

## Molecular fossil evidence for anaerobic ammonium oxidation in the Arabian Sea over the last glacial cycle

Andrea Jaeschke,<sup>1</sup> Martin Ziegler,<sup>2</sup> Ellen C. Hopmans,<sup>1</sup> Gert-Jan Reichart,<sup>2</sup> Lucas J. Lourens,<sup>2</sup> Stefan Schouten,<sup>1</sup> and Jaap S. Sinninghe Damsté<sup>1,2</sup>

Received 29 October 2008; revised 21 January 2009; accepted 6 February 2009; published 8 April 2009.

[1] Anaerobic ammonium oxidation (anammox) has been recognized as an important process converting fixed nitrogen to N<sub>2</sub> in many marine environments, thereby having a major impact on the present-day marine nitrogen cycle. However, essentially nothing is known about the importance of anammox in past marine nitrogen cycles. In this study, we analyzed the distribution of fossil ladderane lipids, derived from bacteria performing anammox, in a sediment core from the northern Arabian Sea. Concentrations of ladderane lipids varied between 0.3 and 5.3 ng g<sup>-1</sup> sediment during the past 140 ka, with high values observed during the Holocene, intervals during the last glacial, and during the penultimate interglacial. Maxima in ladderane lipid abundances correlate with high total organic carbon (4–6%) and elevated δ<sup>15</sup>N (>8‰) values. Anammox activity, therefore, seems enhanced during periods characterized by an intense oxygen minimum zone (OMZ). Low concentrations of ladderanes (<0.5 ng g<sup>-1</sup> sediment), indicating low-anammox activity, coincide with periods during which the OMZ was severely diminished. Since anammox activity covaried with OMZ intensity, it may play an important role in the loss of fixed inorganic nitrogen from the global ocean on glacial-interglacial timescales, which was so far attributed only to heterotrophic denitrification.

**Citation:** Jaeschke, A., M. Ziegler, E. C. Hopmans, G.-J. Reichart, L. J. Lourens, S. Schouten, and J. S. Sinninghe Damsté (2009), Molecular fossil evidence for anaerobic ammonium oxidation in the Arabian Sea over the last glacial cycle, *Paleoceanography*, 24, PA2202, doi:10.1029/2008PA001712.

### 1. Introduction

[2] The global oceanic nitrogen budget is presently not well established with respect to the balance of sources and sinks [Altabet, 2007; Brandes and Devol, 2002; Codispoti et al., 2001]. Oceanic N<sub>2</sub> fixation constitutes the major source for nitrogen while sedimentary and pelagic denitrification is widely thought to be the predominant sink. Total sources and sinks are estimated to be between 200 and ~450 Tg a<sup>-1</sup> [Codispoti et al., 2001; Gruber and Sarmiento, 1997]. One way of establishing the nitrogen budget is to use the stable nitrogen isotope ratio (δ<sup>15</sup>N) of organic matter (OM). The δ<sup>15</sup>N of OM which settles from the surface water is a function of the δ<sup>15</sup>N of the nitrate source and fractionation occurring during uptake of nitrate by phytoplankton [Altabet, 1988]. The average oceanic δ<sup>15</sup>N of dissolved nitrate is around 5‰ [Altabet, 1988; Sigman et al., 1997]. Denitrification occurs under suboxic conditions in the water column resulting in a strong fractionation of ~27‰ [Brandes et al., 1998], leaving the residual nitrate enriched in <sup>15</sup>N. Upwelling of this <sup>15</sup>N-enriched nitrate also influences the isotopic composition of newly produced OM in the surface water by phytoplankton leading to high δ<sup>15</sup>N values

in particulate and sedimentary OM. In contrast, although denitrification taking place within the sediment is changing the overall nitrogen inventory, it is believed to produce no significant change in δ<sup>15</sup>N [Altabet, 2007; Brandes and Devol, 2002]. Thus, OM δ<sup>15</sup>N is mainly thought to reflect the rate of denitrification in the water column. Past changes in the fixed nitrogen budget can be deduced from changes in atmospheric N<sub>2</sub>O concentrations [Flückiger et al., 2004]. Major changes in the extent of denitrification have furthermore been inferred from changes in δ<sup>15</sup>N in sedimentary records on longer timescales [Altabet et al., 1999, 2002; Ganeshram et al., 2000; Suthhof et al., 2001]. Because these changes were parallel with atmospheric CO<sub>2</sub> concentrations, a link between the two has been suggested [Altabet et al., 2002].

[3] Globally, about 30–50% of the total nitrogen loss takes place in oxygen minimum zones (OMZs) and was until recently exclusively attributed to heterotrophic denitrification [Codispoti et al., 2001; Gruber and Sarmiento, 1997]. The Arabian Sea comprises one of the largest OMZs presently found in the oceans, and ~20% of the water column denitrification is estimated to take place here [Bange et al., 2000]. The Arabian Sea, therefore, is believed to have a substantial influence on the global marine fixed nitrogen budget [Gruber and Sarmiento, 1997].

[4] Already in the 1980s several studies suspected other processes than denitrification to contribute to N<sub>2</sub> production without corresponding decrease in the nitrate content [Codispoti and Christensen, 1985; Naqvi, 1987]. Devol et al. [2006] suggested anammox to be responsible for the

<sup>1</sup>Department of Marine Organic Biogeochemistry, Royal Netherlands Institute for Sea Research, Texel, Netherlands.

<sup>2</sup>Department of Earth Sciences, Faculty of Geosciences, Utrecht University, Utrecht, Netherlands.

observed mismatch between denitrification estimated from nitrate deficits and measured excess  $N_2$  concentrations in the OMZ of the Arabian Sea. Recent studies indicate anammox to be the dominant  $N_2$  production pathway in the OMZs off Namibia [Kuypers *et al.*, 2005], Chile [Thamdrup *et al.*, 2006] and Peru [Hamersley *et al.*, 2007], while the contribution of denitrification was only minor or did not exist at all. Recently, we found evidence for anammox in the Arabian Sea by studying the spatial and seasonal distribution of ladderane lipids in OMZ waters off Oman [Jaeschke *et al.*, 2007]. Ladderane lipids are specific membrane lipids unique to anammox bacteria, which mediate the anaerobic oxidation of ammonium [Sinninghe Damsté *et al.*, 2002]. The ladderane core lipid consists of three or five linearly concatenated cyclobutane rings either ester or ether linked to the glycerol backbone. These ladderanes form a dense and highly impermeable membrane surrounding the anammoxosome, an intracellular compartment, where anammox catabolism takes place. Ladderane lipids have so far been successfully applied as biomarkers for anammox bacteria in the suboxic water column of the Black Sea [Kuypers *et al.*, 2003; Wakeham *et al.*, 2007], OMZs off Namibia [Kuypers *et al.*, 2005], Peru [Hamersley *et al.*, 2007], and in the Arabian Sea [Jaeschke *et al.*, 2007].

[5] The dominance and role of anammox in comparison to denitrification in several present-day OMZs, raises the question how past changes in the intensity of anammox impacted the nitrogen budget. In this study we investigate past anammox activity using fossilized ladderane lipids in a sediment core from the northern Arabian Sea (Murray Ridge) spanning the last 140 ka. In conjunction with bulk geochemical tracers (total organic carbon (TOC),  $\delta^{15}N$ ), the abundance of ladderane lipids is used to reconstruct changes in past anammox activity and to assess its potential impact on the oceanic nitrogen budget.

## 2. Material and Methods

### 2.1. Oceanographic Setting and Core Location

[6] The Arabian Sea climate and hydrography is strongly influenced by the seasonal change of monsoonal winds driven by changes in the pressure gradient between the Tibetan plateau and the South Indian Ocean [Wyrski, 1973]. During summer, warm and humid southwest (SW) monsoonal winds induce coastal and open ocean upwelling of nutrient-rich deep waters to the euphotic zone resulting in primary productivity rates estimated to be 200 to 400  $g\ C\ m^{-2}\ a^{-1}$  [Kabanova, 1968; Qasim, 1982]. During winter, dry and relatively cold northeast (NE) monsoonal winds from the Asian landmass are prevailing. Productivity is then generally low as the NE monsoon suppresses upwelling, although deepening of the mixed layer and subsequent nutrient injection in the euphotic zone gives rise to a second, more modest productivity maximum [Sawant and Madhuratap, 1996]. A stable and pronounced OMZ with  $O_2$  values of less than  $2\ \mu mol\ L^{-1}$  persists at water depths between 150 and 1200 m [Wyrski, 1973] as a result of the high biological productivity in combination with moderate ventilation of the thermocline waters.

