slightly different for the coast and open ocean waters (Figs. 3b and 3h). In the former setting the shallow maximum occurs at σ_θ 24.6, at declining oxygen concentrations. The peak is only ~1 µM and rather narrow. The lower peak of ~8 µM is located at σ_θ 26 (i.e. 88 m depth) in the core ODZ (Fig. 3b). The first nitrite maximum in the open ocean reaches1 to 2 µM and spans from fully oxygenated waters to the oxycline (i.e. σ_θ from ~23 to ~25). The deeper maximum on the other hand (i.e. ~6 µM) occurs in the upper ODZ (i.e. σ_θ 26.2) (Fig. 3h). Finally, at

both sampling sites NH_4^+ concentrations are mostly below the detection limit of 0.07 µM (Figs. 3c and 3i) with the exception of a few data points at the costal oxycline (i.e. σ_θ 24.6) and in the open water where the oxygen decreases, and the NH_4^+concentrations are respectively 0.6 µM and ~0.1 µM.

3.2. Biomarker lipid profiles

The SPM for biomarker analysis were collected on 0.7 µm pore size GF filters. Limitations related to the use of 0.7 µm filters to collect archaeal living cells have been reported (Ingalls et al., 2012; Schouten et al., 2012) as the typical size of thaumarchaeotal cells is <0.6 µm (Könneke et al., 2005) and they are suggested to occur predominantly free-living during their lifetime (Ingalls et al., 2012). Although the pore size tends to diminish as the particulate material accumulates, the employment of 0.7 µm filters likely causes an underestimation of the archaeal population, and thus archaeal IPL abundance (Schouten et al., 2012). However, Pitcher et al. (2011b) showed that depth profiles of HPH-crenarchaeol, analyzed on SPM collected using 0.7 µm GFF filters, in the Arabian Sea ODZ were similar to that of thaumarchaeotal genes, analyzed on SPM collected using 0.2 µm filters (Pitcher et al., 2011b). Therefore, our results are likely still suitable to probe the depth habitat of Thaumarchaeota.

Figures 3e and 3m show HPH-crenarchaeol vertical profile for the coastal and the open ocean sites, respectively. In the coastal setting, HPH-crenarchaeol has a maximum in abundance at the interface between the oxycline and the upper ODZ, where σ_θ is ~25 (i.e. ~30 m depth) (Fig. 3e). In the open ocean setting, HPH-crenarchaeol starts to increase at declining oxygen concentrations and peaks at the base of the oxycline (i.e. at σ_θ 25.8 and 100 m depth). Deeper in the water column (i.e. at σ_θ 27.4 corresponding to ~1000 m water depth) a secondary minor maximum in HPH-crenarchaeol was detected (Fig. 3m).

In the coastal waters, the ladderane PC-monoether concentration stays low, except for one data point (i.e.~17 pg L^{-1} at 55 m depth), until the upper ODZ where it starts to increase to its maximum (i.e. ~251 pg L^{-1}) at σ_θ 26.4 in the core ODZ (i.e. 150 m depth) (Fig. 3f). In the open ocean, the PC-monoether maximum in concentration (i.e. ~122 pg L^{-1}) is located at the oxycline (i.e. at σ_θ 25.9 and 105 m depth).

4. Discussion

4.1. Depth distributions and abundance of AOA and anammox bacteria in the ETNP

In this study we have been able to investigate concurrently for the first time, the vertical distribution of AOA and anammox bacteria in both coastal and open waters of the ETNP ODZ. The IPL-biomarker profiles show that AOA and anammox bacteria are present in the region and partially co-exist along the water column (Figs. 3e and 3f; 3m and 3n). Such a distribution was already observed in other dysoxic or anoxic marine systems worldwide such as the Black Sea, and the ODZs of the ESTP and the Benguela upwelling system (Lam et al., 2007, 2009; Woebken et al., 2007), whereas in the southern part of the ETNP ODZ (Podlaska et al., 2012) and in the Arabian Sea (Pitcher et al., 2011b) the two microbial groups are reported to thrive at different water depths. In the northern ETNP our IPLs depth profiles highlight some substantial differences in the distribution and abundance of the two groups between the different settings.

In the coastal setting, the two microbial groups show clear niche segregation in the upper part of the water column. Here, AOA thrive at the bottom of the oxycline, at a σ_θ of ~25, whereas anammox bacteria are just starting to increase in abundance at that point and exhibit a clear maximum only in the core ODZ (Fig. 3e and 3f), where σ_θ has shifted to ~26. The trend of our coastal HPH-crenarchaeol depth profile agrees with previously reported data

for thaumarchaeotal 16SrRNA, archaeal *amoA* gene concentration and rate measurements from the same area (station 3 in Beman et al., 2012), which also revealed an AOA maximum at the base of the oxycline. Consequently, Beman et al. (2012) suggested a prominent role of AOA in performing nitrification in shallow O_2-depleted waters. The observed maximum abundance of anammox bacteria in the core ODZ as based on the ladderane lipid profile is in agreement with previous investigations in the ETSP ODZ, where it has been proposed as a preferential niche for anammox activity (De Brabandere et al., 2014; Hamersley et al., 2007; Ward et al., 2009). Moreover, similarly to De Brabandere et al. (2014), who also reported low anammox rates at the oxycline in one of their sampling stations in the ETSP, we also observed low ladderane concentrations in the ETNP coastal setting.

At the open ocean site, we also find the maximum abundance of anammox bacteria between the base of the oxycline and the upper part of the ODZ. However, here the anammox bacterial abundance displays a concurrent maximum with that of AOA (Figs. 3m and 3n). The segregation of AOA and anammox bacteria niches in the coastal waters of the ETNP ODZ and their contrasting co-occurrence in the open waters clearly indicates a different behavior of the two microbial species at different locations of the ETNP. To the best of our knowledge this is the first study that highlights such different vertical distribution of the two groups on a local scale. We also note that both IPL-biomarkers exhibit higher concentration maxima in coastal waters (Figs. 3e and 3f) than in the open ocean (Figs. 3m and 3n), i.e. the concentration of HPH-crenarchaeol is five times higher and that of the PC-monoether ladderane is more than twice that found in the open ocean. This suggests that both AOA and anammox bacteria are more abundant in the coastal waters of the ETNP. The reasons for such divergence may be various. For instance, the complex and so far not fully resolved upper water circulation in this region may play a role (Fiedler and Talley, 2006; Kessler, 2006). The proximity of the American continent is likely to have a greater influence on the hydrography

of the coastal site (i.e. in a straight line the closest point on the Mexican coastline to our coastal settings is roughly 40 km away), than on the open ocean site. At these latitudes the continental wind forcing is a dominant factor and together with the variations in the coastline influences the local upper circulation (Fiedler and Talley, 2006; Kessler, 2006) and might have an effect on the different vertical distribution and abundance observed in the two microbial species as well. In the same way the nitrogen species profiles are likely to be influenced by variable hydrographical features (Fig. 3).

4.2. Influence of nitrogen species on the abundance and the distribution of Thaumarchaeota and anammox bacteria in the ETNP

Ammonium and nitrite concentrations have been proposed as critical factors in determining the vertical distribution and the abundance of Thaumarchaeota and anammox bacteria (Hamersley et al., 2007; Jaeschke et al., 2007; Jensen et al., 2009; Kuypers et al., 2005; Martens-Habbena et al., 2009; Stahl and de la Torre, 2012; Thamdrup et al., 2006; Ward et al., 2009).

NH_4^+ serves as a substrate for both Thaumarchaeota and anammox bacteria and has been observed to not accumulate in ODZs as a results of efficient turnover between sources and sinks (Kalvelage et al., 2013). In the ETNP ODZ, we found both Thaumarchaeota and anammox bacteria in sub-oxic and anoxic waters. Ammonium concentrations are low and mostly under the detection limit, likely due to the consumption of the nutrient by both (or other) microorganisms (Figs. 3c and 3i). Even at concentrations <1 μM, NH_4^+ may support anammox reaction, which is considered the main sink for this nitrogen species in the core ODZs (Bianchi et al., 2014). Nitrite is the electron acceptor in the anammox process and it has been already described as a limiting factor to anammox bacteria activity in the southern ETNP ODZ (Rush et al., 2012).

