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

M. Sollai[1], E. C. Hopmans[1], S. Schouten[1,3], R. G. Keil[2], and J. 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, Box 355351, Seattle, Washington, 98195-5351, USA
[3]University of Utrecht, Faculty of Geosciences, P.O. Box 80.021, 3508 TA Utrecht, the Netherlands

Correspondence to: M. Sollai (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 ($< 1\,\mu$M) 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, 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). Codispoti et al. (2001) 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 . 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-oxidizing bacteria (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 the electron acceptor, although AOB have

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been reported to perform nitrification in suboxic 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 the observed dominance of AOA over 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 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 the 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 coexist 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).

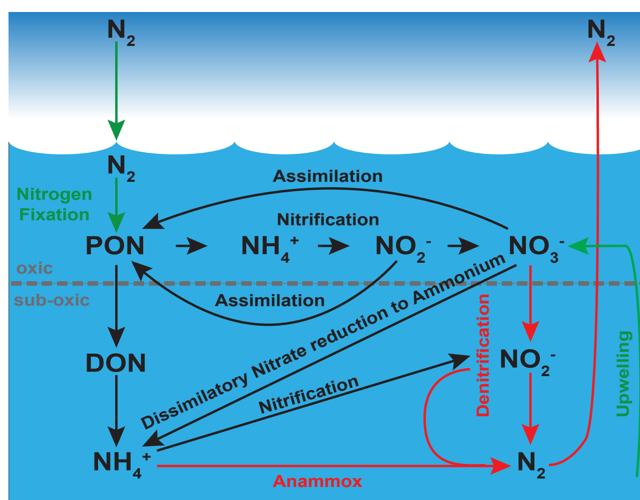

Figure 1. The marine biogeochemical nitrogen cycle. The main redox reactions involved are included: in green are the processes responsible for gains of nitrogen to the ocean, and in red are the losses. Modified from Arrigo (2005).

In this study we investigated the occurrence and depth distribution of Thaumarchaeota and anammox bacteria in the eastern tropical North Pacific (ETNP) ODZ, one of the most extended ODZs in the contemporary ocean. The presence of AOA and anammox bacteria has been reported in the ETNP ODZ 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, nor has 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 concurrently examined 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, c). 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 headgroups comprising phosphocholine (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 in addition to four cyclopentane moieties (de la Torre et al., 2008; Pitcher et al., 2010; Schouten et al., 2008; Sinninghe Damsté et al., 2002b, c). Attached to crenarchaeol are various polar headgroups such as monohexose (MH), dihexose (DH) and 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, we investigated the depth habitat of Thaumarchaeota and anammox bacteria, respectively, in the ETNP ODZ 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 subthermocline 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 ODZ comprises part of the North Pacific subtropical 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 northern Mexico, encounters the NEC is characterized by Ekman transport westward and upwelling mainly off the Californian coast and in a weakened magnitude off the northern 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 yet fully understood (Kessler, 2006). Although ODZs have now been studied for almost a century, it has only recently become possible to determine in situ concentration in these areas to the accuracy of nanomolar O_2 (Revsbech et al., 2009). This has allowed for it to be proven that ODZs, 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. 2a). The cruise route was split into two legs, with the former one comprising six sampling stations (Fig. 2b), in very close proximity to each other, in coastal waters, northwest of the departure port of Manzanillo (Colima, Mexico), and the latter including six sampling stations, clustered closely together, in open-ocean waters southwest of the departure port, around the area known as the Moctezuma Trough (Fig. 2c). 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 into two halves and frozen at −40 °C.

Physical parameters of the water column were recorded using 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, 0.01 and 0.07 µM. The electrochemical oxygen sensor SBE 43 has a detection limit of 1–2 µM (Tiano et al., 2014).

