

The presence of ladderane lipids in the oxygen minimum zone of the Arabian Sea indicates nitrogen loss through anammox

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Abstract

Distributions of ladderane lipids, which are characteristic of membranes of bacteria performing anaerobic ammonium oxidation (anammox), were determined in the northwestern Arabian Sea with respect to season, depth, and distance to the coast of Oman. Ladderane lipids were detected and quantified in suspended particulate matter (SPM) obtained from various water depths along a northwest-to-southeast transect during the spring intermonsoon period. Maximum concentrations of 5–8 pg L⁻¹ generally occurred at 500 m in the upper part of the oxygen minimum zone (OMZ). Fluxes of ladderane lipids obtained from sediment trap material sampled at 500-m water depth ~350 km off the coast reveal a strong seasonal pattern apparently related to the annual monsoon cycle in the northern Arabian Sea, with highest fluxes of 125 ng m⁻² d⁻¹ observed during the southwest monsoon. This fourfold increase in flux during the SW monsoon compared to the spring intermonsoon period may indicate higher anammox bacterial productivity or enhanced export of ladderanes during a period of high particulate matter flux or both. Anammox, in addition to denitrification, seems to be responsible for a significant loss of nutrient nitrogen from OMZ waters in the Arabian Sea.

Anammox, the anaerobic oxidation of ammonium, was first discovered in a wastewater treatment system about a decade ago (Mulder et al. 1995). The microorganisms performing anammox were identified as new members of the order *Planctomycetales*, one of the major and distinct divisions of the domain Bacteria (Strous et al. 1999). Anammox bacteria grow extremely slowly, dividing only once every two weeks (Strous et al. 1999). The anammox reaction involves the oxidation of ammonium under anoxic conditions with nitrite as the electron acceptor and dinitrogen gas as the product. Hydrazine (N₂H₂) and nitric oxide (NO) were found to be important intermediates in this process (Strous et al. 2006). Anammox bacteria have a specific intracytoplasmic compartment where the ana-

mmox reaction takes place, the anammoxosome. It is surrounded by a dense and highly impermeable lipid membrane, consisting of chemotaxonomically unique lipids. These so-called ladderane lipids are composed of up to five linearly concatenated cyclobutane rings (Sinninghe Damsté et al. 2002a). Such a dense membrane is thought to be required to maintain concentration gradients during the exceptionally slow anabolism of anammox bacteria and to protect the cell from the highly toxic intermediate hydrazine (Sinninghe Damsté et al. 2002a).

Evidence for anammox activity in the marine environment was found in the Black Sea (Kuypers et al. 2003), the world's largest anoxic basin, where anammox was detected in the suboxic zone of the water column. Isotopic labelling studies have shown that anammox contributes substantially to N₂ production, i.e., up to 67% in a continental shelf sediment (Thamdrup and Dalsgaard 2002), 19–35% in the anoxic water column of Golfo Dulce, Costa Rica (Dalsgaard et al. 2003), and up to 19% even in Arctic sea ice (Rysgaard and Glud 2004). Recently, anammox was also detected in the oxygen minimum zone (OMZ) of the Benguela upwelling system, one of the most productive regions of the world ocean, indicating that anammox not only takes place in anoxic environments but also in marine regions with low oxygen contents (Kuypers et al. 2005). It has thus been suggested that anammox might be a substantial sink for nitrogen in the OMZ waters off the Namibian coast (Kuypers et al. 2005). Until the discovery

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of anammox, the major (30–50%) sink of the global nutrient N removal occurring in OMZs was mainly attributed to heterotrophic denitrification (Gruber and Sarmiento 1997; Brandes and Devol 2002).

The largest open-ocean OMZ is presently found in the Arabian Sea (Bange et al. 2000). The Arabian Sea is characterized by intense upwelling of nutrient-rich water during the southwest and northeast monsoon periods, which leads to high primary production and enhanced downward particle flux (Honjo et al. 1999). Remineralization of sinking organic matter leads to a high oxygen demand in intermediate waters and causes an extensive OMZ between 150- and 1200-m depth. As much as ~20% of the major water column denitrification in the world ocean is estimated to take place in the Arabian Sea, and thus this area has a significant influence on the global oceanic nitrogen budget (Codispoti 1989; Gruber and Sarmiento 1997). However, the contribution of anammox to loss of nitrogen in this area has yet to be established.