[7] Sediment core MD04-2879 ( $22^{\circ}32.9'N$ ,  $64^{\circ}02.8'E$ ) was recovered with R/V *Marion Dufresne* in 2004 from the Murray Ridge in the northern Arabian Sea, from a water depth of 920 m (Figure 1) well within the present-day OMZ. Oxygen concentration in the bottom waters is below  $2\ \mu mol\ L^{-1}$  [Van Bennekom *et al.*, 1995]. The sediment generally consists of homogeneous, dark greenish to light greenish/gray hemipelagic muds.

### 2.2. Chronostratigraphy

[8] It has been shown in several studies [Reichert *et al.*, 1998, 2004] that short-lived occurrences of deep-dwelling planktic foraminifera *Globorotalia truncatulinoides* and *Globorotalia crassaformis* match very well with cooling events in the North Atlantic. These species are not present in the modern Arabian Sea as they rely for their reproductive cycle on deep overturning during intense winter mixing, which is associated with an enhanced NE monsoon during cold periods. Their high relative abundances during those short periods make them accurate biostratigraphic markers for Arabian Sea sediment records.

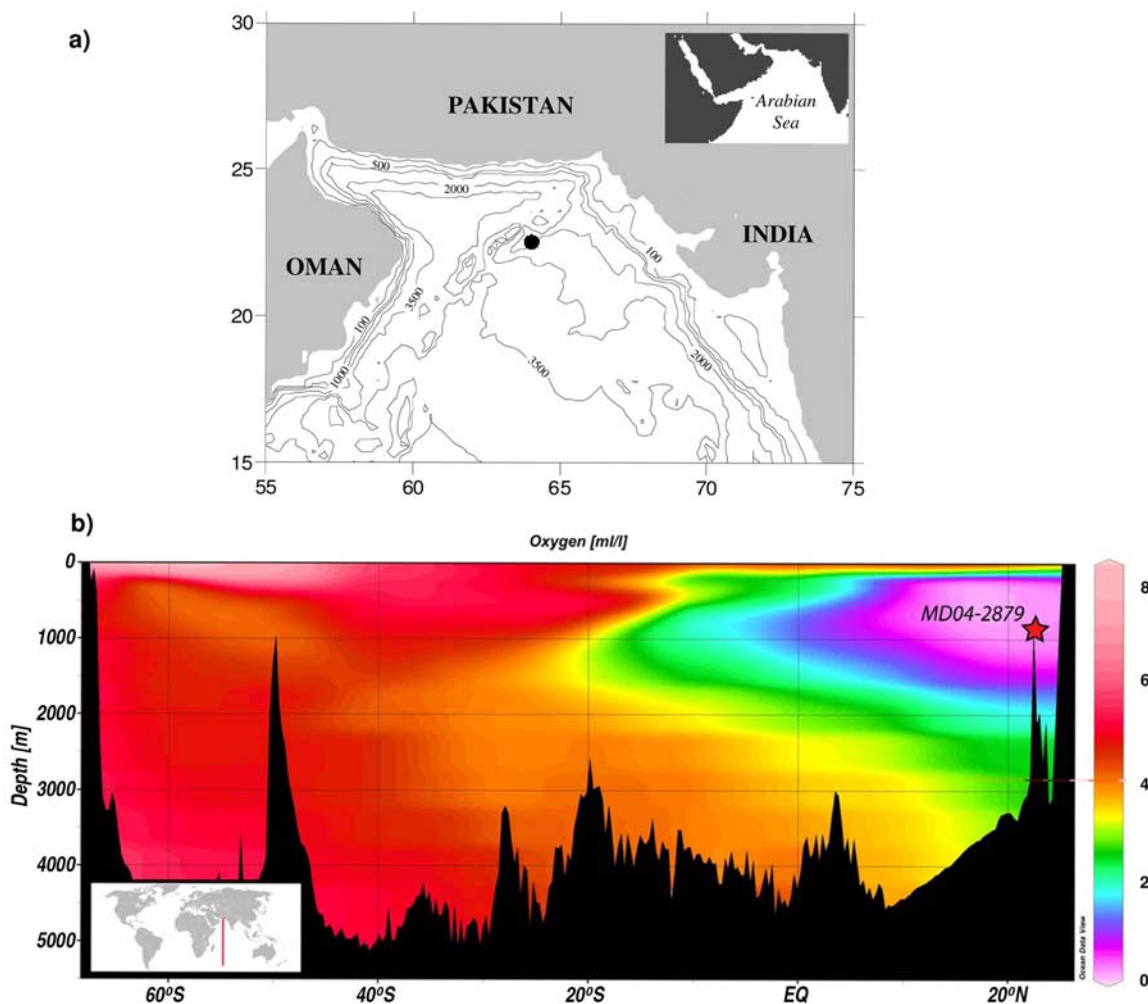
[9] Species of *G. truncatulinoides* and *G. crassaformis* have been counted on split samples from the 150–600  $\mu m$  size fraction and are expressed as specimen per gram sediment (Figure 2). The occurrence peaks in MD04-2879 have been tuned to extreme North Atlantic cooling events as recorded in an alkenone record from the Iberian margin [Martrat *et al.*, 2007]. The age model is based on 15 age control points (Figure 2). Ages for each individual sample depth have been derived by linear interpolation between calibration points. A second-order polynomial fit gives a correlation coefficient ( $r$ ) of 0.989. Sedimentation rates varied between 2 and 30  $cm\ ka^{-1}$ . Sedimentation rates in the Northern Arabian Sea covary in general with changes in calcium carbonate preservation, which is strongly influenced by supralysocline dissolution related to postdepositional organic matter degradation under dysoxic conditions [den Dulk *et al.*, 2000].

### 2.3. Total Organic Carbon and $\delta^{15}N$ Analysis

[10] Freeze-dried and powdered sediment samples were decalcified with dilute (2N) HCl, rinsed with dematerialized water to remove  $CaCl_2$  and freeze-dried again afterward. Total organic carbon (TOC) was measured against a benzoic acid laboratory standard ( $C = 68.80\%$ ),  $\delta^{15}N$  against the lab standards acetanilide ( $\delta^{15}N = 1.3\%$  versus air) and glycine ( $\delta^{15}N = 2.43\%$  versus air) on an elemental analyzer (Carlo Erba Flash) coupled online to a mass spectrometer (ThermoFinnigan Delta<sup>plus</sup>). On the basis of analyzing standards and duplicate analysis of samples the analytical reproducibility for TOC was found to be better than 0.2%.  $\delta^{15}N$  values are expressed in per mil relative to atmospheric  $N_2$ , with reproducibility better than 0.3%.

### 2.4. Lipid Extraction and Analysis

[11] Samples of ca. 4 g of freeze-dried and homogenized sediment were repeatedly (5x) extracted with a dichloromethane (DCM) methanol mixture (2:1 by volume). The extracts were combined and the bulk of the solvent was removed by rotary evaporation under vacuum, and the extract was dried over a  $Na_2SO_4$  column. An aliquot of



**Figure 1.** (a) Map showing the location of core MD04-2879 (22°32.9'N, 64°02.8'E, 920 m below sea surface). (b) Dissolved oxygen concentrations in the water column ( $\text{mL L}^{-1}$  shown in color) and seafloor topography along a transect (indicated by red line in inset map).

the lipid extract was saponified with aqueous 1 N KOH in methanol for 2 h at 100°C. Nonsaponifiable lipids (neutral lipids) were extracted out of the basic solution ( $\text{pH} > 13$ ) using DCM. Fatty acids were obtained by acidifying the residue to pH 3 and subsequent extraction with DCM. The fatty acid fraction was methylated by adding diazomethane ( $\text{CH}_2\text{N}_2$ ) to convert fatty acids into their corresponding methyl esters (FAMES). Excess  $\text{CH}_2\text{N}_2$  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 FAMES were removed by eluting the aliquots with ethyl acetate over a small  $\text{AgNO}_3$  (5%) impregnated silica column, yielding a saturated FAME fraction. These fractions were dissolved in acetone and then filtered through a 0.45  $\mu\text{m}$ , 4 mm diameter PTFE filter.