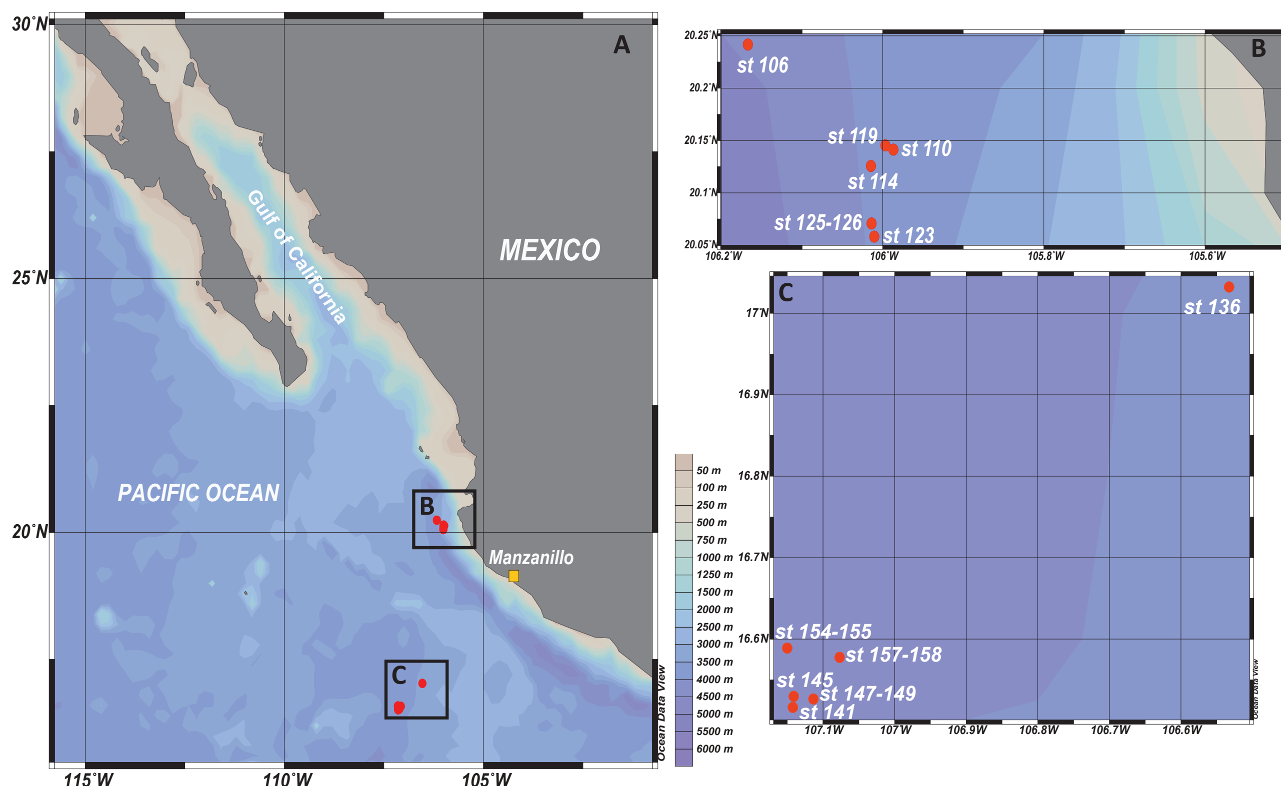

Figure 2. **(a)** Map of the sampling area of the eastern tropical North Pacific cruise (March–April 2012) and sampling stations (red dots); panel **(b)** shows the coastal site and panel **(c)** the open-ocean site. The coastal sampling site (i.e., st 106, st 110, st 114, st 119, st 123, and st 125–126) is placed in the area between 20°25.00′ N and 105°60.00′ W; 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°00.00′ N and 106°60.00′ W.

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 mass spectrometry (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 specifically targeting 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 per liter (i.e., r.u. L^{-1}), revealed 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 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).

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, and 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. n.d.: not detected

Station	Location	Temperature CTD (°C)	Depth of SPM sampling (m)	Water filtered (L)	O_2 (µM)	Depth of nutrients sampling (m)	NO_3^- (µM)	NO_2^- (µM)	NH_4^+ (µM)
106	20°14.49′ N 106°10.00′ W	15	70	1627.72	0.75	n.d.	n.d.	n.d.	n.d.
		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 105°59.18′ W	14	70	128.50	0.67	50	20.14	2.32	0.02
		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 106°00.84′ W	18	25	104.10	26.20	n.d.	n.d.	n.d.	n.d.
		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 105°59.77′ W	14	80	211.60	0.72	100	16.55	5.74	0.00
		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 106°00.59′ W	19	20	41.50	51.16	n.d.	n.d.	n.d.	n.d.
		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 106°00.81′ W	20	15[a]		72.05	15	12.55	0.96	0.36
		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 106°31.96′ W	14	110	755.19	0.74	110	21.54	1.07	0.00
		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 107°08.52′ W	23	60	482.64	108.13	60	7.00	1.14	0.16
		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 107°08.45′ W	26	50	368.00	189.01	n.d.	n.d.	n.d.	n.d.
		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 107°06.80′ W	13	170	668.00	0.84	175	27.54	4.04	0.00
		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 107°08.98′ W	25	55	382.80	176.12	n.d.	n.d.	n.d.	n.d.
		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 107°04.61′ W	19	80	3287.62	3.49	n.d.	n.d.	n.d.	n.d.
		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.