In this study, we investigate whether anammox bacteria are present in the water column of the Arabian Sea and the extent to which anammox might be responsible for the removal of fixed inorganic nitrogen from oxygen-depleted parts of the water column. We focus on the spatial and seasonal distribution of anammox-specific ladderane lipids in the northwestern Arabian Sea.

Material and methods

Suspended particulate matter and sediment traps—Suspended particulate matter (SPM) samples were collected at seven sites in the northwestern Arabian Sea (Fig. 1) during U. S. Joint Global Ocean Flux Study (JGOFS) Arabian Sea Process Study (ASPS) cruise TN047 of the R/V *Thomas G. Thompson*. All stations (except station 13) formed a transect perpendicular to the coast of Oman, and the most remote station was ~570 km off the coast. SPM samples used for this study were taken during the spring intermonsoon (SI) period in mid-May 1995, which is generally a period of low primary productivity. SPM samples were collected at specific depths of the water column (Table 1) by filtration of large volumes of water (~2,400 to 3,000 liters) through 292-mm-diameter, precombusted glass fiber filters (nominal pore size 0.7 μm) with a Challenger Oceanic Mark II in situ pump. Filters were stored frozen (-20°C) until they were used for extraction.

Sinking particulate matter was collected in sediment traps (IRS-C traps; Peterson et al. 1993) deployed over an annual cycle along the southern U. S. JGOFS section off the coast of Oman. The traps were deployed in November 1994 during cruise TN047 of R/V *Thomas G. Thompson* and recovered in January 1996 during cruise TN050. Samples used for this study derived from an ~500-m-deep sediment trap at site MS-3 ($17^{\circ}12'\text{N}$, $59^{\circ}36'\text{E}$; Fig. 1), which was located ~350 km offshore in 3,000 m of water (Honjo et al. 1999; Wakeham et al. 2002). Particulate matter was collected over variable time intervals, ranging from 9 to 34 d, with shorter intervals set for monsoon periods when high flux was expected, giving a total of 22 time-resolved samples for the study period of 408 d.

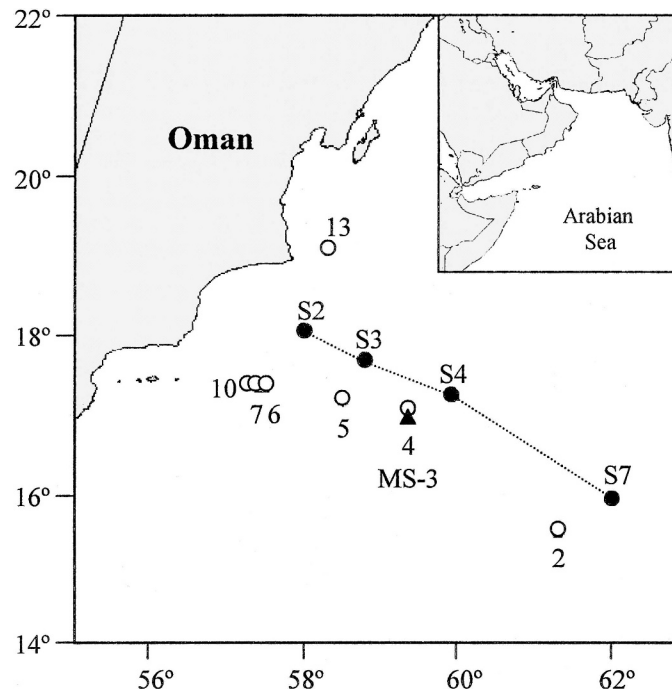


Fig. 1. Site locations (open circles) of the seven hydrographic stations where suspended particulate matter (SPM) was obtained during the U. S. JGOFS Arabian Sea Process Study cruise TTN047 in May 1995, and mooring site of IRS-C sediment trap MS-3 (filled triangle). The dotted line indicates part of the southern U. S. JGOFS Arabian Sea Process Study transect where nutrient data were collected in April 1995. The filled circles indicate stations S2–S7.

