Minireview

Lipids as paleomarkers to constrain the marine nitrogen cycle

Darci Rush1,2 and Jaap S. Sinninghe Damsté1,3*

1Department of Marine Microbiology and Biogeochemistry, NIOZ Royal Netherlands Institute for Sea Research, and Utrecht University, Den Burg, P.O. Box 59 1790 AB, The Netherlands
2School of Civil Engineering and Geosciences, Newcastle University, Newcastle-upon-Tyne, NE1 7RU, United Kingdom
3Department of Earth Sciences, Faculty of Geosciences, Utrecht University, TA Utrecht, P.O. Box 80.121, 3508, The Netherlands

Summary

Global climate is, in part, regulated by the effect of microbial processes on biogeochemical cycling. The nitrogen cycle, in particular, is driven by microorganisms responsible for the fixation and loss of nitrogen, and the reduction-oxidation transformations of bio-available nitrogen. Within marine systems, nitrogen availability is often the limiting factor in the growth of autotrophic organisms, intrinsically linking the nitrogen and carbon cycles. In order to elucidate the state of these cycles in the past, and help envisage present and future variability, it is essential to understand the specific microbial processes responsible for transforming bio-available nitrogen species. As most microorganisms are soft-bodied and seldom leave behind physical fossils in the sedimentary record, recalcitrant lipid biomarkers are used to unravel microbial processes in the geological past. This review emphasises the recent advances in marine nitrogen cycle lipid biomarkers, underlines the missing links still needed to fully elucidate past shifts in this biogeochemically-important cycle, and provides examples of biomarker applications in the geological past.

Biomarker lipids and the marine nitrogen cycle

Nitrogen is vital to the building blocks of life (e.g. nitrogen is a key element in amino acids, nucleic acids, and membrane lipids). The largest marine pool of nitrogen is dissolved dinitrogen (N₂). However, few organisms in the ocean are capable of utilising N₂; non-diazotrophic microbes must depend on the less substantial pools of bio-available nitrogen [i.e. nitrate (NO₃⁻); nitrite (NO₂⁻); ammonium (NH₄⁺)] to sustain their activity (Fig. 1). The concentration of these different forms of nitrogen varies spatially and temporally in the ocean. In general, the highest concentrations of NO₂⁻ and NH₄⁺ are found in the surface waters where they are assimilated by phytoplankton to form a pool of organic nitrogen. The concentrations of these two species decrease significantly at the base of the euphotic zone, as they are converted into NO₃⁻ by nitrifying microorganisms. NO₃⁻ on the other hand accumulates with water depth and is usually present an order of magnitude higher than NO₂⁻ and NH₄⁺ (Gruber, 2008). The fate of nitrogen in deeper waters can either be towards burial as particulate matter, or complete loss as it is converted back to N₂ in anoxic conditions. Nevertheless, it must be emphasised that the presence of these bio-available forms of nitrogen in the ocean is inconsistent, and their concentrations in the water column are habitually low.

Nitrogen is often the limiting nutrient for primary productivity in marine environments, and our understanding of the marine carbon pump inherently depends on what we know of the nitrogen cycle. Since the global inception of the Haber-Bosh process in the early 20th century, anthropogenic activity has significantly influenced the marine nitrogen cycle. Run-off from fertilisers in coastal areas causes eutrophication of the water column, leading to oceanic dead zones (Altieri and Gedan, 2015), and changes in the extent and vertical structure of oxygen minimum zones (OMZs) (Paulmier and Ruiz-Pino, 2009; Wright et al., 2012). Besides the obvious repercussions on ecology and climate, anthropogenic nitrogen input also greatly affects local economies by decreasing the available stock of commercial fish (Lavin et al., 2006; Rabotyagov et al., 2014).

Received 21 November, 2016; Revised: 24 January, 2017; accepted 25 January, 2017. *For correspondence. E-mail: J.S.SinningheDamste@uu.nl; Tel. +31 (0)222 369 550; Fax +31 (0)222 319 674.