In the coastal waters, thaumarchaeotal nitrification is probably taking place at the bottom of the oxycline, as indicated by the HPH-crenarchaeol maximum (Fig. 3e) and the concurrent ammonium concentration peak (Fig. 3c), most likely resulting from the mineralization of organic matter. Moreover, thaumarchaeotal nitrification, which converts ammonium into nitrite (Arrigo, 2005), may cause the observed minor primary peak in the nitrite concentration profile (the so-called primary nitrite maximum or PNM) occurring at the bottom of the oxycline in these waters (Fig. 3b), which coincides with the maximum of AOA abundance (Figs. 3b and 3e). In the core ODZ a clear secondary nitrite maximum (SNM) co-occurs with the maximum in anammox bacteria concentration (Figs. 3b and 3f). Although heterotrophic denitrification represents the obvious candidate as main provider of nitrite to anammox bacteria in oceanic settings (Ward et al., 2009), the two processes are usually not found coupled together (Dalsgaard et al., 2012). Alternatively, a combination of several pathways including dissimilatory NO_3^- reduction to NO_2^- (DNRN) or NH_4^+ (DNRA) plays a role, as has been observed in other ODZs (Canfield et al., 2010; Kartal et al., 2007; Lam et al., 2009, 2011; Lipschultz et al., 1990; Ward et al., 2009). The extent of the contribution of these processes as nutrients providers to anammox bacteria is still unclear (Lam and Kuypers, 2011). Finally, a recent study has also brought into attention zooplankton migrators as alternative source of substrates to the anammox metabolism, previously overlooked in ODZs (Bianchi et al., 2014). In total, these things suggest that those mechanisms are all feasible to feed the anammox process in the coastal waters of the core ETNP ODZ with the nutrients required.

In the open ocean ETNP, AOA and anammox bacteria maxima may be coupled in the upper ODZ because of the (partial) overlap of the ecological niches of the two groups in this setting (Figs. 3m and 3n). In this case, AOA and anammox bacteria could either compete or co-operate for the substrates they require, as already proved in laboratory-scale models (Yan

et al., 2010, 2012). The nutrient profiles are also consistent with the co-occurrence of the two metabolic processes (Figs. 3h and 3i): the secondary nitrite maximum is concurrent with the two biomarker maxima (Figs. 3h; 3m and 3n), whereas NH_4^+ is either possibly consumed by anammox as the water column turns sub-oxic or by nitrification by AOA successfully adapted to low nutrients and oxygen conditions (Coolen et al., 2007; Francis et al., 2005; Lam et al., 2007, 2009; Martens-Habbena et al., 2009; Park et al., 2010; Pitcher et al., 2011b; Schouten et al., 2004; Sinninghe Damsté et al., 2002a; Stahl and de la Torre, 2012; Stolper et al., 2010; Tiano et al., 2014; Woebken et al., 2007) and potentially to a broad variety of different environmental conditions (Qin et al., 2014).

The role of NO_2^- and NH_4^+ in differentiating the distribution of Thaumarchaeota and anammox bacteria observed at the coastal and at the open water sites of the ETNP ODZ is not clear; when the NO_2^- and NH_4^+ concentrations were compared with those of the specific biomarkers studied at both sites, no evident relationship was apparent (data not shown).

In conclusion, further investigation is required to establish the contribution of the single processes to the N cycle occurring in the settings investigated in this study and to explain the divergence between the two. Other studies have called attention to the relevance of organic matter fluxes as a control over these metabolic pathways and ultimately over the balance between the two mainly responsible for the N_2 removal from the oceans, i.e. anammox and denitrification (Babbin et al., 2014; Chang et al., 2014; Kalvelage et al., 2013; Koeve and Kähler, 2010; Ward, 2013; Ward et al., 2008, 2009). Specifically, variations in the C/N ratio content of the particulate organic matter (POM) entering the ODZ may play a prominent role in determining anammox and heterotrophic denitrification rates, with anammox being favorite by nitrogen-rich OM (Babbin et al., 2014).

4.3. The role of the oxygen

As the features of ODZs suggest, oxygen might play a pivotal role in controlling the abundance and the special distribution of Thaumarchaeota and anammox bacteria in those areas. Previous studies have already pointed to this in other ODZs (Jaeschke et al., 2007; Kuypers et al., 2005; Stahl and de la Torre, 2012; Thamdrup et al., 2006) and in the southern ETNP ODZ itself (Rush et al., 2012). To investigate if oxygen concentration is influencing the abundance of Thaumarchaeota and anammox bacteria in our study sites in the ETNP ODZ, we compared our biomarker concentrations with oxygen concentrations in both coastal and open ocean site (Figs. 4a and 4b). Figure 4a and 4b shows how PC-monoether ladderane and HPH-crenarchaeol are distributed according to O_2 concentration at the two sites. The two distributions appear rather similar, with both biomarkers being more abundant at an oxygen concentration below the detection limit, i.e. ca. 1 µM, which is even overestimated by the CTD sensor employed for the measurements, as suggested by O_2 measurements taken with the STOX microsensor during the same cruise (Tiano et al., 2014). The only evident difference between the two plots is found in one HPH-crenarchaeol data point from the coastal site, corresponding to an oxygen concentration of ~26 µM, which reflects the much broader range of tolerance of AOA to O_2 compared to the strictly anaerobic anammox bacteria (Tiano et al., 2014). However, the relation revealed by our plots suggests that both microbial species are potentially able to cope with low oxygen concentrations and O_2 plays a primary role in controlling the distribution of the two microbial species, as shown previously (Martens-Habbena et al., 2009; Park et al., 2010; Pitcher et al., 2011b; Rush et al., 2012; Stahl and de la Torre, 2012; Tiano et al., 2014). The high relative abundance of HPH-crenarchaeol in the poorly oxygenated waters of the ETNP is consistent with the ability of AOA to thrive and perform nitrification under low oxygen conditions (Coolen et al., 2007; Francis et al., 2005; Lam et al., 2007, 2009; Park et al., 2010; Pitcher et al., 2011b; Schouten et al., 2004; Sinninghe Damsté et al., 2002a; Stolper et al., 2010; Woebken et al., 2007). In the open ocean

site a secondary minor peak of HPH-crenarchaeol at the lower oxycline, i.e. 1100 m depth (Fig. 3m), supports the hypothesis. Pitcher et al. (2011b) also observed a secondary maximum of AOA at the bottom of the Arabian Sea ODZ. Our findings thus confirm oxygen concentration as an important environmental control in determining the distribution of Thaumarchaeota and anammox bacteria in the water column of the ETNP ODZ.

4.4. Conclusions

In this study high resolution profiles of the two specific IPL-biomarkers of AOA and anammox bacteria, i.e. HPH-crenarchaeol and PC-monoether ladderane, allowed us to have a detailed insight on the vertical distribution of these microbial groups in the ETNP ODZ. It shows that AOA and anammox bacteria are abundant at both shallow coastal and open ocean waters of the ETNP oxygen deficient zone. Our findings also indicate that the ecological niches of the two species diverge on a local scale in the ETNP. Different O_2 concentration and water stratification features between the two study sites play an important role in determining such differences; whereas the role of NO_2^- and NH_4^+ is not clear. Further studies are needed to elucidate potential interactions between AOA and anammox in this ODZ. However future investigations on the N-cycle in the ETNP and other ODZs might take into a greater account the importance of regional differences in the ecological niches of these microbial species.

Acknowledgements

We thank the captain and the crew of the R/V Thomas G. Thompson for help and hospitality. The chief scientists, Allan Devol and Bess Ward, are thanked for organizing and supervising the ETNP (TN278) cruise. We thank Stuart G. Wakeham for the invitation to take part to this cruise. Allison Myers-Pigg, Jaqui Neibauer and Rodolfo Alatorre-Gutierrez are thanked for assistance in the sampling. We also thank Rachel Horack for providing potential density data

and for helpful discussion. Additional thanks are due to Laura Villanueva, Anna Rabitti, Laura Tiano, Yvonne Lipsewers and Borja Aguiar-González for helpful discussion and comments. This research was funded by a grant to JSSD from the Darwin Center for Biogeosciences (project nr. 3012). In-situ pump sampling and nutrient analyses were funded by grants to RGK (USA NSF #1153935) and Allan Devol (USA NSF #1029951).

References

Arrigo, K. R.: Marine microorganisms and global nutrient cycles., Nature, 437(7064), 349–356, doi:10.1038/nature04159, 2005.

Babbin, A. R., Keil, R. G., Devol, A. H. and Ward, B. B.: Organic matter stoichiometry, flux, and oxygen control nitrogen loss in the ocean., Science, 344(6182), 406–408, doi:10.1126/science.1248364, 2014.

Bale, N. J., Villanueva, L., Hopmans, E. C., Schouten, S. and Sinninghe Damsté, J. S.: Different seasonality of pelagic and benthic Thaumarchaeota in the North Sea., Biogeosciences Discuss., 10(8), 12593–12624, doi:10.5194/bgd-10-12593-2013, 2013.