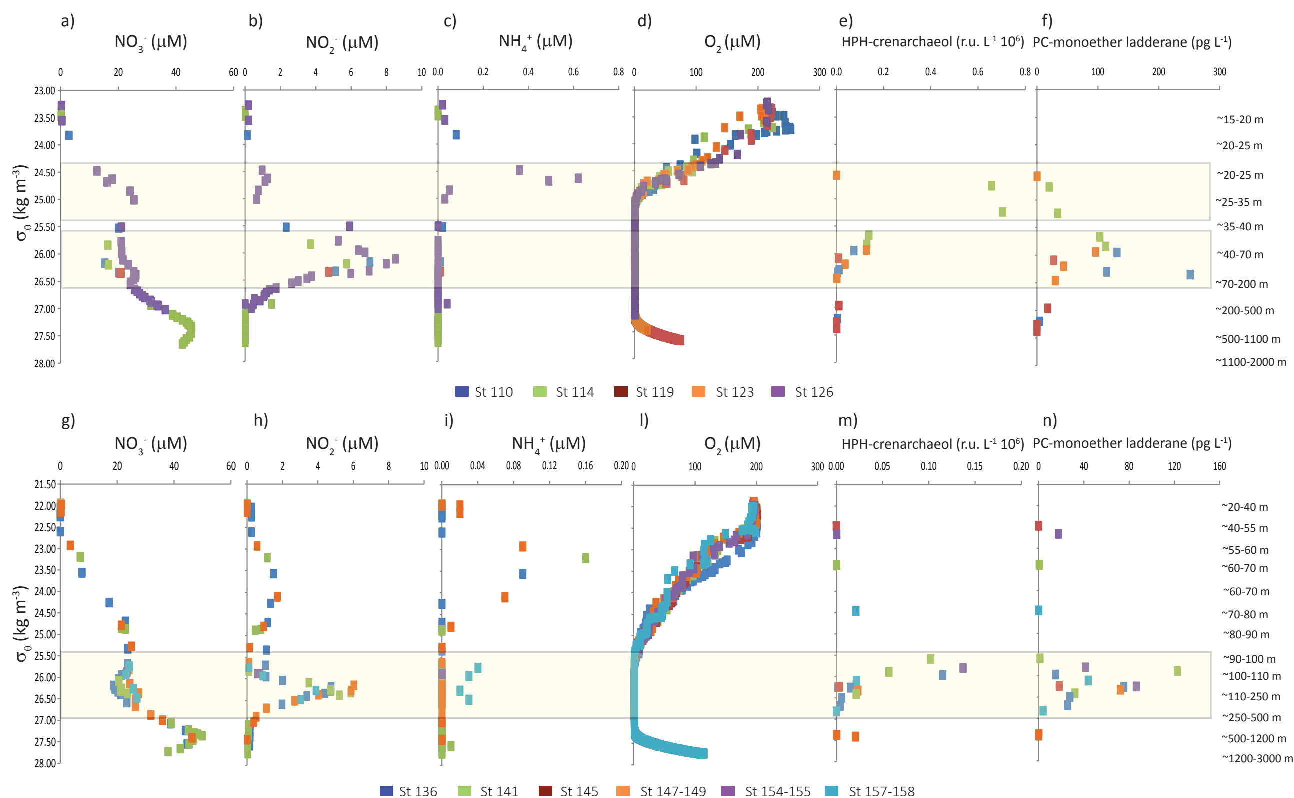

Figure 3. Concentration profiles of (**a**, **g**) nitrate (NO_3^-), (**b**, **h**) nitrite (NO_2^-), (**c**, **i**) ammonium (NH_4^+), (**d**, **l**) oxygen, (**e**, **m**) hexose-phosphohexose (HPH)-crenarchaeol, and (**f**, **n**) phosphocholine (PC)-monoether ladderane according to the potential 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. The upper panels **(a–f)** provide an overview of the complete water column (2000 m) of the coastal sampling site; the lower panels **(g–n)** show the complete water column (3000 m) relative to the open-ocean sampling site. All profiles are obtained by combining 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.

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 (Fig. 3a–c, g–i), oxygen (Fig. 3d and l), and lipid SPM (Fig. 3e, f and m, n) 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. 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 (Fig. 3d and l) 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., $\sim$30 m depth, while for the in open-ocean waters it is located at σ_θ 25.5, which corresponds to $\sim$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 and 50 μM until σ_θ 23.5 (i.e., $\sim$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 $\sim$35 and $\sim$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 $\sim 75\,\mu M$, at 2000 m depth (Fig. 3d). In the open-ocean waters, oxygen concentration is stable at $\sim 200\,\mu M$ 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 $\sim 120\,\mu M$ 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 (Fig. 3a–c, g–i). In both coastal and open waters, nitrate is the most abundant nitrogen species and shows two maxima at different σ_θ (Fig. 3a and g). In coastal waters the first maximum of $\sim 25\,\mu M$ 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 $\sim 45\,\mu M$ 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., $\sim 25\,\mu M$) 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., $\sim 50\,\mu M$) also 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 (Fig. 3b and h). In the former setting the shallow maximum occurs at σ_θ 24.6, at declining oxygen concentrations. The peak is only $\sim 1\,\mu M$ and rather narrow. The lower peak of $\sim 8\,\mu M$ 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 reaches 1 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., $\sim 6\,\mu M$) 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 (Fig. 3c and i), 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 and $\sim 0.1\,\mu M$.