[12] The saturated FAME fractions were analyzed by high-performance liquid chromatography coupled to positive ion atmospheric pressure chemical ionization tandem

mass spectrometry (HPLC/APCI-MS/MS) as described by *Hopmans et al.* [2006] with some modifications. Specifically, separation was achieved using a Zorbax Eclipse XDB-C<sub>8</sub> column ( $3.0 \times 250$  mm, 5  $\mu\text{m}$ ; Agilent) and a flow rate of 0.18  $\text{mL min}^{-1}$  MeOH. The source settings were: vaporizer temperature 475°C, discharge current 2.5  $\mu\text{A}$ , sheath gas ( $\text{N}_2$ ) pressure 30 (arbitrary units), auxiliary gas ( $\text{N}_2$ ) 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 done by using an external calibration curve using previously obtained standards [*Hopmans et al.*, 2006; *Sinninghe Damsté et al.*, 2002] of isolated methylated ladderane fatty acids containing the [3]- and [5]-ladderane moieties (Figure 3, structures I–IV). A detection limit (defined by a signal-to-noise ratio of 3) of 30–35 pg injected was achieved with this technique. The reproducibility of ladderane lipid concentration based on duplicate analysis of the samples was better than 8%.

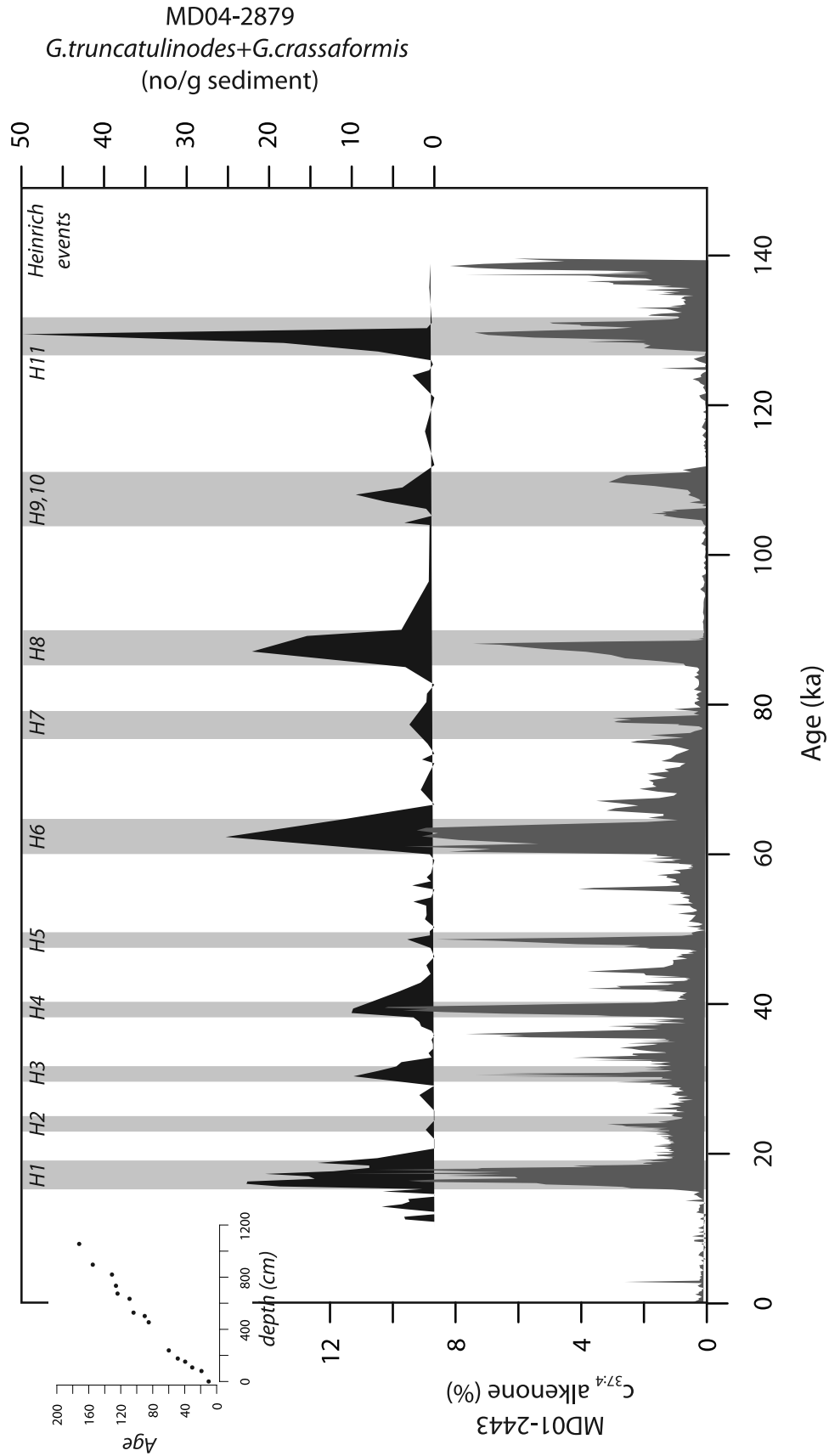
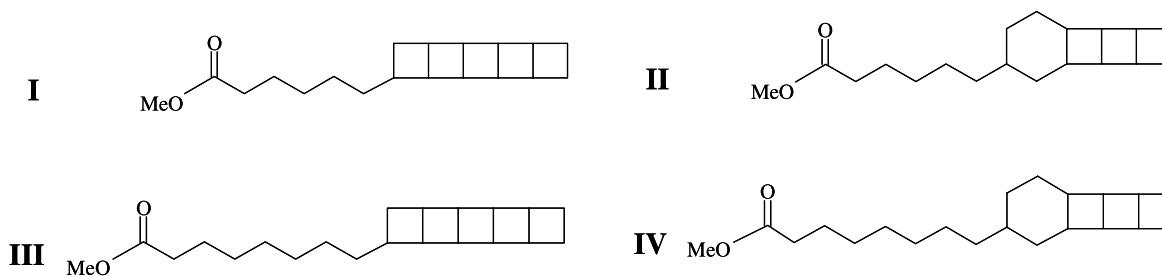
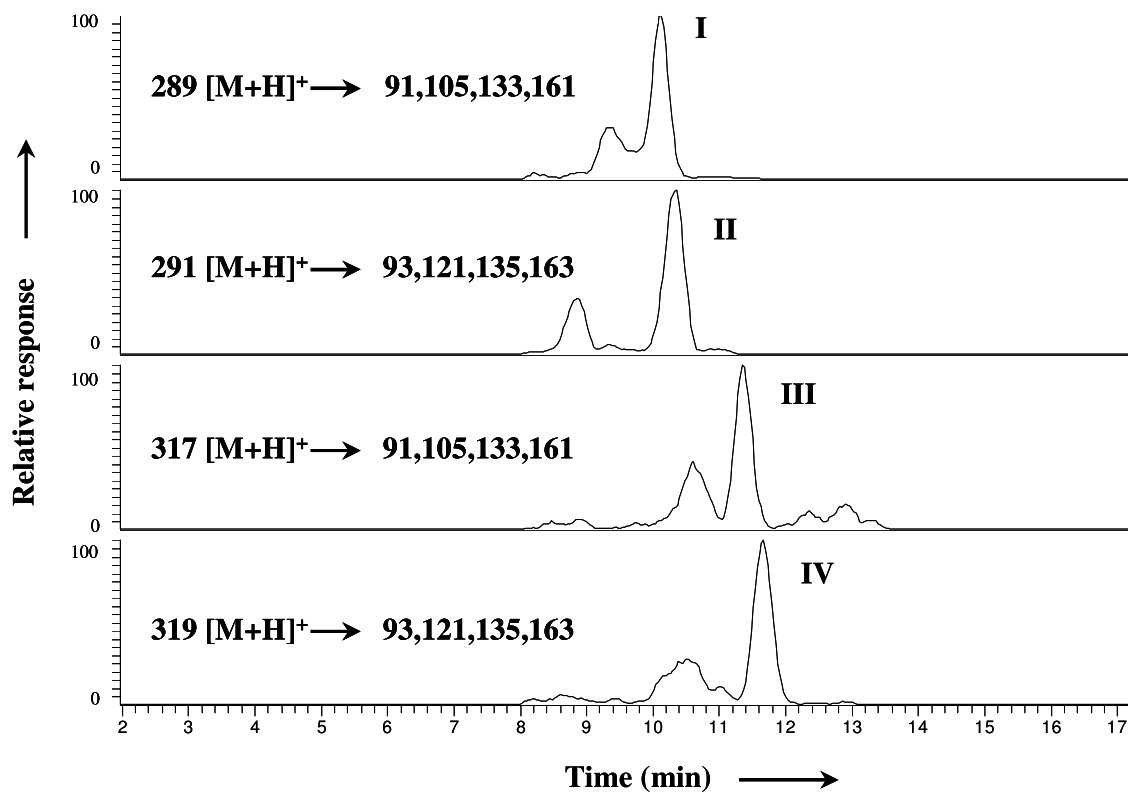