Beman, J. M., Popp, B. N. and Alford, S. E.: Quantification of ammonia oxidation rates and ammonia-oxidizing archaea and bacteria at high resolution in the Gulf of California and eastern tropical North Pacific Ocean., Limnol. Oceanogr., 57(3), 711–726, doi:10.4319/lo.2012.57.3.0711, 2012.

Beman, J. M., Popp, B. N. and Francis, C. A.: Molecular and biogeochemical evidence for ammonia oxidation by marine Crenarchaeota in the Gulf of California., ISME J., 2(4), 429–41, doi:10.1038/ismej.2007.118, 2008.

Beman, J. M., Shih, J. L. and Popp, B. N.: Nitrite oxidation in the upper water column and oxygen minimum zone of the eastern tropical North Pacific Ocean., ISME J., 7(11), 2192–2205, doi:10.1038/ismej.2013.96, 2013.

Bianchi, D., Babbin, A. R. and Galbraith, E. D.: Enhancement of anammox by the excretion of diel vertical migrators., Proc. Natl. Acad. Sci. U. S. A., 111(44), 15653–15658, doi:10.1073/pnas.1410790111, 2014.

Boumann, H. A., Hopmans, E. C., van de Leemput, I., Op den Camp, H. J. M., van de Vossenberg, J., Strous, M., Jetten, M. S. M., Sinninghe Damsté, J. S. and Schouten, S.: Ladderane phospholipids in anammox bacteria comprise phosphocholine and phosphoethanolamine headgroups., FEMS Microbiol. Lett., 258(2), 297–304, doi:10.1111/j.1574-6968.2006.00233.x, 2006.

De Brabandere, L., Canfield, D. E., Dalsgaard, T., Friederich, G. E., Revsbech, N. P., Ulloa, O. and Thamdrup, B.: Vertical partitioning of nitrogen-loss processes across the oxic-anoxic interface of an oceanic oxygen minimum zone., Environ. Microbiol., 16, 3041–3054, doi:10.1111/1462-2920.12255, 2014.

Brochier-Armanet, C., Boussau, B., Gribaldo, S. and Forterre, P.: Mesophilic Crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota., Nat. Rev. Microbiol., 6(3), 245–52, doi:10.1038/nrmicro1852, 2008.

Buckles, L. K., Villanueva, L., Weijers, J. W. H., Verschuren, D. and Sinninghe Damsté, J. S.: Linking isoprenoidal GDGT membrane lipid distributions with gene abundances of ammonia-oxidizing Thaumarchaeota and uncultured crenarchaeotal groups in the water

column of a tropical lake (Lake Challa, East Africa)., Environ. Microbiol., 2, doi:10.1111/1462-2920.12118, 2013.

Canfield, D. E., Stewart, F. J., Thamdrup, B., De Brabandere, L., Dalsgaard, T., Delong, E. F., Revsbech, N. P. and Ulloa, O.: A cryptic sulfur cycle in oxygen-minimum-zone waters off the Chilean coast., Science, 330(6009), 1375–8, doi:10.1126/science.1196889, 2010.

Chang, B. X., Rich, J. R., Jayakumar, A., Naik, H., Pratihary, A. K., Keil, R. G., Ward, B. B. and Devol, A. H.: The effect of organic carbon on fixed nitrogen loss in the eastern tropical South Pacific and Arabian Sea oxygen deficient zones., Limnol. Oceanogr., 59(4), 1267–1274, doi:10.4319/lo.2014.59.4.1267, 2014.

Codispoti, L. A., Brandes, J. A., Christensen, J. P., Devol, A. H., Naqvi, S. W. A., Paerl, H. W. and Yoshinari, T.: The oceanic fixed nitrogen and nitrous oxide budgets: moving targets as we enter the anthropocene?, Sci. Mar., 65, 85–105, doi:10.3989/scimar.2001.65s285, 2001.

Codispoti L.A.: The limits to growth, Nature, 387, 237–238, doi:10.1046/j.1463-1326.1999.00023.x, 1997.

Coolen, M. J. L., Abbas, B., van Bleijswijk, J., Hopmans, E. C., Kuypers, M. M. M., Wakeham, S. G. and Sinninghe Damsté, J. S.: Putative ammonia-oxidizing Crenarchaeota in suboxic waters of the Black Sea: a basin-wide ecological study using 16S ribosomal and functional genes and membrane lipids., Environ. Microbiol., 9(4), 1001–16, doi:10.1111/j.1462-2920.2006.01227.x, 2007.

Dalsgaard, T., Canfield, D. E., Petersen, J., Thamdrup, B. and Acuña-González, J.: N_2 production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica., Nature, 422(6932), 606–8, doi:10.1038/nature01526, 2003.

Dalsgaard, T., Thamdrup, B., Farías, L. and Revsbech, N. P.: Anammox and denitrification in the oxygen minimum zone of the eastern South Pacific., Limnol. Oceanogr., 57(5), 1331–1346, doi:10.4319/lo.2012.57.5.1331, 2012.

Fiedler, P. C. and Lavín, M. F.: Introduction: a review of eastern tropical Pacific oceanography., Prog. Oceanogr., 69, 94–100, doi:10.1016/j.pocean.2006.03.006, 2006.

Fiedler, P. C. and Talley, L. D.: Hydrography of the eastern tropical Pacific: a review., Prog. Oceanogr., 69(2-4), 143–180, doi:10.1016/j.pocean.2006.03.008, 2006.

Field, C. B., Behernefeld, M. J., Randerson, J. T. and Falkowski, P. G.: Primary production of the biosphere: integrating terrestrial and oceanic components., Science (80-.)., 281(5374), 237–240, doi:10.1126/science.281.5374.237, 1998.

Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E. and Oakley, B. B.: Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean., Proc. Natl. Acad. Sci. U. S. A., 102(41), 14683–8, doi:10.1073/pnas.0506625102, 2005.

Galán, A., Molina, V., Thamdrup, B., Woebken, D., Lavik, G., Kuypers, M. M. M. and Ulloa, O.: Anammox bacteria and the anaerobic oxidation of ammonium in the oxygen minimum

zone off northern Chile., Deep Sea Res. Part II Top. Stud. Oceanogr., 56(16), 1021–1031, doi:10.1016/j.dsr2.2008.09.016, 2009.

Van de Graaf, A. A., Mulder, A., de Bruijn, P., Jetten, M. S. M., Robertson, L. A. and Kuenen, J. G.: Anaerobic oxidation of ammonium is a biologically mediated process., Appl. Environ. Microbiol., 61(4), 1246–1251, 1995.

Gruber, N. and Galloway, J. N.: An Earth-system perspective of the global nitrogen cycle., Nature, 451(7176), 293–6, doi:10.1038/nature06592, 2008.

Hamersley, M. R., Lavik, G., Woebken, D., Rattray, J. E., Lam, P., Hopmans, E. C., Sinninghe Damsté, J. S., Krüger, S., Graco, M., Gutierrez, D. and Kuypers, M. M. M.: Anaerobic ammonium oxidation in the Peruvian oxygen minimum zone., Limnol. Oceanogr., 52(3), 923–933, doi:10.4319/lo.2007.52.3.0923, 2007.

Ingalls, A. E., Huguet, C. and Truxal, L. T.: Distribution of intact and core membrane lipids of archaeal glycerol dialkyl glycerol tetraethers among size-fractionated particulate organic matter in Hood canal, Puget Sound., Appl. Environ. Microbiol., 78(5), 1480–90, doi:10.1128/AEM.07016-11, 2012.

Jaeschke, A., Hopmans, E. C., Wakeham, S. G., Schouten, S. and Sinninghe Damsté, J. S.: The presence of ladderane lipids in the oxygen minimum zone of the Arabian Sea indicates nitrogen loss through anammox., Limnol. Oceanogr., 52(2), 780–786, doi:10.4319/lo.2007.52.2.0780, 2007.

Jaeschke, A., Rooks, C., Trimmer, M., Nicholls, J. C., Hopmans, E. C., Schouten, S. and Sinninghe Damsté, J. S.: Comparison of ladderane phospholipid and core lipids as indicators for anaerobic ammonium oxidation (anammox) in marine sediments., Geochim. Cosmochim. Acta, 73(7), 2077–2088, doi:10.1016/j.gca.2009.01.013, 2009.

Jensen, M. M., Lam, P., Revsbech, N. P., Nagel, B., Gaye, B., Jetten, M. S. M. and Kuypers, M. M. M.: Intensive nitrogen loss over the Omani Shelf due to anammox coupled with dissimilatory nitrite reduction to ammonium., ISME J., 5(10), 1660–70, doi:10.1038/ismej.2011.44, 2011.