3.2 Biomarker lipid profiles

The SPM for biomarker analysis was 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 $< 0.6\,\mu m$ (Könneke et al., 2005) and they are suggested to be 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 the profile 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.

Figure 3e and m 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., $\sim 17\,pg\,L^{-1}$ at 55 m depth), until the upper ODZ, where it starts to increase to its maximum (i.e., $\sim 251\,pg\,L^{-1}$) 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., $\sim 122\,pg\,L^{-1}$) 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 coexist along the water column (Fig. 3e, f and m, n). Such a distribution has already been observed in other dysoxic or anoxic marine systems worldwide such as the Black Sea and the ODZs of the eastern tropical South Pacific (ETSP) 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 IPL 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

$\sim$26. The trend of our coastal HPH-crenarchaeol depth profile agrees with previously reported data for thaumarchaeotal 16S rRNA, 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, similar 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 (Fig. 3m and n). 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 (Fig. 3e and f) than in the open ocean (Fig. 3m and n), i.e., the concentration of HPH-crenarchaeol is 5 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 suboxic 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 (Fig. 3c and i). Even at concentrations $< 1\,\mu M$, 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 (Fig. 3b and e). In the core ODZ a clear secondary nitrite maximum (SNM) co-occurs with the maximum in anammox bacteria concentration (Fig. 3b and f). 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 to attention zooplankton migrators as an 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

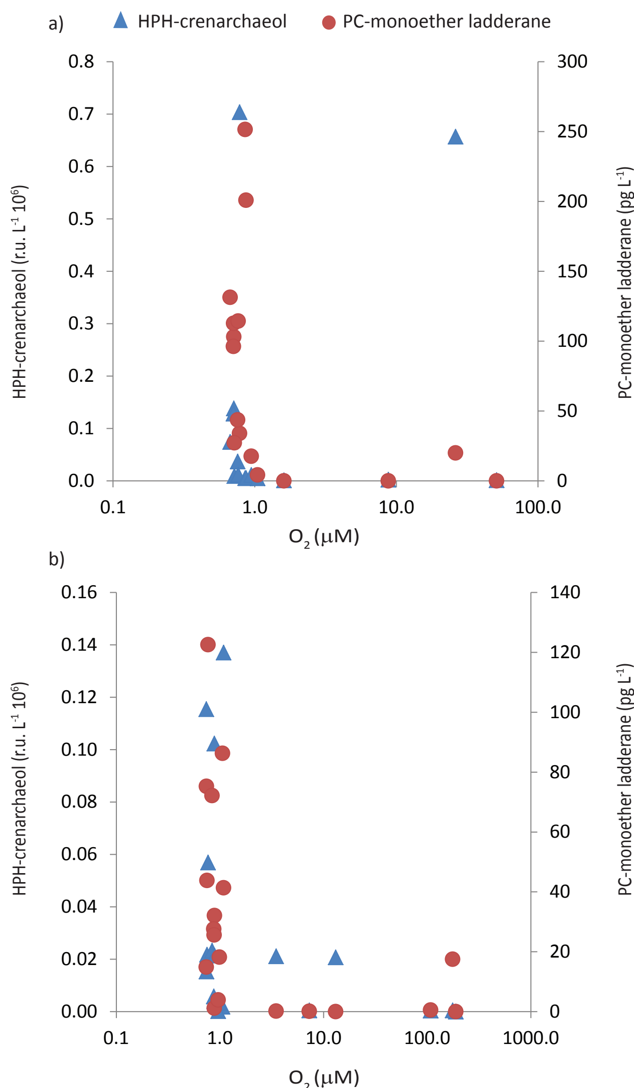

Figure 4. Abundance of HPH-crenarchaeol (r.u. L^{-1}, i.e., response units per liter) and PC-monoether ladderane lipids (pg L^{-1}) according to the concentration of oxygen (µM). The upper panel **(a)** shows the response of the two biomarkers at the coastal site, while the lower panel shows **(b)**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

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 (Fig. 3m and n). In this case, AOA and anammox bacteria could either compete or cooperate 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 (Fig. 3h and i): the secondary nitrite maximum is concurrent with the two biomarker maxima (Fig. 3h, m and n), whereas NH_4^+ is either possibly consumed by anammox as the water column turns suboxic or by nitrification by AOA successfully adapted to conditions of low nutrients and oxygen (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; Koevef 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 favored 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 the ETNP ODZ. 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 whether 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 the coastal and open-ocean sites (Fig. 4a and b). Figure 4a and b show 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, cor-

responding 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.

5 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 gain a detailed insight into 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 ODZ. 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.

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