Figure 2

## MD04-2879 (143 cm)



**Figure 3.** HPLC/APCI-MS/MS chromatograms generated using the transition of the protonated molecules to selected product ions for ladderane lipids analyzed in this study. I–IV correspond to structures of ladderane lipids shown at the bottom of the figure. I,  $C_{18}$ -[5]-ladderane FAME; II,  $C_{20}$ -[5]-ladderane FAME; III,  $C_{18}$ -[3]-ladderane FAME; IV,  $C_{20}$ -[3]-ladderane FAME.

**Figure 2.** Age model for MD04-2879. Tuning of deep-dwelling planktic foraminifera in MD04-2879 to North Atlantic cold events: (top) *G. truncatulinodes* and *G. crassaformis* per gram sediment in MD04-2879; (bottom) relative proportion of tetraunsaturated  $C_{37}$  alkenone to total  $C_{37}$  alkenones ( $C_{37:4}$ ) in MD01-2443 [Martrat et al., 2007]. Dots are calibration points. Gray bars indicate timing of Heinrich events according to Rashid et al. [2003].

[13] The index of ladderane lipids with 5 cyclobutane rings, an index for the relative chain length of ladderane lipids, was calculated according to *Rattray* [2008]:

$$NL_5 = \frac{C_{20}[5]\text{fatty acid}}{(C_{18}[5]\text{fatty acid} + C_{20}[5]\text{fatty acid})}$$

The  $NL_5$ , based on culture experiments with anammox bacteria, water column and surface sediment samples, was then converted to a temperature estimate using the equation of *Rattray* [2008]:

$$NL_5 = 0.2 + \frac{0.7}{1 + e^{-\left(\frac{\text{Temp}-16.0}{1.6}\right)}}$$

The reproducibility of the  $NL_5$  based on duplicate measurements was better than 0.07.

### 3. Results

#### 3.1. Organic Carbon and $^{15}\text{N}$ Content

[14] The TOC record of core MD04-2879 shows changes between 0.6 and 5.9% over the last 140 ka (Figure 4a). Highest TOC values were observed during the early Holocene (4.8%), the penultimate interglacial (marine isotope stage MIS 5e) (5.5%), substages 5d and 5a (5.9%) as well as during MIS 3 (4.7%). TOC values increased by about a factor of five over the last deglaciation. Overall, the TOC pattern is in line with those from previous studies from the Murray Ridge area [*Reichart et al.*, 1997].

[15] The  $\delta^{15}\text{N}$  record exhibits values ranging from 4 to 9.6‰ during the last 140 ka (Figure 4b). Maxima in the  $\delta^{15}\text{N}$  record characterize the early Holocene ( $>7.5\text{‰}$ ), the penultimate interglacial (7.5–8.8‰) and several warmer episodes within the last glacial ( $>8\text{‰}$ ). An increase in  $\delta^{15}\text{N}$  of  $>2\text{‰}$  from the Last Glacial Maximum (LGM) to the early Holocene is observed. These results are consistent with other studies from different parts of the Arabian Sea [*Altabet et al.*, 1999; *Altabet*, 2007; *Reichart et al.*, 1998].

#### 3.2. Ladderane Lipids

[16] Ladderane lipids were detected in all sediment samples studied of core MD04-2879 (Figure 4c). Ladderane lipid concentrations (total concentrations of the four components analyzed, structures I–IV in Figure 3) ranged from 0.3 to 5.3  $\text{ng g}^{-1}$  dry weight sediment. Maxima in the summed ladderane lipid concentrations are found during the early Holocene and the penultimate interglacial as well as during several intervals within the last glacial with values of 2.5 to 5.3  $\text{ng g}^{-1}$ . Concentration minima coincide with the LGM and colder episodes within the last glacial with ladderane lipid concentrations generally below 1  $\text{ng g}^{-1}$ . During the last deglaciation, ladderane lipid concentrations increased by a factor of 4.

### 4. Discussion

#### 4.1. Evidence for Past Anammox in the Arabian Sea

[17] The fossil ladderane lipid record from sediment core MD04-2879 gives the first evidence for past anammox

activity. The record provides evidence that anammox occurred continuously over at least the last 140,000 years in the Arabian Sea (Figure 4c). Variations in the concentration of ladderanes further suggest that significant changes in anammox activity took place over that period. Ladderane lipid concentration and TOC content reveal similar patterns, suggesting that both are associated with the same mechanism. *Reichart et al.* [1998] showed that variations in TOC are mainly controlled by variations in primary productivity. Their evidence for a productivity signal is based on covarying patterns of *G. bulloides*, an indicator for upwelling, Ba/Al ratios which are linked to surface water productivity driven by the summer monsoon, and similar TOC variations in sediment cores from different water depths. Furthermore, fluctuations in TOC and biomarker concentrations in the Arabian Sea sediment cores have also been interpreted as resulting primarily from changing productivity in surface waters [*Schubert et al.*, 1998; *Schulte et al.*, 1999]. On the basis of stable nitrogen isotopes ( $\delta^{15}\text{N}$ ), benthic foraminiferal assemblages and trace metals, OMZ intensity and thickness have been shown to fluctuate strongly in the past in line with changes in primary productivity, resulting in changes in bottom and pore water conditions [*Altabet et al.*, 1999; *den Dulk et al.*, 2000; *Reichart et al.*, 1998; *Schulte et al.*, 1999; *Suthhof et al.*, 2001; *van der Weijden et al.*, 2006]. This correspondence suggests that anammox activity was enhanced during times of higher surface productivity and a strong OMZ. However, biomarker lipids have also been shown to have differential preservation in response to changes in the extent of the OMZ intensity [*Sinninghe Damsté et al.*, 2002]. Thus, although it seems likely that the observed variations in ladderane lipid concentration primarily reflect changes in anammox productivity, we cannot exclude that changes in bottom water redox conditions affected the preservation of the primary ladderane lipid signal. However, when we normalize the ladderane lipid concentration on OC to account for this effect, the general pattern remains the same suggesting that production rather than preservation was the main control on the ladderane lipid distributions (Figure 5).