Mercuric chloride was added as a biocide (Lee et al. 1992), and upon recovery, trap samples were sealed in their collection tubes and stored refrigerated until processing (Wakeham et al. 2002).

Lipid analysis—Trap material was extracted with dichloromethane (DCM)-methanol (2 : 1 by volume; Wakeham et al. 2002). Lipid extracts were archived in solvent and stored at -20°C from the time of the original studies until the ladderane lipid analyses were made in this study. Filters were ultrasonically extracted with methanol, DCM-methanol (1 : 1, vol/vol), and DCM (three times). The bulk of the solvent was removed by rotary evaporation under vacuum, and the extract was further dried over a Na_2SO_4 column. An aliquot of the lipid extract was saponified under N_2 with aqueous 0.5 mol L^{-1} KOH in methanol for 2 h at 100°C . Non-saponifiable lipids (neutral lipids) were extracted out of the basic solution ($\text{pH} > 13$) using hexane. Fatty acids were obtained by acidifying the residue to pH 2 and extraction with hexane thereafter. The fatty acid fraction was methylated by adding diazomethane (CH_2N_2) to convert fatty acids into their corresponding methyl esters (FAMES). The excess CH_2N_2 was removed by evaporation. To remove very polar components, aliquots of the FAMES were eluted with ethyl acetate over a small column filled with silica. Polyunsaturated fatty acids were removed by eluting the aliquots with ethyl acetate over a small AgNO_3 (5%) silica column, yielding a saturated fatty acid fraction. These fractions were dissolved

Table 1. Description of field stations, ladderane lipids, and nutrients.

Ladderane lipids					Nutrients*					
Station	Location	Date	Depth (m)	FAME I–III (pg L ⁻¹)	Station	Location	Date	[O ₂] (mg L ⁻¹)	[NO ₂ ⁻] (μmol L ⁻¹)	[NH ₄ ⁺] (μmol L ⁻¹)
2	15°58'N, 61°29'E	05–06 May 95	60	n.d. [†]	S7	15°99'N, 61°98'E	31 Mar 95	4.20	0.81	0.00
			500	5.10				0.05	0.01	0.03
			1,000	n.d.				0.16	0.00	0.00
			1,500	n.d.				1.00	0.00	0.00
4	17°12'N, 59°35'E	07–08 May 95	70	n.d.	S4	17°29'N, 59°93'E	03 Apr 95	2.80	0.06	0.00
			1,000	1.00				0.15	0.00	0.00
			1,500	0.20				1.00	0.00	0.00
5	17°24'N, 58°49'E	10–11 May 95	90	3.40	S3	17°73'N, 58°80'E	05 Apr 95	~0.50	0.03	0.00
			500	8.10				0.18	0.00	0.00
			1,500	6.00				0.89	0.00	0.00
6	17°41'N, 57°50'E	13 May 95	500	5.50	S2	18°08'N, 58°00'E	07 Apr 95	0.20	0.00	0.00
			1,000	0.30				0.23	0.00	0.00
7	17°40'N, 57°40'E	14 May 95	85	0.60	S2	18°08'N, 58°00'E	07 Apr 95	3.20	0.06	0.00
10	17°44'N, 57°29'E	16 May 95	80	n.d.	S2	18°08'N, 58°00'E	07 Apr 95	3.20	0.06	0.00
			450	4.00				0.12	0.01	0.02
13	19°13'N, 58°31'E	16–17 May 95	35	n.d.	NA	NA	NA	NA [‡]	NA	NA
			500	6.60				NA	NA	NA
			1,000	3.10				NA	NA	NA

* Data are from nearby stations S7 (for station 2), S4 (for station 4), S3 (for station 5), and S2 (for stations 6, 7, and 10) of the U. S. JGOFS Arabian Sea Process Study cruise 2 (see <http://usjgofs.whoi.edu>) in early April 1995.