Jensen, M. M., Petersen, J., Dalsgaard, T. and Thamdrup, B.: Pathways, rates, and regulation of N_2 production in the chemocline of an anoxic basin, Mariager Fjord, Denmark., Mar. Chem., 113(1-2), 102–113, doi:10.1016/j.marchem.2009.01.002, 2009.

Kalvelage, T., Lavik, G., Lam, P., Contreras, S., Arteaga, L., Löscher, C. R., Oschlies, A., Paulmier, A., Stramma, L. and Kuypers, M. M. M.: Nitrogen cycling driven by organic matter export in the South Pacific oxygen minimum zone., Nat. Geosci., 6(3), 228–234, doi:10.1038/ngeo1739, 2013.

Karl, D., Letelier, R., Tupas, L., Dore, J., Christian, J. and Hebel, D.: The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean., Nature, 388(6642), 533–538, doi:10.1038/41474, 1997.

Karner, M. B., DeLong, E. F. and Karl, D. M.: Archaeal dominance in the mesopelagic zone of the Pacific Ocean., Nature, 409(6819), 507–10, doi:10.1038/35054051, 2001.

Karstensen, J., Stramma, L. and Visbeck, M.: Oxygen minimum zones in the eastern tropical Atlantic and Pacific oceans., Prog. Oceanogr., 77(4), 331–350, doi:10.1016/j.pocean.2007.05.009, 2008.

Kartal, B., Kuypers, M. M. M., Lavik, G., Schalk, J., Op den Camp, H. J. M., Jetten, M. S. M. and Strous, M.: Anammox bacteria disguised as denitrifiers: nitrate reduction to dinitrogen gas via nitrite and ammonium., Environ. Microbiol., 9(3), 635–42, doi:10.1111/j.1462-2920.2006.01183.x, 2007.

Kessler, W. S.: The circulation of the eastern tropical Pacific: a review., Prog. Oceanogr., 69(2-4), 181–217, doi:10.1016/j.pocean.2006.03.009, 2006.

Koeve, W. and Kähler, P.: Heterotrophic denitrification vs. autotrophic anammox – quantifying collateral effects on the oceanic carbon cycle., Biogeosciences, 7(8), 2327–2337, doi:10.5194/bg-7-2327-2010, 2010.

Könneke, M., Bernhard, A. E., de la Torre, J. R., Walker, C. B., Waterbury, J. B. and Stahl, D. A.: Isolation of an autotrophic ammonia-oxidizing marine archaeon., Nature, 437(7058), 543–6, doi:10.1038/nature03911, 2005.

Könneke, M., Schubert, D. M., Brown, P. C., Hügler, M., Standfest, S., Schwander, T., Schada von Borzyskowski, L., Erb, T. J., Stahl, D. A. and Berg, I. A.: Ammonia-oxidizing archaea use the most energy-efficient aerobic pathway for CO_2 fixation., Proc. Natl. Acad. Sci. U. S. A., 111(22), 8239–8244, doi:10.1073/pnas.1402028111, 2014.

Kuypers, M. M. M., Lavik, G., Woebken, D., Schmid, M., Fuchs, B. M., Amann, R., Jørgensen, B. B. and Jetten, M. S. M.: Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation., Proc. Natl. Acad. Sci. U. S. A., 102(18), 6478–83, doi:10.1073/pnas.0502088102, 2005.

Kuypers, M. M. M., Sliekers, A. O., Lavik, G., Schmid, M., Jørgensen, B. B., Kuenen, J. G., Sinninghe Damsté, J. S., Strous, M. and Jetten, M. S. M.: Anaerobic ammonium oxidation by anammox bacteria in the Black Sea., Nature, 422(6932), 608–611, doi:10.1038/nature01526.1., 2003.

De la Torre, J. R., Walker, C. B., Ingalls, A. E., Könneke, M. and Stahl, D. A.: Cultivation of a thermophilic ammonia oxidizing archaeon synthesizing crenarchaeol., Environ. Microbiol., 10(3), 810–8, doi:10.1111/j.1462-2920.2007.01506.x, 2008.

Lam, P., Jensen, M. M., Kock, A., Lettmann, K. A., Plancherel, Y., Lavik, G., Bange, H. W. and Kuypers, M. M. M.: Origin and fate of the secondary nitrite maximum in the Arabian Sea., Biogeosciences, 8(6), 1565–1577, doi:10.5194/bg-8-1565-2011, 2011.

Lam, P., Jensen, M. M., Lavik, G., McGinnis, D. F., Müller, B., Schubert, C. J., Amann, R., Thamdrup, B. and Kuypers, M. M. M.: Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea., Proc. Natl. Acad. Sci. U. S. A., 104(17), 7104–9, doi:10.1073/pnas.0611081104, 2007.

Lam, P. and Kuypers, M. M. M.: Microbial nitrogen cycling processes in oxygen minimum zones., Ann. Rev. Mar. Sci., 3(1), 317–345, doi:10.1146/annurev-marine-120709-142814, 2011.

Lam, P., Lavik, G., Jensen, M. M., van de Vossenberg, J., Schmid, M., Woebken, D., Gutiérrez, D., Amann, R., Jetten, M. S. M. and Kuypers, M. M. M.: Revising the nitrogen cycle in the Peruvian oxygen minimum zone., Proc. Natl. Acad. Sci. U. S. A., 106(12), 4752–7, doi:10.1073/pnas.0812444106, 2009.

Lavín, M. F., Fiedler, P. C., Amador, J. A., Ballance, L. T., Färber-Lorda, J. and Mestas-Nuñez, A. M.: A review of eastern tropical Pacific oceanography: summary., Prog. Oceanogr., 69(2-4), 391–398, doi:10.1016/j.pocean.2006.03.005, 2006.

Lengger, S. K., Hopmans, E. C., Reichart, G.-J., Nierop, K. G. J., Sinninghe Damsté, J. S. and Schouten, S.: Intact polar and core glycerol dibiphytanyl glycerol tetraether lipids in the Arabian Sea oxygen minimum zone. Part II: Selective preservation and degradation in sediments and consequences for the TEX_{86}., Geochim. Cosmochim. Acta, 98, 244–258, doi:10.1016/j.gca.2012.05.003, 2012.

Lipschultz, F., Wofsy, S. C., Ward, B. B., Codispoti, L. A., Friedrich, G. and Elkins, J. W.: Bacterial transformations of inorganic nitrogen in the oxygen-deficient waters of the eastern tropical South Pacific Ocean., Deep Sea Res., 37(10), 1513–1541, doi:10.1016/0198-0149(90)90060-9, 1990.

Martens-Habbena, W., Berube, P. M., Urakawa, H., de la Torre, J. R. and Stahl, D. A.: Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria., Nature, 461(7266), 976–9, doi:10.1038/nature08465, 2009.

Park, B.-J., Park, S.-J., Yoon, D.-N., Schouten, S., Sinninghe Damsté, J. S. and Rhee, S.-K.: Cultivation of autotrophic ammonia-oxidizing archaea from marine sediments in coculture with sulfur-oxidizing bacteria., Appl. Environ. Microbiol., 76(22), 7575–87, doi:10.1128/AEM.01478-10, 2010.

Paulmier, A. and Ruiz-Pino, D.: Oxygen minimum zones (OMZs) in the modern ocean., Prog. Oceanogr., 80(3-4), 113–128, doi:10.1016/j.pocean.2008.08.001, 2009.

Pester, M., Schleper, C. and Wagner, M.: The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology., Curr. Opin. Microbiol., 14(3), 300–6, doi:10.1016/j.mib.2011.04.007, 2011.

Pitcher, A., Hopmans, E. C., Mosier, A. C., Park, S.-J., Rhee, S.-K., Francis, C. A., Schouten, S. and Sinninghe Damsté, J. S.: Core and intact polar glycerol dibiphytanyl glycerol tetraether lipids of ammonia-oxidizing archaea enriched from marine and estuarine sediments., Appl. Environ. Microbiol., 77(10), 3468–77, doi:10.1128/AEM.02758-10, 2011a.

Pitcher, A., Rychlik, N., Hopmans, E. C., Spieck, E., Rijpstra, W. I. C., Ossebaar, J., Schouten, S., Wagner, M. and Sinninghe Damsté, J. S.: Crenarchaeol dominates the membrane lipids of Candidatus Nitrososphaera gargensis, a thermophilic group I.1b archaeon., ISME J., 4(4), 542–52, doi:10.1038/ismej.2009.138, 2010.

Pitcher, A., Villanueva, L., Hopmans, E. C., Schouten, S., Reichart, G.-J. and Sinninghe Damsté, J. S.: Niche segregation of ammonia-oxidizing archaea and anammox bacteria in the Arabian Sea oxygen minimum zone., ISME J., 5(12), 1896–904, doi:10.1038/ismej.2011.60, 2011b.