[18] Important for the interpretation of the ladderane record is the location of the source of these lipids, i.e., anammox occurring in the water column (OMZ) or in the anoxic sediment. Evidence for anammox in the present-day OMZ comes from the presence of ladderane lipids in suspended particulate matter (SPM), and analysis of sediment trap material revealing that these ladderane lipids produced in the water column are transported down to the ocean floor [*Jaeschke et al.*, 2007]. However, a sedimentary source for ladderane lipids cannot be excluded, as anammox is also a widespread process in organic-rich sediments of coastal and shelf areas [*Dalsgaard and Thamdrup*, 2002; *Thamdrup and Dalsgaard*, 2002; *Jaeschke et al.*, 2009]. Since the core location presently is in the OMZ, high organic carbon contents and low bottom water oxygen could also result here in elevated anammox activity in the sediment. To discriminate between ladderane lipids derived from water column and sedimentary anammox, we applied the index of ladderane lipids with 5 cyclobutane rings, termed  $NL_5$  [*Rattray*, 2008]. Anammox bacteria have been

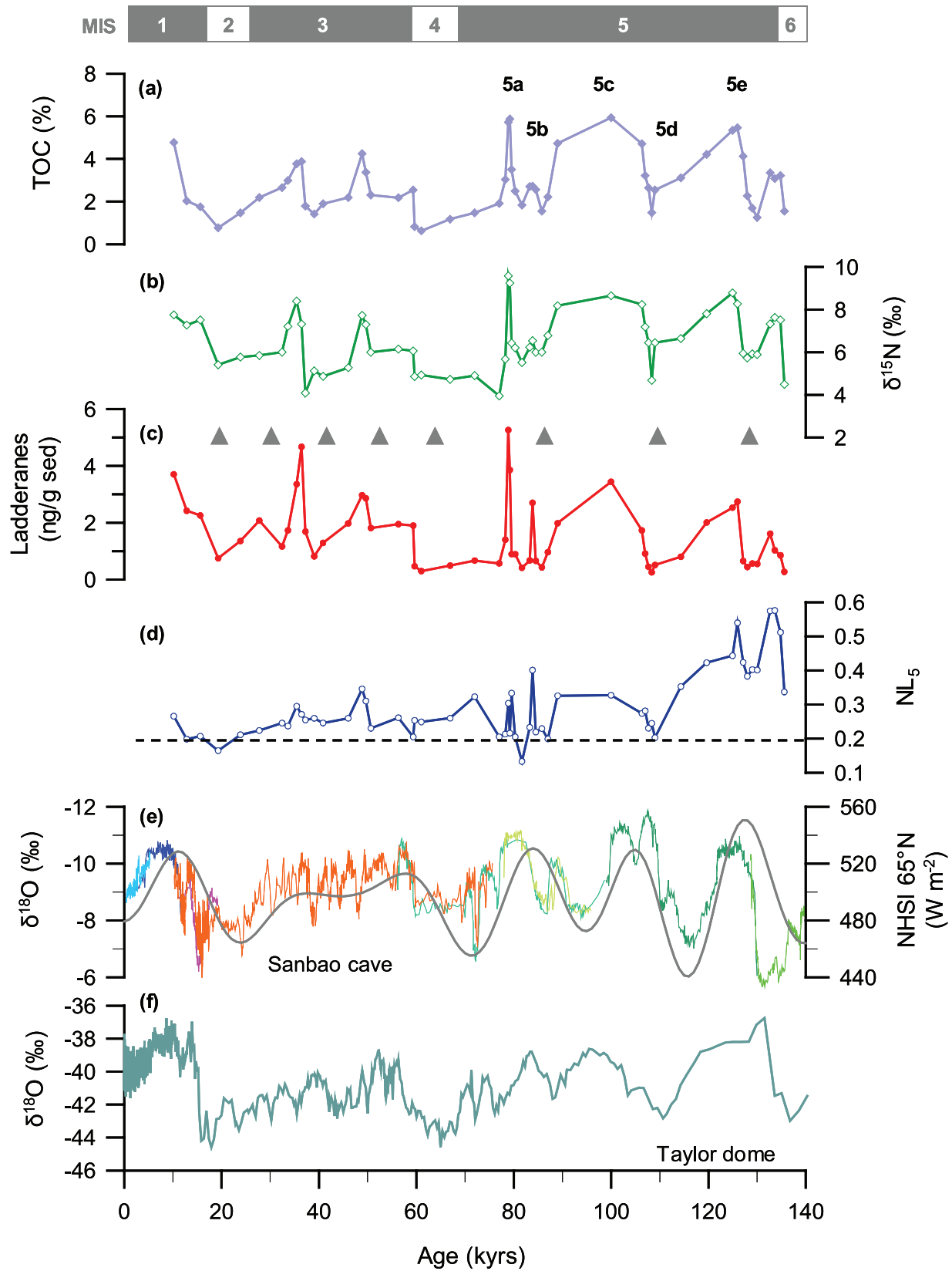
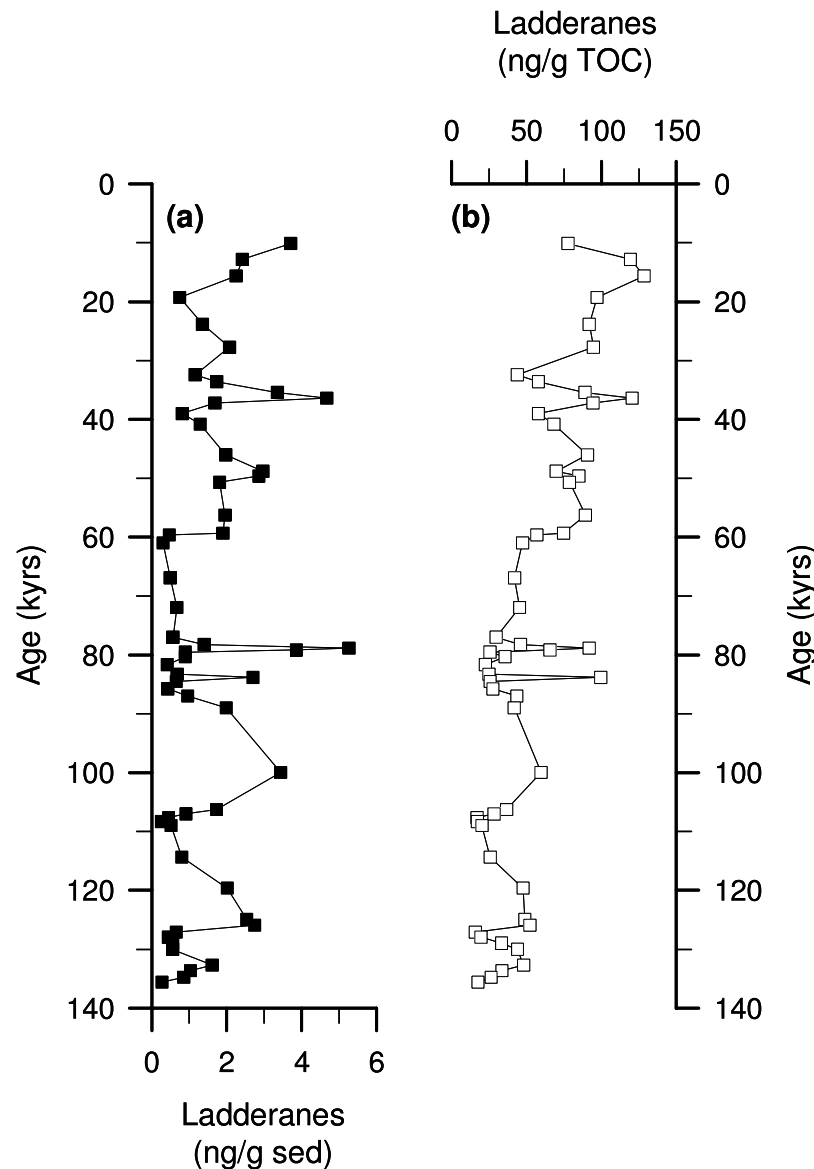


Figure 4



**Figure 5.** Concentration of ladderane lipids (a) per gram sediment and (b) normalized to TOC for sediment core MD04-2879.

found to alter their membrane composition in response to temperature changes; that is, the amount of shorter-chain ladderane fatty acids increases relative to the amount of longer-chain fatty acids at lower temperatures and vice versa. Most changes in ladderane lipid chain length take

place between 12 and 20°C, while no significant change in chain length has been observed at temperatures <12°C and >20°C. The NL<sub>5</sub> index has been proposed to quantify this relative change [Ratray, 2008]. The NL<sub>5</sub> index of the ladderane lipids at the Murray Ridge varied between 0.16