[†] n.d., not detected.

[‡] NA, not available.

in acetone and then filtered through a 0.45-μm, 4-mm-diameter polytetrafluorethylen (PTFE) filter.

An aliquot was analyzed by using high-performance liquid chromatography coupled to positive ion-atmospheric pressure-chemical ionization tandem mass spectrometry (HPLC/APCI-MS/MS) under conditions described recently by Hopmans et al. (2006). This method was developed for the detection of three specific and most abundantly occurring ladderane fatty acids. These fatty acids (expressed as FAMES) contain the biochemically unique [3]- and [5]-ladderane moieties composed of up to five linearly fused cyclobutane rings (see Fig. 2, structures I–III). Analyses of ladderane lipids were performed using an Agilent 1100 LC system, which consisted of an inline membrane degassing unit, thermostatted auto injector and column compartment, coupled to a Quantum TSQ Ultra EM triple quadrupole mass spectrometer equipped with an Ion max source with atmospheric pressure-chemical ionization (APCI) probe. Separation was achieved on two Zorbax Eclipse XDB-C₈ columns (4.6 × 150 mm, 5 μm, Agilent) coupled in series and maintained at 30°C. Ladderane lipids were eluted with 0.4 mL min⁻¹ methanol with a total run time of 20 min. Detection was achieved by positive ion APCI and selective reaction monitoring (SRM) of four specific fragments for each ladderane lipid. The source settings were: vaporizer temperature 475°C, discharge current 2.5 μA, sheath gas (N₂) pressure 50 (arbitrary units), auxiliary gas (N₂) pressure 5 (arbitrary units), capillary temperature 350°C, source CID -10 V. Argon pressure was maintained at 1.5 mTorr in the second quadrupole. Quantification of ladderane lipids was achieved by using an external calibration curve with

standards of isolated methylated ladderane fatty acids containing the [3]- and [5]-ladderane moieties (Sinninghe Damsté et al. 2002a; Hopmans et al. 2006). A detection limit (defined by a signal-to-noise ratio of 3) of 30–35 pg injected on-column was achieved with this technique.

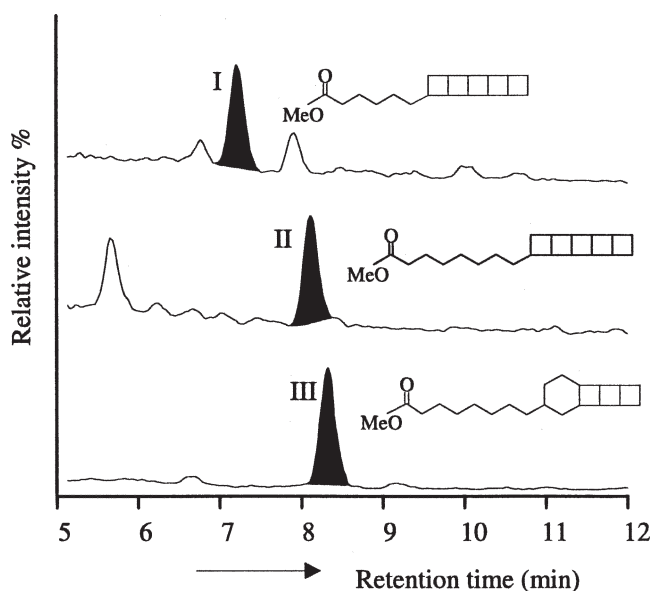


Fig. 2. Partial SRM traces of three ladderane FAMES obtained by HPLC/APCI-MS/MS analysis of suspended particulate matter at station 5 (500-m water depth), and their corresponding structures. Traces I, II, and III show the pentyl-[5]-, the heptyl-[5]-, and the heptyl-[3]-ladderane FAMES, respectively.