Pitcher, A., Wuchter, C., Siedenberg, K., Schouten, S. and Sinninghe Damsté, J. S.: Crenarchaeol tracks winter blooms of ammonia-oxidizing Thaumarchaeota in the coastal North Sea., Limnol. Oceanogr., 56(6), 2308–2318, doi:10.4319/lo.2011.56.6.2308, 2011c.

Podlaska, A., Wakeham, S. G., Fanning, K. A. and Taylor, G. T.: Microbial community structure and productivity in the oxygen minimum zone of the eastern tropical North Pacific., Deep Sea Res. Part I Oceanogr. Res. Pap., 66, 77–89, doi:10.1016/j.dsr.2012.04.002, 2012.

Qin, W., Amin, S. A., Martens-Habbena, W., Walker, C. B., Urakawa, H., Devol, A. H., Ingalls, A. E., Moffett, J. W., Armbrust, E. V. and Stahl, D. A.: Marine ammonia-oxidizing archaeal isolates display obligate mixotrophy and wide ecotypic variation., Proc. Natl. Acad. Sci., 111(34), 12504–12509, doi:10.1073/pnas.1324115111, 2014.

Rattray, J. E., van de Vossenberg, J., Hopmans, E. C., Kartal, B., van Niftrik, L., Rijpstra, W. I. C., Strous, M., Jetten, M. S. M., Schouten, S. and Sinninghe Damsté, J. S.: Ladderane lipid distribution in four genera of anammox bacteria., Arch. Microbiol., 190(1), 51–66, doi:10.1007/s00203-008-0364-8, 2008.

Revsbech, N. P., Larsen, L. H., Gundersen, J., Dalsgaard, T., Ulloa, O. and Thamdrup, B.: Determination of ultra-low oxygen concentrations in oxygen minimum zones by the STOX sensor., Limnol. Oceanogr. Methods, 7, 371–381, doi:10.4319/lom.2009.7.371, 2009.

Rusch, A., Hannides, A. K. and Gaidos, E.: Diverse communities of active Bacteria and Archaea along oxygen gradients in coral reef sediments, Coral Reefs, 28(1), 15–26, doi:10.1007/s00338-008-0427-y, 2009.

Rush, D., Wakeham, S. G., Hopmans, E. C., Schouten, S. and Sinninghe Damsté, J. S.: Biomarker evidence for anammox in the oxygen minimum zone of the eastern tropical North Pacific., Org. Geochem., 53, 80–87, doi:10.1016/j.orggeochem.2012.02.005, 2012.

Schmidt, I. and Bock, E.: Anaerobic ammonia oxidation with nitrogen dioxide by Nitrosomonas eutropha., Arch. Microbiol., 167(2/3), 106–11, 1997.

Schouten, S., Hopmans, E. C., Baas, M., Boumann, H., Standfest, S., Könneke, M., Stahl, D. A. and Sinninghe Damsté, J. S.: Intact membrane lipids of "Candidatus Nitrosopumilus maritimus," a cultivated representative of the cosmopolitan mesophilic group I Crenarchaeota., Appl. Environ. Microbiol., 74(8), 2433–40, doi:10.1128/AEM.01709-07, 2008.

Schouten, S., Pitcher, A., Hopmans, E. C., Villanueva, L., van Bleijswijk, J. and Sinninghe Damsté, J. S.: Intact polar and core glycerol dibiphytanyl glycerol tetraether lipids in the Arabian Sea oxygen minimum zone: I. Selective preservation and degradation in the water column and consequences for the TEX_{86}., Geochim. Cosmochim. Acta, 98, 228–243, doi:10.1016/j.gca.2012.05.002, 2012.

Schouten, S., Strous, M., Kuypers, M. M. M., Rijpstra, W. I. C., Baas, M., Schubert, C. J., Jetten, S. M. and Sinninghe Damsté, J. S.: Stable carbon isotopic fractionations associated with inorganic carbon fixation by anaerobic ammonium-oxidizing Bacteria., Appl. Environ. Microbiol., doi:10.1128/AEM.70.6.3785, 2004.

Sinninghe Damsté, J. S., Rijpstra, W. I. C., Hopmans, E. C., Prahl, F. G., Wakeham, S. G. and Schouten, S.: Distribution of membrane lipids of planktonic Crenarchaeota in the Arabian Sea., Appl. Environ. Microbiol., doi:10.1128/AEM.68.6.2997, 2002a.

Sinninghe Damsté, J. S., Schouten, S., Hopmans, E. C., van Duin, A. C. T. and Geenevasen, J. A. J.: Crenarchaeol: the characteristic core glycerol dibiphytanyl glycerol tetraether membrane lipid of cosmopolitan pelagic crenarchaeota., J. Lipid Res., 43(10), 1641–1651, doi:10.1194/jlr.M200148-JLR200, 2002b.

Sinninghe Damsté, J. S., Strous, M., Rijpstra, W. I. C., Hopmans, E. C., Geenevasen, J. A. J., van Duin, A. C. T., van Niftrik, L. and Jetten, M. S. M.: Linearly concatenated cyclobutane lipids form a dense bacterial membrane., Nature, 419(October), 708–712, doi:10.1038/nature01067.1., 2002c.

Spang, A., Hatzenpichler, R., Brochier-Armanet, C., Rattei, T., Tischler, P., Spieck, E., Streit, W., Stahl, D. A., Wagner, M. and Schleper, C.: Distinct gene set in two different lineages of ammonia-oxidizing archaea supports the phylum Thaumarchaeota., Trends Microbiol., 18(8), 331–40, doi:10.1016/j.tim.2010.06.003, 2010.

Stahl, D. A. and de la Torre, J. R.: Physiology and diversity of ammonia-oxidizing archaea., Annu. Rev. Microbiol., 66, 83–101, doi:10.1146/annurev-micro-092611-150128, 2012.

Stolper, D. A., Revsbech, N. P. and Canfield, D. E.: Aerobic growth at nanomolar oxygen concentrations., Proc. Natl. Acad. Sci. U. S. A., 107(44), 18755–60, doi:10.1073/pnas.1013435107, 2010.

Strous, M., Fuerst, J., Kramer, E. H. M., Logemann, S., Muyzer, G., van de Pas-Schoonen, K., Webb, R., Kuenen, J. G. and Jetten, M. S. M.: Missing lithotroph identified as new planctomycete., Nature, 400(July), 446–449, doi:10.1038/22749, 1999.

Sturt, H. F., Summons, R. E., Smith, K., Elvert, M. and Hinrichs, K.-U.: Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry–new biomarkers for biogeochemistry and microbial ecology., Rapid Commun. Mass Spectrom., 18(6), 617–28, doi:10.1002/rcm.1378, 2004.

Thamdrup, B. and Dalsgaard, T.: Production of N_2 through Anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments., Appl. Environ. Microbiol., 68(3), 1312–1318, doi:10.1128/AEM.68.3.1312, 2002.

Thamdrup, B., Dalsgaard, T., Jensen, M. M., Ulloa, O., Farías, L. and Escribano, R.: Anaerobic ammonium oxidation in the oxygen-deficient waters off northern Chile., Limnol. Oceanogr., 51(5), 2145–2156, doi:10.4319/lo.2006.51.5.2145, 2006.

Thamdrup, B., Dalsgaard, T. and Revsbech, N. P.: Widespread functional anoxia in the oxygen minimum zone of the eastern South Pacific., Deep Sea Res. Part I Oceanogr. Res. Pap., 65, 36–45, doi:10.1016/j.dsr.2012.03.001, 2012.

Tiano, L., Garcia-Robledo, E., Dalsgaard, T., Devol, A. H., Ward, B. B., Ulloa, O., Canfield, D. E. and Peter Revsbech, N.: Oxygen distribution and aerobic respiration in the north and south eastern tropical Pacific oxygen minimum zones, Deep Sea Res. Part I Oceanogr. Res. Pap., 94, 173–183, doi:10.1016/j.dsr.2014.10.001, 2014.

Venter, J. C., Remington, K., Heidelberg, J. F., Halpern, A. L., Rusch, D., Eisen, J. A., Wu, D., Paulsen, I., Nelson, K. E., Nelson, W., Fouts, D. E., Levy, S., Knap, A. H., Lomas, M. W., Nealson, K., White, O., Peterson, J., Hoffman, J., Parsons, R., Baden-Tillson, H., Pfannkoch, C., Rogers, Y.-H. and Smith, H. O.: Environmental genome shotgun sequencing of the Sargasso Sea., Science, 304(5667), 66–74, doi:10.1126/science.1093857, 2004.