**Figure 4.** (a) Weight percentage of total organic carbon (TOC); (b) stable nitrogen isotope ratio ( $\delta^{15}\text{N}$ ) for sediment core MD04-2879; (c) total ladderane lipid concentrations (i.e., I–IV in Figure 3) for core MD04-2879; (d) NL<sub>5</sub> as a temperature proxy, used in this study to locate the origin of the anammox signal; (e)  $\delta^{18}\text{O}$  records from the Sanbao cave, China [Wang *et al.*, 2008] (light blue, stalagmite SB26; dark blue, SB10; pink, SB3; turquoise, SB22; gray, SB25-1; dark green, SB23; light green, SB11), and Hulu cave [Wang *et al.*, 2001] (orange) and NHSI (Northern Hemisphere summer insolation, 21 July) at 65°N; and (f)  $\delta^{18}\text{O}$  from the Taylor dome ice core as a proxy for Antarctic air temperatures [Grootes *et al.*, 2001]. The dashed line represents a NL<sub>5</sub> value below that expected for lipids produced only by sedimentary anammox bacteria. Gray triangles indicate the occurrence of deep-dwelling planktic foraminifera *G. truncatulinoides* and *G. crassaformis*. MIS, marine isotope stage. Here 5a–5e indicate MIS 5 substages.



and 0.58 over the last 140 ka (Figure 4d). Converted to temperatures using the correlation between the  $NL_5$  and temperature proposed by *Ratray* [2008], this suggests that ladderanes were synthesized at temperatures of up to 17°C. Analysis of SPM samples from a transect offshore Oman at water depths of 450–500 m [*Jaeschke et al.*, 2007] showed  $NL_5$  values between 0.45 and 0.49, which suggested that they were synthesized at temperatures of ca 15°C, which is in good agreement with today's temperatures at these depths. The bottom water temperature at the core site (920m) is around 9°C, which would correspond to a  $NL_5$  value below 0.2. Most  $NL_5$  values in our record are above 0.2 (indicated by the dashed line in Figure 4d), suggesting that the ladderane lipids are to a large extent derived from the water column. Furthermore, bottom water temperatures might have been even lower during the glacial and thus the  $NL_5$  threshold value for ladderanes synthesized might have been even lower. During the penultimate interglacial  $NL_5$  values increase to 0.4–0.6 which corresponds to water temperature estimates of 15–17°C. This increase in the  $NL_5$  during this period follows the general global warming observed in ice cores from Antarctica (Figure 4f) and is in line with global climate change. Finally, our ladderane lipid record follows the  $\delta^{18}O$  records from Sanbao cave and Hulu cave speleothems (Figure 4e) [*Wang et al.*, 2001, 2008] which are indicators for monsoon intensity and vary in response to changes in Northern Hemisphere summer solar radiation on orbital scales (Figure 4e, gray curve). Variations in monsoon intensity triggers upwelling and primary productivity, again causing changes in OMZ strength and likely also anammox activity (Figure 4c). Taken together, our ladderane lipid record thus seems to primarily reflect past changes in anammox activity in the OMZ.

#### 4.2. Implications for Nitrogen Cycling in the Ocean

[19] The down-core  $\delta^{15}N$  record from the Murray Ridge (Figure 4b) shows a similar range in  $\delta^{15}N$  (4–9‰) as observed in other records from the Arabian Sea, indicating that these variations are caused by large-scale changes in nitrogen cycling in the Arabian Sea [*Altabet et al.*, 1999; *Suthhof et al.*, 2001]. Strikingly, high  $\delta^{15}N$  values (Figure 4b) in our record correspond to high concentrations of ladderane lipids (Figure 4c), suggesting that anammox may also leave an imprint on the  $\delta^{15}N$  signature. Since the isotopic fractionation of anammox in the water column and in sediments is not known, it is currently not possible to assess the extent to which anammox contributes to the observed  $\delta^{15}N$  signal, which is required for a better interpretation of sedimentary  $\delta^{15}N$  records. However, in areas where anammox rather than denitrification was found to be the dominant process [*Hamersley et al.*, 2007; *Kuypers et al.*, 2005; *Thamdrup et al.*, 2006] the observed  $^{15}N$  fractionations are similar to those attributed to denitrification [*Galbraith et al.*, 2004; *Ganeshram et al.*, 2000]. Therefore, it is likely that anammox leaves a similar nitrogen isotopic imprint as denitrification, although further studies like cultivation experiments are required to provide evidence for this hypothesis. The general coincidence of high ladderane lipid concentrations with enriched  $\delta^{15}N$  values may thus be indicative of enhanced anammox activity and/or denitri-

fication during periods characterized by a pronounced OMZ. Strong SW monsoon driven upwelling of nutrient-rich waters leading to high surface productivity and subsequent remineralization of OM is resulting in strong oxygen deficiency at intermediate water depths which is driving the anammox process in the OMZ. During the coldest glacial episodes, the extent and intensity of the OMZ is thought to have been much reduced or even absent as is indicated by low  $\delta^{15}N$  values approaching 4‰, which is slightly lower than average modern values for marine nitrate [*Altabet et al.*, 1999]. Reduced SW monsoonal winds and more intense cooling of the Asian continent during the last glacial period leading to stronger and colder NE monsoonal winds [*Duplessy*, 1982; *Reichart et al.*, 1998] caused intensified winter mixing and a better ventilation of the OMZ. High water column oxygenation is also evidenced by decreased Sr/Ca ratios as well as the basin-wide occurrence of *G. truncatulinoides* and *G. crassaformis*. The occurrence events of the deep-dwelling planktic foraminifera (Figure 2) correlate with minima in  $\delta^{15}N$  and ladderane lipid concentrations in our record derived from the same location (Figure 4, gray triangles). Our results, however, suggest that there was still some minor anammox activity. However, during these phases, anammox activity in the sediment might have contributed to a larger extent to the ladderane lipids detected in our record as suggested by the low  $NL_5$  values (Figure 4d).

[20] During several intervals  $\delta^{15}N$  values up to 9.6‰ are observed which is 3–4‰ higher compared to average glacial values and which coincides with maxima in ladderane lipid concentrations  $>5 \text{ ng g}^{-1}$  sediment, indicating conditions comparable to the Holocene. Maxima in  $\delta^{15}N$  and ladderanes showed that anammox played a prominent role during the last glacial contributing to the net loss of fixed nitrogen from the ocean. This alternative mechanism for the removal of fixed inorganic nitrogen via the anammox reaction, suggested by the ladderane lipid record is in line with previous studies indicating suboxic conditions and a well established OMZ in the Arabian Sea during interstadials [*Altabet et al.*, 1999, 2002; *Suthhof et al.*, 2001]. During the penultimate interglacial warm period, high TOC and  $\delta^{15}N$  values indicate enhanced productivity and a strong OMZ although the abundance of ladderane lipids is somewhat lower compared to the Holocene and values associated with the warmer glacial episodes. However, we cannot exclude that at these depths of the core (654–808 cm, 115–130 ka) ladderane lipids might have been partly lost through ongoing diagenesis resulting in lower ladderane lipid concentrations (Figure 5).

[21] Anammox and denitrification could, by changing the oceanic combined nitrogen inventory, influence marine productivity, thereby modulating atmospheric  $pCO_2$  [*Altabet et al.*, 2002]. Changes in the marine nitrogen inventory controlling past changes in  $CO_2$  levels is, however, thought to be unlikely [*Gruber*, 2004]. On the other hand the greenhouse gas nitrous oxide ( $N_2O$ ) is an intermediate in the oxidation of ammonium to nitrate (nitrification) and reduction of nitrate to  $N_2$  (denitrification) in the ocean, and could thus potentially also directly influence Earth's climate.  $N_2O$  has undergone large variations syn-

chronous with rapid climatic changes [Flückiger *et al.*, 2004]. Denitrification in the Arabian Sea was proposed to be one possible source contributing to the observed changes in atmospheric N<sub>2</sub>O [Suthhof *et al.*, 2001]. The anammox reaction, however, does not involve the liberation of N<sub>2</sub>O as an intermediate. Assuming a substantial contribution of anammox in converting fixed nitrogen into gaseous N<sub>2</sub> in the Arabian Sea in the past makes it thus unlikely that oceanic denitrification has contributed solely to the changes in the atmospheric N<sub>2</sub>O. Possibly, terrestrial denitrification and nitrification processes especially in the tropics may account for a large fraction of N<sub>2</sub>O released to the atmosphere, in addition to oceanic nitrification and denitrification. [Bouwman *et al.*, 1993; Flückiger *et al.*, 2004].