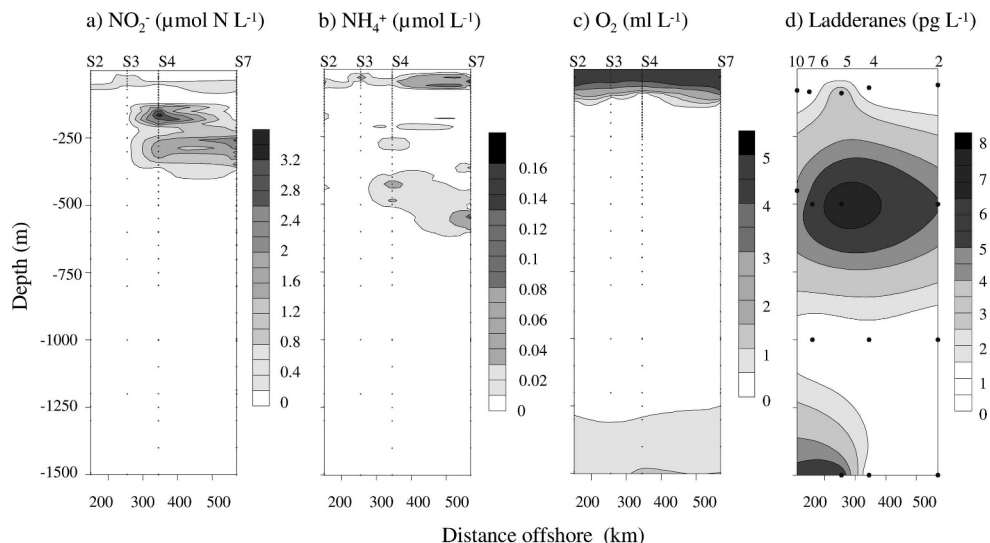


Fig. 3. Contour plots of (a) NO_2^- , (b) NH_4^+ , (c) dissolved oxygen, and (d) ladderane lipids at various depths in the water column on a northwest-to-southeast transect offshore of Oman in the northern Arabian Sea. The black dots in panel (d) indicate positions where samples were collected for ladderane lipid analysis (stations 2, 4, 5, 6, 7, and 10). NO_2^- , NH_4^+ , and dissolved oxygen data are from nearby stations (S2, S3, S4, and S7) of the U. S. JGOFS Arabian Sea Process Study cruise 2 (see <http://usjgofs.whoi.edu>) in early April 1995. Grid points for the calculation of the contour plots are indicated.

Statistical data treatment—Contour plots were generated using the Kriging interpolation technique in Surfer (version 7.04, 2001).

Results

We analyzed a set of 18 SPM samples from different water depths along a northwest-to-southeast transect in the Arabian Sea off Oman, as well as a set of 22 trap samples from ~480-m water depth covering a whole monsoon cycle from November 1994 to January 1996. In nearly all samples, ladderane fatty acids were detected in the saponified extracts of water filtrates and sediment trap material. Their relative distribution was similar in all samples, i.e., it was dominated by the heptyl-[3]-ladderane FAME (Fig. 2, III), followed by the pentyl-[5]- and heptyl-[5]-ladderane FAMES (Fig. 2, I and II), both of which were two to five times lower in concentration. The concentrations of the three isomers were summed and are reported in Table 1.

Distribution of ladderane lipids in the water column—Figure 3 shows contour plots based on the concentrations of ladderane lipids (summed signal of the three ladderane fatty acids) in the water column offshore Oman during the spring intermonsoon, together with those based on nitrite, ammonium, and dissolved oxygen concentrations from nearby stations taken along the southern U. S. JGOFS ASPS section during R/V *Thomas G. Thompson* cruise TN045 in early April 1995 (see <http://usjgofs.whoi.edu>), about 1 month earlier than the SPM sampling on cruise TN047.

Ladderane lipids were found in most of the samples from the oxygen-depleted parts of the water column along the

sample transect of the Arabian Sea (Table 1). Concentrations at ~100 m were below 1 pg L^{-1} , except at station 5, where concentrations of 3.4 pg L^{-1} were detected. Concentrations were highest at a water depth of around 500 m at all sites, and they varied from 5 to 8 pg L^{-1} (Fig. 3d). Concentrations at 1,000-m water depth were approximately 1 pg L^{-1} or lower, and only near the coast (station 13) was a higher concentration of 3 pg L^{-1} found. Abundances of ladderanes at 1,500-m water depth were $<0.5 \text{ pg L}^{-1}$, except at station 5, where concentrations were around 6 pg L^{-1} .