Ward, B. B.: How nitrogen is lost., Science, 341(6144), 352–3, doi:10.1126/science.1240314, 2013.

Ward, B. B., Devol, A. H., Rich, J. J., Chang, B. X., Bulow, S. E., Naik, H., Pratihary, A. and Jayakumar, A.: Denitrification as the dominant nitrogen loss process in the Arabian Sea., Nature, 461(7260), 78–81, doi:10.1038/nature08276, 2009.

Ward, B. B., Tuit, C. B., Jayakumar, A., Rich, J. J., Moffett, J. and Naqvi, S. W. A.: Organic carbon, and not copper, controls denitrification in oxygen minimum zones of the ocean., Deep Sea Res. Part I Oceanogr. Res. Pap., 55(12), 1672–1683, doi:10.1016/j.dsr.2008.07.005, 2008.

Woebken, D., Fuchs, B. M., Kuypers, M. M. M. and Amann, R.: Potential interactions of particle-associated anammox bacteria with bacterial and archaeal partners in the Namibian upwelling system., Appl. Environ. Microbiol., 73(14), 4648–57, doi:10.1128/AEM.02774-06, 2007.

Wuchter, C., Abbas, B., Coolen, M. J. L., Herfort, L., van Bleijswijk, J., Timmers, P., Strous, M., Teira, E., Herndl, G. J., Middelburg, J. J., Schouten, S. and Sinninghe Damsté, J. S.: Archaeal nitrification in the ocean., Proc. Natl. Acad. Sci. U. S. A., 103(33), 12317–22, doi:10.1073/pnas.0600756103, 2006.

Yan, J., Haaijer, S. C. M., Op den Camp, H. J. M., van Niftrik, L., Stahl, D. A., Könneke, M., Rush, D., Sinninghe Damsté, J. S., Hu, Y. Y. and Jetten, M. S. M.: Mimicking the oxygen minimum zones: stimulating interaction of aerobic archaeal and anaerobic bacterial ammonia oxidizers in a laboratory-scale model system., Environ. Microbiol., 14(12), 3146–58, doi:10.1111/j.1462-2920.2012.02894.x, 2012.

Yan, J., Op den Camp, H. J. M., Jetten, M. S. M., Hu, Y. Y. and Haaijer, S. C. M.: Induced cooperation between marine nitrifiers and anaerobic ammonium-oxidizing bacteria by incremental exposure to oxygen., Syst. Appl. Microbiol., 33(7), 407–15, doi:10.1016/j.syapm.2010.08.003, 2010.

Table 1. Sampling locations for SPM during the Eastern Tropical North Pacific cruise aboard the R/V Thomas G. Thompson (March-April 2012). For each sampling location the table reports the depth of SPM sampling, the volume of water filtered by each pump deployed as well as physical parameters of the water at these depths, i.e. temperature and oxygen concentration (O_2). Nutrients concentrations, i.e. nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+) and corresponding station and depth of sampling, when available, are also reported.

Station	Location	Temperature CTD	Depth of SPM sampling	Water filtered	O_2	Depth of nutrients sampling	NO_3^-	NO_2^-	NH_4^+
		(°C)	(m)	(L)	(µM)	(m)	(µM)	(µM)	(µM)
106	20°14.49' N	15	70	1627.72	0.75	n.d.	n.d.	n.d.	n.d.
	106°10.00' W	13	105	200.00	0.81	n.d.	n.d.	n.d.	n.d.
		10	365	699.50	0.97	n.d.	n.d.	n.d.	n.d.
110	20° 08.48' N	14	70	128.50	0.67	50	20.14	2.32	0.02
	105° 59.18' W	13	125	336.14	0.77	100	15.27	7.06	0.01
		12	150	511.79	0.86	150	20.13	5.12	0.00
		6	710	999.50	1.05	n.d.	n.d.	n.d.	n.d.
114	20° 07.54' N	18	25	104.10	26.20	n.d.	n.d.	n.d.	n.d.
	106° 00.84' W	17	35	97.40	0.78	n.d.	n.d.	n.d.	n.d.
		15	45	255.51	0.71	n.d.	n.d.	n.d.	n.d.
		15	55	230.50	0.71	n.d.	n.d.	n.d.	n.d.
119	20° 08.72' N	14	80	211.60	0.72	100	16.55	5.74	0.00
	105° 59.77' W	8	500	932.50	0.95	501	31.27	1.50	0.00
		5	800	595.50	1.61	800	43.66	0.00	0.00
		5	1000	1069.38	8.78	1001	45.42	0.00	0.00

123	20° 03.50’ N	19	20	41.50	51.16	n.d.	n.d.	n.d.	n.d.
	106° 00.59’ W	14	60	181.70	0.71	n.d.	n.d.	n.d.	n.d.
		13	90	179.81	0.76	n.d.	n.d.	n.d.	n.d.
		12	200	210.50	0.87	n.d.	n.d.	n.d.	n.d.
125-126	20° 04.26’ N	20	15[a]		72.05	15	12.55	0.96	0.36
	106° 00.81’ W	n.d.	1300	1003.00	n.d.	n.d.	n.d.	n.d.	n.d.
		n.d.	1600[a]		n.d.	n.d.	n.d.	n.d.	n.d.
		n.d.	2000	859.66	n.d.	n.d.	n.d.	n.d.	n.d.
136	17° 01.95’ N	14	110	755.19	0.74	110	21.54	1.07	0.00
	106° 31.96’ W	13	150	747.50	0.74	160	19.24	4.74	0.00
		11	250	987.99	0.87	200	21.30	3.37	0.00
		10	350	714.90	0.88	300	23.39	1.97	0.00
141	16° 30.98’ N	23	60	482.64	108.13	60	7.00	1.14	0.16
	107° 08.52 W	16	90	997.00	0.88	80	22.91	0.45	0.00
		15	105	496.00	0.77	100	24.20	0.09	0.00
		12	200	864.96	0.89	181	23.62	5.21	0.00
145	16° 31.78’ N	26	50	368.00	189.01	n.d.	n.d.	n.d.	n.d.
	107° 08.45’W	21	65[a]		69.83	n.d.	n.d.	n.d.	n.d.
		13	155	768.44	0.99	n.d.	n.d.	n.d.	n.d.
		n.d.	710	1417.63	n.d.	n.d.	n.d.	n.d.	n.d.
147-149	16° 31.60’ N	13	170	668.00	0.84	175	27.54	4.04	0.00
	107° 06.80’ W	5	990	1249.18	7.30	n.d.	n.d.	n.d.	n.d.
		4	1100	689.50	13.11	1100	46.27	0.01	0.00
		n.d.	2500	567.81	n.d.	n.d.	n.d.	n.d.	n.d.
154-155	16° 35.34’ N	25	55	382.80	176.12	n.d.	n.d.	n.d.	n.d.

	107° 08.98’ W	15	100	686.29	1.09	104	23.40	0.65	0.00
		13	145	334.50	1.06	n.d.	n.d.	n.d.	n.d.
		n.d.	160	612.86	n.d.	n.d.	n.d.	n.d.	n.d.
157-158	16° 34.66’N	19	80	3287.62	3.49	n.d.	n.d.	n.d.	n.d.
	107° 04.61’ W	13	140	1178.02	0.75	141	25.70	3.89	0.02
		9	450	3484.84	0.96	n.d.	n.d.	n.d.	n.d.
		8	550[a]		1.02	n.d.	n.d.	n.d.	n.d.

[a] Sample not analyzed for lipids.