## 5. Conclusions

[22] The detection of fossil ladderane lipids in our record shows for the first time that anaerobic ammonium oxidation

has been occurring in the Arabian Sea at least over the last 140 ka. Concurrent variations in δ<sup>15</sup>N and abundances of ladderane lipids reflect changes in anammox intensity in the OMZ. Anammox was, therefore, also responsible for losses of fixed nitrogen from the Arabian Sea, and might to some degree be responsible for the suggested imbalance in the local marine nitrogen budget. Further studies including better constraints on nitrogen isotopic fractionation during anammox are needed to assess the extent to which anammox contributes to the production of N<sub>2</sub> in the present-day OMZ, and thus to better understand past, present, and future changes in the ocean's nitrogen cycle.

[23] **Acknowledgments.** This study is supported by the Research Council for Earth and Life Sciences with financial aid from the Netherlands Organization for Scientific Research (NWO) to J. S. Sinninghe Damsté (grant 853.00.032). G.-J. Reichart and L. J. Lourens acknowledge NWO for funding IODP Cruise 144 to the Arabian Sea. The crew and technicians of Royal NIOZ and R/V *Marion Dufresne* are thanked for shipboard support.

## References

- Altabet, M. A. (1988), Variations in nitrogen isotopic composition between sinking and suspended particles: Implications for nitrogen cycling and particle transformation in the open ocean, *Deep Sea Res., Part A*, 35(4), 535–554, doi:10.1016/0198-0149(88)90130-6.
- Altabet, M. A. (2007), Constraints on oceanic N balance/imbalance from sedimentary <sup>15</sup>N records, *Biogeosciences*, 4(1), 75–86.
- Altabet, M. A., D. W. Murray, and W. L. Prell (1999), Climatically linked oscillations in Arabian Sea denitrification over the past 1 m.y.: Implications for the marine N cycle, *Paleoceanography*, 14(6), 732–743, doi:10.1029/1999PA900035.
- Altabet, M. A., M. J. Higginson, and D. W. Murray (2002), The effect of millennial-scale changes in Arabian Sea denitrification on atmospheric CO<sub>2</sub>, *Nature*, 415(6868), 159–162, doi:10.1038/415159a.
- Bange, H. W., T. Rixen, A. M. Johansen, R. L. Siefert, R. Ramesh, V. Ittekkot, M. R. Hoffmann, and M. O. Andreae (2000), A revised nitrogen budget for the Arabian Sea, *Global Biogeochem. Cycles*, 14(4), 1283–1297, doi:10.1029/1999GB001228.
- Bouwman, A. F., I. Fung, E. Matthews, and J. John (1993), Global analysis of the potential for N<sub>2</sub>O production in natural soils, *Global Biogeochem. Cycles*, 7(3), 557–597, doi:10.1029/93GB01186.
- Brandes, J. A., and A. H. Devol (2002), A global marine-fixed nitrogen isotopic budget: Implications for Holocene nitrogen cycling, *Global Biogeochem. Cycles*, 16(4), 1120, doi:10.1029/2001GB001856.
- Brandes, J. A., A. H. Devol, T. Yoshinari, D. A. Jayakumar, and S. W. A. Naqvi (1998), Isotopic composition of nitrate in the central Arabian Sea and eastern tropical North Pacific: A tracer for mixing and nitrogen cycles, *Limnol. Oceanogr.*, 43(7), 1680–1689.
- Codispoti, L. A., and J. P. Christensen (1985), Nitrification, denitrification and nitrous-oxide cycling in the eastern tropical South Pacific Ocean, *Mar. Chem.*, 16(4), 277–300, doi:10.1016/0304-4203(85)90051-9.
- Codispoti, L. A., J. A. Brandes, J. P. Christensen, A. H. Devol, S. W. A. Naqvi, H. W. Paerl, and T. Yoshinari (2001), 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.
- Dalsgaard, T., and B. Thamdrup (2002), Factors controlling anaerobic ammonium oxidation with nitrite in marine sediments, *Appl. Environ. Microbiol.*, 68(8), 3802–3808, doi:10.1128/AEM.68.8.3802-3808.2002.
- den Dulk, M., G.-J. Reichart, S. van Heyst, W. J. Zachariasse, and G. J. Van der Zwaan (2000), Benthic foraminifera as proxies of organic matter flux and bottom water oxygenation? A case history from the northern Arabian Sea, *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 161(3–4), 337–359, doi:10.1016/S0031-0182(00)00074-2.
- Devol, A. H., A. G. Uhlenhopp, S. W. A. Naqvi, J. A. Brandes, D. A. Jayakumar, H. Naik, S. Gaurin, L. A. Codispoti, and T. Yoshinari (2006), Denitrification rates and excess nitrogen gas concentrations in the Arabian Sea oxygen deficient zone, *Deep Sea Res., Part I*, 53(9), 1533–1547, doi:10.1016/j.dsr.2006.07.005.
- Duplessy, J. C. (1982), Glacial to interglacial contrasts in the northern Indian Ocean, *Nature*, 295(5849), 494–498, doi:10.1038/295494a0.
- Flückiger, J., T. Blunier, B. Stauffer, M. Chappellaz, R. Spahni, K. Kawamura, J. Schwander, T. F. Stocker, and D. Dahl-Jensen (2004), N<sub>2</sub>O and CH<sub>4</sub> variations during the last glacial epoch: Insight into global processes, *Global Biogeochem. Cycles*, 18, GB1020, doi:10.1029/2003GB002122.
- Galbraith, E. D., M. Kienast, T. F. Pedersen, and S. E. Calvert (2004), Glacial-interglacial modulation of the marine nitrogen cycle by high-latitude O<sub>2</sub> supply to the global thermocline, *Paleoceanography*, 19, PA4007, doi:10.1029/2003PA001000.
- Ganeshram, R. S., T. F. Pedersen, S. E. Calvert, G. W. McNeill, and M. R. Fontugne (2000), Glacial-interglacial variability in denitrification in the world's oceans: Causes and consequences, *Paleoceanography*, 15(4), 361–376, doi:10.1029/1999PA000422.
- Grootes, P. M., E. J. Steig, M. Stuiver, E. D. Waddington, D. L. Morse, and M.-J. Nadeau (2001), The Taylor dome Antarctic <sup>18</sup>O record and globally synchronous changes in climate, *Quat. Res.*, 56(3), 289–298, doi:10.1006/qres.2001.2276.
- Gruber, N. (2004), The dynamics of the marine nitrogen cycle and its influence on atmospheric CO<sub>2</sub> variations, in *The Ocean Carbon Cycle and Climate*, edited by M. Follows and T. Ogunz, pp. 97–148, Kluwer Acad., Dordrecht, Netherlands.
- Gruber, N., and J. L. Sarmiento (1997), Global patterns of marine nitrogen fixation and denitrification, *Global Biogeochem. Cycles*, 11(2), 235–266, doi:10.1029/97GB00077.
- Hammersley, M. R., et al. (2007), Anaerobic ammonium oxidation in the Peruvian oxygen minimum zone, *Limnol. Oceanogr.*, 52(3), 923–933.
- Hopmans, E. C., M. V. M. Kienhuis, J. E. Rattray, A. Jaeschke, S. Schouten, and J. S. Sinninghe Damsté (2006), Improved analysis of ladderane lipids in biomass and sediments using high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry, *Rapid Commun. Mass Spectrom.*, 20(14), 2099–2103, doi:10.1002/rcm.2572.
- Jaeschke, A., E. C. Hopmans, S. G. Wakeham, S. Schouten, and J. S. Sinninghe Damsté (2007), 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.
- Jaeschke, A., C. Rooks, M. Trimmer, J. C. Nicholls, E. C. Hopmans, S. Schouten, and J. S. Sinninghe Damsté (2009), Comparison of ladderane phospholipid and core lipids as indicators for anaerobic ammonium oxidation (anammox) in marine sediments, *Geochim. Cosmochim. Acta*, 73, 2077–2088.
- Kabanova, Y. G. (1968), Primary production of northern part of Indian Ocean, *Oceanology*, Engl. Transl., 8(2), 214–225.