Seasonal variations in ladderane lipid fluxes—Figure 4 shows a time series of the ladderane lipid flux (summed fluxes of the three ladderane fatty acids) obtained from sinking particulate matter at station MS-3. A marked seasonality in ladderane lipid flux was observed, with maximum fluxes of $125 \text{ ng m}^{-2} \text{ d}^{-1}$ during the beginning of the SW monsoon. Strong winds from the southwest during the SW monsoon induce upwelling of nutrient-rich bottom waters during the late spring and summer, which leads to enhanced primary production in surface waters and enhanced particulate matter fluxes down the water column. During that SW monsoon, ladderane lipid fluxes increased by a factor of about three within four weeks. During the spring and fall intermonsoon (SI and FI, respectively) periods, when wind strengths were relatively low, ladderane lipid fluxes were significantly reduced, varying between 3 and $20 \text{ ng m}^{-2} \text{ d}^{-1}$. The NE monsoon is characterized by moderate winds from the northeast during late fall and winter, and ladderane lipid fluxes of $65 \text{ ng m}^{-2} \text{ d}^{-1}$ were observed at the beginning of the NE monsoon period, but then values decreased to as low as $\sim 2 \text{ ng m}^{-2} \text{ d}^{-1}$ toward the end of the NE monsoon.

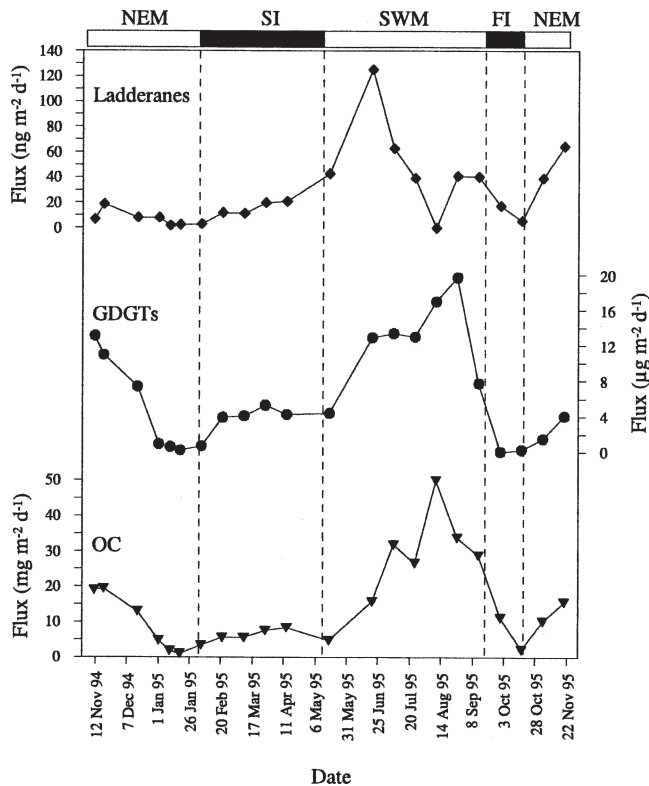


Fig. 4. Summed ladderane lipid, GDGT (Wuchter et al. 2006b), and organic carbon (OC) (Wakeham et al. 2002) fluxes for IRS-C trap MS-3 deployed at ~500-m water depth. Data points represent the centers of collection intervals. Bars at the top of the plot and dotted vertical lines indicate the NE monsoon (NEM), spring intermonsoon (SI), SW monsoon (SWM), and fall intermonsoon (FI) periods of 1994/1995 after Weller et al. (1998).

Overall, average ladderane lipid fluxes during the intermonsoon periods were $12.6 \text{ ng m}^{-2} \text{ d}^{-1}$ for the SI and $11.5 \text{ ng m}^{-2} \text{ d}^{-1}$ for the FI, whereas the average flux during the SW monsoon increased about four times to $50 \text{ ng m}^{-2} \text{ d}^{-1}$. The NW monsoon period had an average flux of about $19 \text{ ng m}^{-2} \text{ d}^{-1}$.