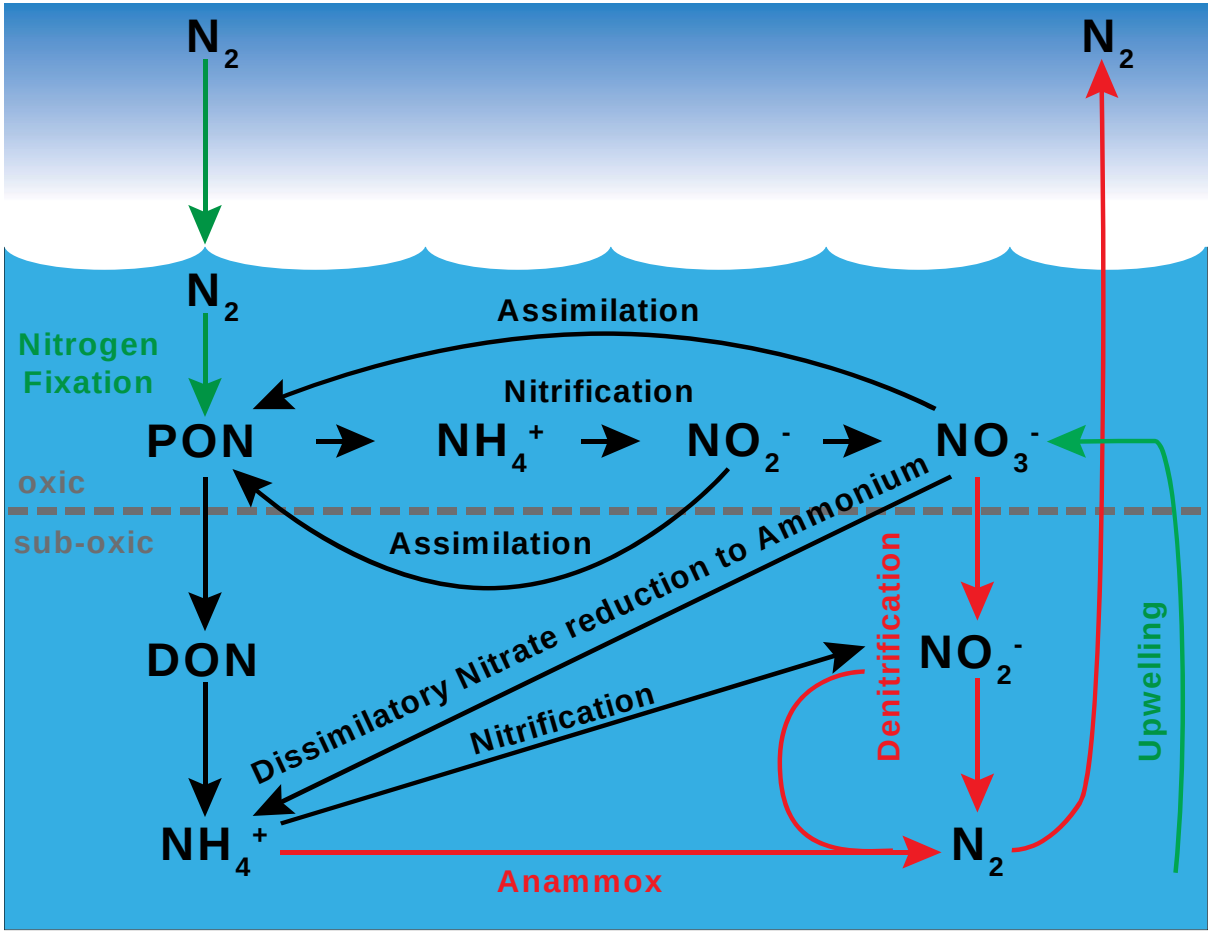


Figure 1. The marine biogeochemical nitrogen cycle. The main redox reactions involved are included: in green are the processes responsible for gains of nitrogen to the ocean, in red are the losses. Modified from Arrigo (2005).

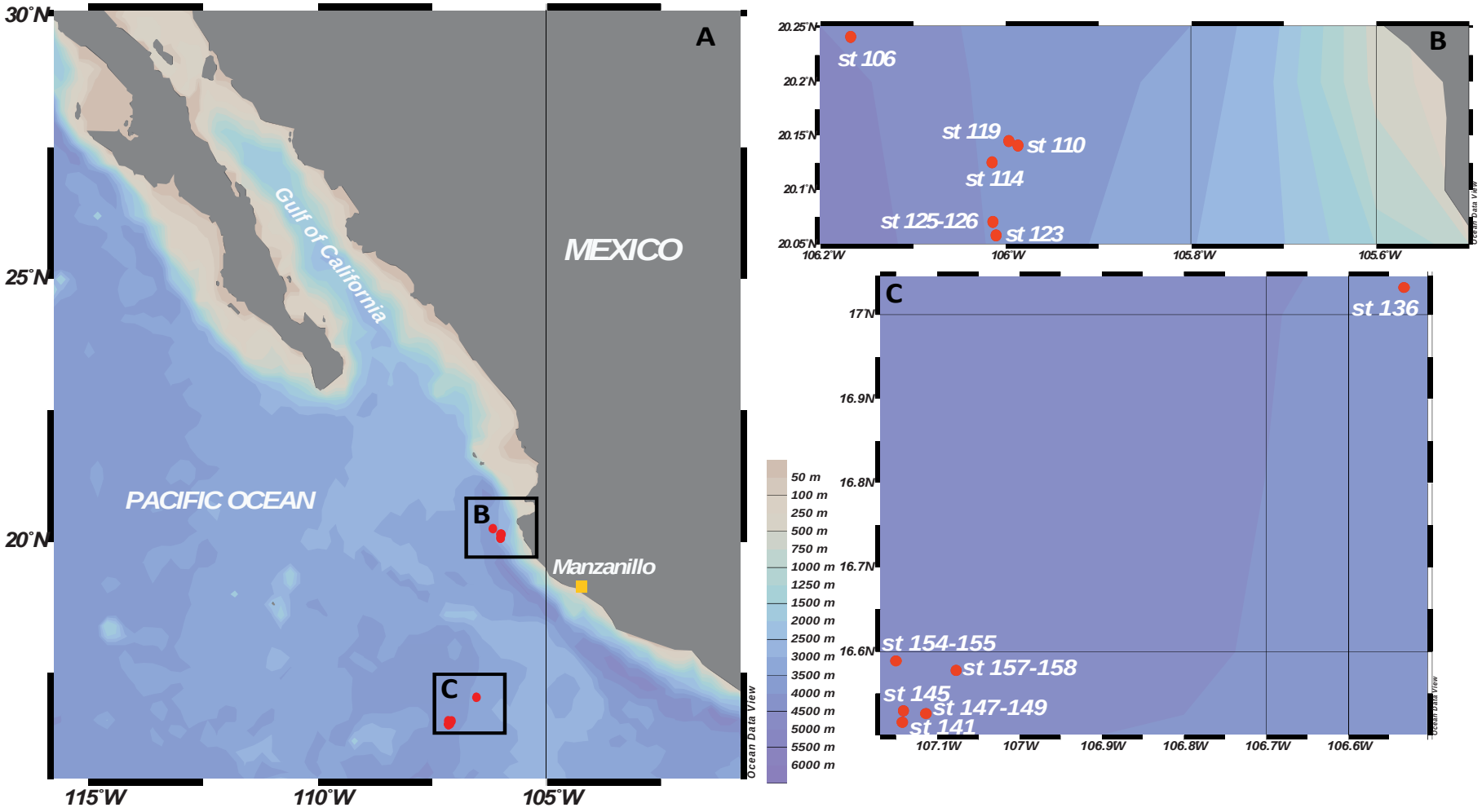
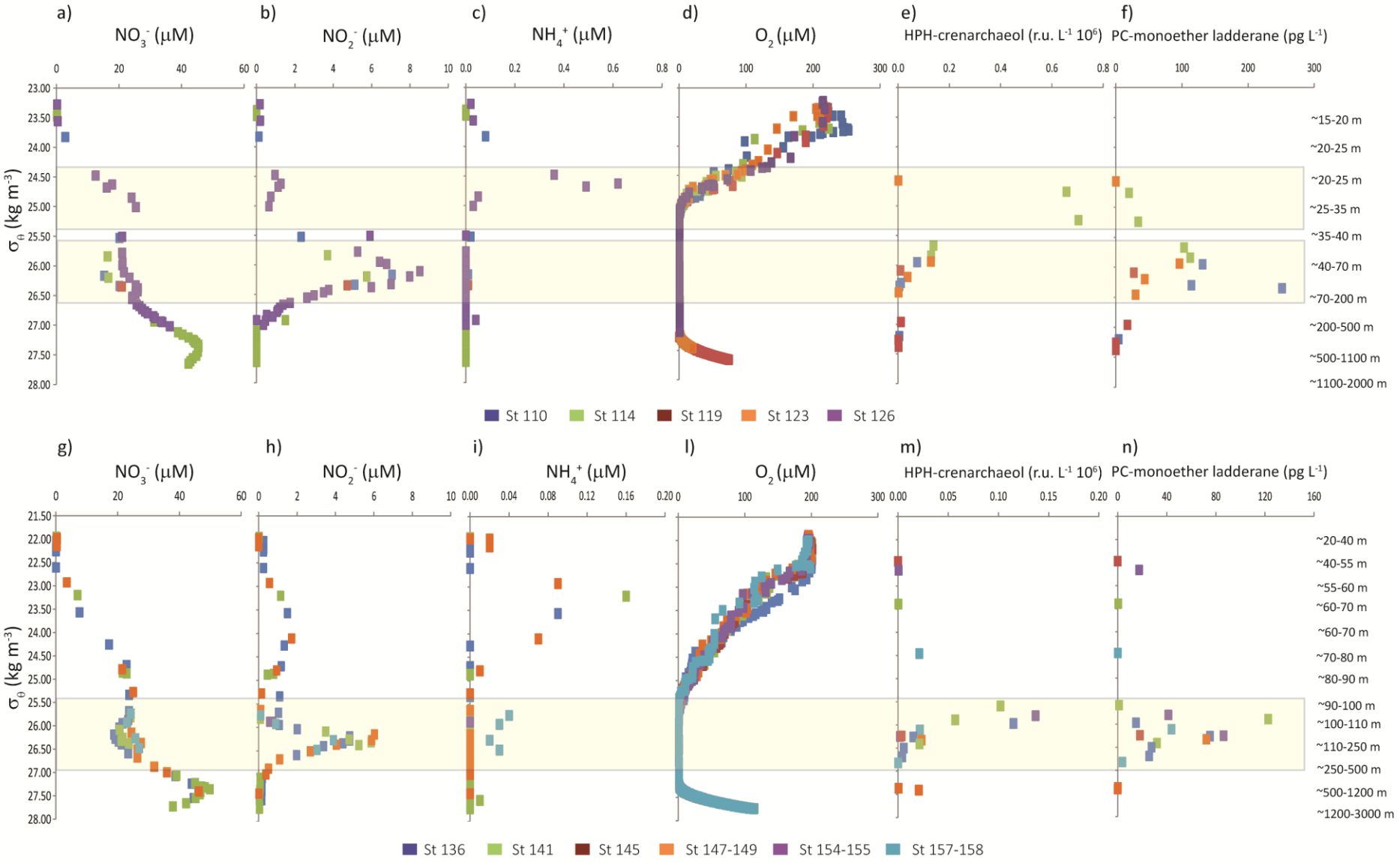