- Kuypers, M. M. M., A. O. Slikkers, G. Lavik, M. Schmid, B. B. Jørgensen, J. G. Kuenen, J. S. Sinninghe Damsté, M. Strous, and M. S. M. Jetten (2003), Anaerobic ammonium oxidation by anammox bacteria in the Black Sea, *Nature*, 422(6932), 608–611, doi:10.1038/nature01472.
- Kuypers, M. M. M., G. Lavik, D. Woebken, M. Schmid, B. M. Fuchs, R. Amann, B. B. Jørgensen, and M. S. M. Jetten (2005), Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation, *Proc. Natl. Acad. Sci. U. S. A.*, 102(18), 6478–6483, doi:10.1073/pnas.0502088102.
- Martrat, B., J. O. Grimalt, N. J. Shackleton, L. de Abreu, M. A. Hutterli, and T. F. Stocker (2007), Four climate cycles of recurring deep and surface water destabilizations on the Iberian margin, *Science*, 317(5837), 502–507, doi:10.1126/science.1139994.
- Naqvi, S. W. A. (1987), Some aspects of the oxygen-deficient conditions and denitrification in the Arabian Sea, *J. Mar. Res.*, 45, 1049–1072.
- Qasim, S. Z. (1982), Oceanography of the northern Arabian Sea, *Deep Sea Res., Part A*, 29(9), 1041–1068, doi:10.1016/0198-0149(82)90027-9.
- Rashid, H., R. Hesse, and D. J. W. Piper (2003), Evidence for an additional Heinrich event between H5 and H6 in the Labrador Sea, *Paleoceanography*, 18(4), 1077, doi:10.1029/2003PA000913.
- Rattray, J. E. (2008), *Ladderane Lipids in Anammox Bacteria: Occurrence, Biosynthesis and Application as Environmental Markers*, Univ. of Utrecht, Utrecht, Netherlands.
- Reichart, G.-J., L. J. Lourens, and W. J. Zachariasse (1998), Temporal variability in the northern Arabian Sea oxygen minimum zone (OMZ) during the last 225,000 years, *Paleoceanography*, 13(6), 607–621, doi:10.1029/98PA02203.
- Reichart, G.-J., M. den Dulk, H. J. Visser, C. H. van der Weijden, and W. J. Zachariasse (1997), A 225 kyr record of dust supply, paleoproductivity and the oxygen minimum zone from the Murray Ridge (northern Arabian sea), *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 134(1–4), 149–169, doi:10.1016/S0031-0182(97)00071-0.
- Reichart, G.-J., H. Brinkhuis, F. Huiskamp, and W. J. Zachariasse (2004), Hyperstratification following glacial overturning events in the northern Arabian Sea, *Paleoceanography*, 19, PA2013, doi:10.1029/2003PA000900.
- Sawant, S., and M. Madhupratap (1996), Seasonality and composition of phytoplankton in the Arabian Sea, *Curr. Sci.*, 71(11), 869–873.
- Schubert, C. J., J. Villanueva, S. E. Calvert, G. L. Cowie, U. Von Rad, H. Schulz, U. Berner, and H. Erlenkeuser (1998), Stable phytoplankton community structure in the Arabian Sea over the past 200,000 years, *Nature*, 394(6693), 563–566, doi:10.1038/29047.
- Schulte, S., F. Rostek, E. Bard, J. Rullkötter, and O. Marchal (1999), Variations of oxygen-minimum and primary productivity recorded in sediments of the Arabian Sea, *Earth Planet. Sci. Lett.*, 173(3), 205–221, doi:10.1016/S0012-821X(99)00232-0.
- Sigman, D. M., M. A. Altabet, R. Michener, D. C. McCorkle, B. Fry, and R. M. Holmes (1997), Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: An adaptation of the ammonia diffusion method, *Mar. Chem.*, 57(3–4), 227–242, doi:10.1016/S0304-4203(97)00009-1.
- Sinninghe Damsté, J. S., M. Strous, W. I. C. Rijppstra, E. C. Hopmans, J. A. J. Geenevasen, A. C. T. van Duin, L. A. van Niftrik, and M. S. M. Jetten (2002), Linearly concatenated cyclobutane lipids form a dense bacterial membrane, *Nature*, 419(6908), 708–712, doi:10.1038/nature01128.
- Suthhof, A., V. Ittekkot, and B. Gaye-Haake (2001), Millennial-scale oscillation of denitrification intensity in the Arabian Sea during the late Quaternary and its potential influence on atmospheric N<sub>2</sub>O and global climate, *Global Biogeochem. Cycles*, 15(3), 637–649, doi:10.1029/2000GB001337.
- Thamdrup, B., and T. Dalsgaard (2002), Production of N<sub>2</sub> through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments, *Appl. Environ. Microbiol.*, 68(3), 1312–1318, doi:10.1128/AEM.68.3.1312-1318.2002.
- Thamdrup, B., T. Dalsgaard, M. M. Jensen, O. Ulloa, L. Farias, and R. Escobedo (2006), Anaerobic ammonium oxidation in the oxygen-deficient waters off northern Chile, *Limnol. Oceanogr.*, 51(5), 2145–2156.
- Van Bennekom, A. J., M. Hiehle, J. van Ooyen, E. van Weerlee, and M. van Koutrik (1995), CTD and hydrography, in *Tracing a Seasonal Upwelling*, edited by J. E. Van Hinte, pp. 41–54, Neth. Geosci. Found., The Hague, Netherlands.
- van der Weijden, C. H., G.-J. Reichart, and B. J. H. van Os (2006), Sedimentary trace element records over the last 200 kyr from within and below the northern Arabian Sea oxygen minimum zone, *Mar. Geol.*, 231(1–4), 69–88, doi:10.1016/j.margeo.2006.05.013.
- Wakeham, S. G., R. Amann, K. H. Freeman, E. C. Hopmans, B. B. Jørgensen, I. F. Putnam, S. Schouten, J. S. Sinninghe Damsté, H. M. Talbot, and D. Woebken (2007), Microbial ecology of the stratified water column of the Black Sea as revealed by a comprehensive biomarker study, *Org. Geochem.*, 38, 2070–2097, doi:10.1016/j.orggeochem.2007.08.003.
- Wang, Y. J., H. Cheng, R. L. Edwards, Z. S. An, J. Y. Wu, C. C. Shen, and J. A. Dorale (2001), A high-resolution absolute-dated late Pleistocene monsoon record from Hulu cave, China, *Science*, 294(5550), 2345–2348, doi:10.1126/science.1064618.
- Wang, Y. J., H. Cheng, R. L. Edwards, X. G. Kong, X. H. Shao, S. T. Chen, J. Y. Wu, X. Y. Jiang, X. F. Wang, and Z. S. An (2008), Millennial- and orbital-scale changes in the East Asian monsoon over the past 224,000 years, *Nature*, 451(7182), 1090–1093, doi:10.1038/nature06692.
- Wyrski, K. (1973), Physical oceanography of the Indian Ocean, in *The Biology of the Indian Ocean*, edited by B. Zeitschel, pp. 18–36, Springer, Berlin.

E. C. Hopmans, A. Jaeschke, S. Schouten, and J. S. Sinninghe Damsté, Department of Marine Organic Biogeochemistry, Royal Netherlands Institute for Sea Research, P.O. Box 59, NL-1790 AB Den Burg, Texel, Netherlands. (jaeschke@nioz.nl)

L. J. Lourens, G.-J. Reichart, and M. Ziegler, Department of Earth Sciences, Faculty of Geosciences, Utrecht University, Budapestlaan 4, P.O. Box 80.021, NL-3508 TA Utrecht, Netherlands.