Discussion

Distribution of ladderane lipids in the water column—The presence of ladderane lipids in nearly all SPM samples strongly indicates that anammox bacteria occur throughout the water column of the northwestern Arabian Sea. Their distribution in the water column generally ranges from 85-m to 1,000-m, and even to 1,500-m, water depth at station 5, which may reflect the dynamic and heterogeneous nature of the OMZ waters offshore of Oman. Our data suggest that anammox bacteria are most abundant in the upper part of the OMZ at about 500-m water depth, but the low resolution of the SPM sampling (see grid points in Fig. 3d) precludes pinpointing the exact depth of maximum abundance. The observation that ladderane lipid and anammox bacteria abundances appear to be localized at 500 m rather than distributed throughout the OMZ (given

our sampling resolution) is likely related to the distribution of nitrogen species in the OMZ. Both ammonium and nitrite are required for anammox. In the thermocline, where oxygen is still present, nitrite originates from phytoplankton reduction of nitrate, nitrification, or both (Codispoti and Christensen 1985), and a weak nitrite maximum is present in the thermocline (~100 m) along the whole transect (Fig. 3a). A quasi-permanent secondary nitrite maximum in the suboxic waters of the OMZ at ~200- to 350-m water depth with concentrations up to $3.4 \mu\text{mol L}^{-1}$ is associated with denitrification (Naqvi 1991). This secondary nitrite maximum is reported to be strongest offshore the coast of Oman in the eastern and central Arabian Sea, whereas it is generally lacking along the northern and western boundaries due to re-oxygenation of subsurface waters (Naqvi 1991). Ammonium concentrations of maximum $0.17 \mu\text{mol L}^{-1}$ are found within the oxygenated thermocline, but a secondary ammonium maximum is present offshore at a water depth of around 500 m (Fig. 3b). Although the nutrient data reflect conditions of the water column during the intermonsoon period, about four weeks earlier than the SPM sampling, temporal variability of suboxic conditions is thought to have been minimal at these depths below the thermocline (Morrison et al. 1999). The highest abundances of ladderane lipids were below the depth of the secondary nitrite maximum and close to the ammonium maximum, and this depth distribution strongly indicates active anammox at these depths. Anammox, in addition to denitrification, likely contributes to the loss of nitrogen from the OMZ. Anammox might be coupled to reduction of nitrate to nitrite by denitrifying bacteria (Dalsgaard and Thamdrup 2002). The co-occurrence of suboxic conditions and nitrite maxima suggests that denitrification is the dominant respiratory pathway (Morrison et al. 1998). However, Crenarchaeota have also been found to cope with extremely low oxygen levels, and concentrations of glycerol dibiphytanyl glycerol tetraethers (GDGTs) and crenarchaeol, the characteristic membrane lipid of Crenarchaeota (Sinninghe Damsté et al. 2002c), have been shown to be abundant at 500-m depth in these Arabian Sea samples as well (Sinninghe Damsté et al. 2002b). As at least some of the Crenarchaeota are likely involved in nitrification (Könneke et al. 2005, Wuchter et al. 2006a), they may, in addition to denitrifying bacteria, provide nitrite that could subsequently be used by anammox bacteria to transform ammonium to dinitrogen gas. However, further studies are needed to confirm this hypothesis.

Concentrations of ladderane lipids in the water column of the Arabian Sea are much lower than those previously reported from the Black Sea ($1\text{--}4 \text{ ng L}^{-1}$; Kuypers et al. 2003) and the Benguela upwelling area ($0.1\text{--}4 \text{ ng L}^{-1}$; Kuypers et al. 2005). This might be explained by the fact that the main zone where anammox potentially takes place, i.e., the zone of the secondary nitrite maximum at 200–400 m, was not sampled in this study. Furthermore, the main zone of denitrification (Naqvi 1991; Morrison et al. 1998) where anammox might be more prevalent lies further offshore and to the southwest of the transect we sampled. Overall, the rate of water column denitrification in the

Arabian Sea is generally believed to be globally significant and is thought to represent a major oceanic sink for reactive nitrogen (Codispoti 1989; Gruber and Sarmiento 1997). The imbalance between the nitrate deficit and the much higher excess of nitrogen gas probably from denitrification remains unexplained (Devol et al. 2006). One possible explanation could be that oxidation of ammonium by anammox bacteria is a significant potential source of excess N_2 . Future resampling of the Arabian Sea at higher resolution and using different transects could resolve this important issue.