Figure 2. (**A**) Map of the sampling area of the Eastern Tropical North Pacific cruise (March-April 2012) and sampling stations (red dots), in the coastal (**B**) and in the open ocean site (**C**).The coastal sampling site (i.e. st 106, st 110, st 114, st 119, st 123, and st 125-126) is placed in the area

between 20 25.00N and 105 60.00W; the open ocean sampling site (i.e. st 136, st 141, st 145, st 147-149, st 154-155, st 157-158) is placed in the area between 17 00.00N and 106 60.00W.


a)
b)
c)
d)
e)
f)
NO_3^- (µM)
NO_2^- (µM)
NH_4^+ (µM)
O_2 (µM)
HPH-crenarchaeol (r.u. L^{-1} 10^6)
PC-monoether ladderane (pg L^{-1})
σ_θ (kg m^{-3})
~15-20 m
~20-25 m
~20-25 m
~25-35 m
~35-40 m
~40-70 m
~70-200 m
~200-500 m
~500-1100 m
~1100-2000 m
St 110
St 114
St 119
St 123
St 126
g)
h)
i)
l)
m)
n)
NO_3^- (µM)
NO_2^- (µM)
NH_4^+ (µM)
O_2 (µM)
HPH-crenarchaeol (r.u. L^{-1} 10^6)
PC-monoether ladderane (pg L^{-1})
σ_θ (kg m^{-3})
~20-40 m
~40-55 m
~55-60 m
~60-70 m
~60-70 m
~70-80 m
~80-90 m
~90-100 m
~100-110 m
~110-250 m
~250-500 m
~500-1200 m
~1200-3000 m
St 136
St 141
St 145
St 147-149
St 154-155
St 157-158

53

Figure 3. Concentration profiles of (**a**, **g**) nitrate (NO_3^-); (**b**, **h**) nitrite (NO_2^-); (**c, i**) ammonium (NH_4^+); (**d**, **l**) oxygen; (**e**, **m**) hexose-phosphohexose (HPH)-crenarchaeol; and (**f**, **n**) phosphocholine (PC)-monoether ladderane according to the potential density anomalies (σ_θ) of the water column of the ETNP. The corresponding depths intervals are reported on the right side of the figure as a reference. Upper four panels (**a-f**) provide an overview of the complete water column (2000 m) of the coastal sampling site; lower four panels (**g-n**) show the complete water column (3000 m) relative to the open ocean sampling site. All profiles are obtained by combining respectively the coastal and the open ocean stations sampled. The yellow bars highlight the main differences observed between the two sampling sites with respect to the distribution of the two microbial species: i.e. on the one hand a clear niche segregation of AOA and anammox bacteria in the coastal waters of the ETNP and on the other hand a partial overlap of the two niches of these microbial species in the open water setting.

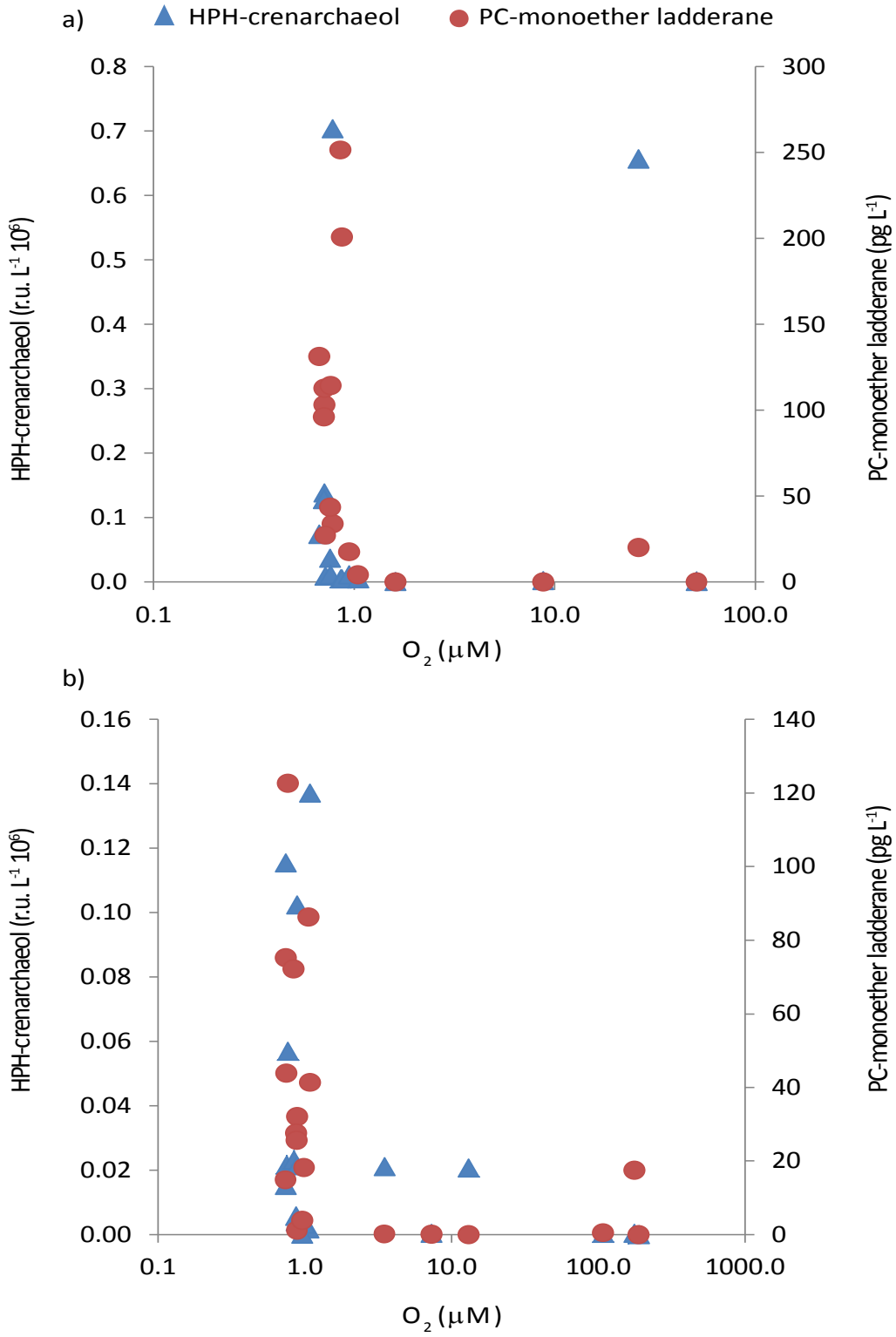


Figure 4. Abundance of HPH-crenarchaeol (r.u. L^{-1}, i.e. response units L^{-1}) and PC-monoether ladderane lipids (pg L^{-1}) according to the concentration of oxygen (µM). In the upper panel (**a**) the response of the two biomarkers at the coastal site; in the lower panel (**b**) the response at the open ocean site.