Seasonal variations in ladderane lipid fluxes—The sediment trap ladderane lipid flux data show a marked seasonal variability in the amount of ladderane lipids being transported vertically to the sediment trap at 500-m water depth. Ladderane fluxes generally show the same seasonal pattern as other biomarkers measured in sediment trap MS-3 (see Wakeham et al. 2002 for additional biomarker flux data), and the highest fluxes occurred during the SW monsoon, low fluxes occurred during the intermonsoon periods, and moderate fluxes occurred during the NE monsoon, although the timing of maximal flux varied among individual compounds. Ladderane lipid flux peaked near the start of the SW monsoon (Fig. 4), which was about 2–4 weeks earlier than, for example, fluxes of alkenones or C_{30} -diols produced by photoautotrophic algae in surface waters (Wakeham et al. 2002). In contrast, the flux of organic carbon (OC, Fig. 4) was highest during the mid-to-late SW monsoon period, presumably at the time of maximum export of phytoplankton-derived material following the high-productivity SW monsoon period. The flux of GDGTs (Wuchter et al. 2006b; Fig. 4), possibly derived from nitrifying Crenarchaeota, increased at the same time as the ladderane flux, but continued to increase toward the end of the SW monsoon, parallel with organic carbon.

Anammox bacterial cells are probably too small to settle themselves and other mechanisms (e.g., marine snow aggregation and faecal pellet formation) have to be invoked to explain the presence of ladderane lipids in descending particles. One such mechanism is increased primary productivity during the SW monsoon leading to enhanced particle export through the water column (Honjo et al. 1999) due to a shift in enhanced food web dynamics and, consequently, in an increased marine snow aggregation and faecal pellet formation. Vertical migration by zooplankton in the Arabian Sea, even into the OMZ (Smith et al. 1998; Wishner et al. 1998) could also provide an active-transport mechanism by which ladderanes are injected directly as faecal pellets into the trap during times of high productivity. In these cases, the maximum ladderane flux would be expected to coincide with the maximum in the organic carbon flux, which is clearly not the case (Fig. 3). One of the factors complicating a comparison of lipid and organic carbon fluxes is that ladderanes are likely produced at much greater depths than other biomarkers and the bulk of the organic carbon, leading to differences in settling times and likely temporal offsets. Furthermore, there may also be changes in anammox bacterial productivity due to

changing nutrient concentrations. Clearly, more work involving temporal studies of the water column is needed to investigate the seasonality of anammox activity. Regardless, the fact that ladderane lipids were detected in settling particles that would eventually reach the seafloor makes them potential biomarkers that can be used to assess anammox activity in the geological past. For this, however, further research on the stability of ladderane lipids throughout sediment burial is required.

Ladderane lipids derived from membranes of anammox bacteria have been identified in suspended particulate matter in the water column of the Arabian Sea during the spring intermonsoon period, where maximum concentrations were found in the upper part of the OMZ. Fluxes of ladderane lipids obtained by sediment trapping over an annual cycle revealed a strong seasonal pattern, with highest fluxes in the beginning of the SW monsoon period. Enhanced vertical flux during the SW monsoon may be related to a combination of increased ladderane lipid biosynthesis due to enhanced anammox activity and enhanced sedimentation of ladderanes during the monsoon-driven high-productivity, high-vertical flux period. The occurrence of ladderane lipids in the oxygen-deficient part of the water column throughout the whole year indicates that, in addition to denitrification, anammox may be an important sink for fixed inorganic nitrogen in the OMZ of the northern Arabian Sea.

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