© 2017 The Authors. Environmental Microbiology published by Society for Applied Microbiology and John Wiley & Sons Ltd This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
The transformations between chemical species of nitrogen are primarily regulated by microbial activity, with specialised organisms performing each process (Fig. 1). Nitrogen cycle microorganisms are often taxonomically divided, and many have structurally unique lipids, known as biomarkers (Peters et al., 2005). By employing biomarker lipids, we can determine the contributions of different nitrogen cycle processes through Earth’s history. Biomarkers are especially useful in reconstructing paleo-environments where genetic information has been degraded (cf. Hofreiter et al., 2001). Subsequently, this biomarker information can be used to better predict modern and future nitrogen and carbon cycle variations by constraining biogeochemical models of the past [e.g. during the Cenomanian-Turonian Ocean Anoxic Event (OAE 2); Monteiro et al., 2012].

Biomarkers exist for many of the key nitrogen biogeochemical processes. However, there are some glaring gaps, notably on the anoxic side of the nitrogen cycle. OMZs are found in the marine water column underlying productive photic zones, and are hotbeds for anaerobic nitrogen cycling processes, most of which result in net marine nitrogen loss through nitrogenous gas production. It has been estimated that half of the nitrogen loss in the ocean occurs within these OMZs (Codispoti et al., 2001; Lam et al., 2009). In our current dynamic ocean climate of increasing temperature and decreasing ventilation, OMZs are expanding (Wright et al., 2012). It is imperative that we be able to predict how past perturbations in ocean biogeochemistry, especially within OMZs, affected the sensitivity of the system.

Several review papers have highlighted the marine nitrogen cycle (Gruber, 2004; 2008; Arrigo, 2005;
Bringing nitrogen in: Diazotrophic organisms in the marine system

N₂ accounts for 94% (10⁷ Tg N) of the nitrogen in the ocean (Gruber, 2008). Diazotrophy, or nitrogen-fixation, is essential to the fertilisation of ocean surface waters in oligotrophic regions, where there are no other significant inputs of nitrogen (Capone, 2001; Mahaffey et al., 2005). N₂-fixation has been estimated to contribute 36–50% of the nitrogen used by microbes in the Pacific and Atlantic gyres (Carpenter et al., 1999; Dore et al., 2002). The most important marine diazotrophs belong to the phylum Cyanobacteria (Carpenter and Romans, 1991; Capone et al., 1997; Karl et al., 1997; Gallon, 2001), and by far the most well-studied species are the filamentous Trichodesmium spp. However, unicellular cyanobacteria (Martinez-Perez et al., 2016) and non-cyanobacteria diazotrophs (Farnelid et al., 2011) may also play an important role in N₂-fixation in marine systems. Diazotrophic organisms use the enzyme nitrogenase to break the triple bonds between the N atoms of N₂. Nitrogenase is inactive in the presence of oxygen, a fact that is problematic to the surface dwelling, primarily photoautotrophic, microorganisms using nitrogenase. Diazotrophs compensate for this in different ways. Some use the light/dark cycle to spatially separate these processes. Others use physical separation by compartmentalising diazotrophy into specialised cells. Below, we discuss two groups of diazotrophy biomarkers.

Bacteriohopanepolyols of cyanobacteria

Cyanobacteria are considered to be the most environmentally significant source of bacteriohopanepolyol lipids (BHPs) (Farrimond et al., 1998; Talbot and Farrimond, 2007). BHPs are the precursors to the most ubiquitous lipids found in geological materials: geohopanoids (e.g. Ourisson and Albrecht, 1992). BHPs are pentacyclic C₃₀ triterpenoids linked to a polyfunctionalised side chain with e.g. hydroxyl, amine, or cyclic groups (Fig. 2). Although not all cyanobacteria synthesise BHPs, and many synthesise non-source specific BHPs, [e.g. bacteriohopanetetrol (BHT) and aminobacteriohopanetriol (aminotriol)], some marine N₂-fixing species of cyanobacteria have been shown to synthesise more characteristic BHPs. C-2 methylated BHPs (Fig. 2) were found in Trichodesmium and Crocosphaera, both ubiquitous marine species (Talbot et al., 2008). C-2 methylated hopanes (the diagenetic product of BHPs) were originally thought to be a biomarker for cyanobacteria, and have been used previously to indicate the rise of oxygenic photosynthesis (Summons et al., 1999) as well as the importance of N₂-fixation at times of stratified oceans (e.g. Cretaceous black shales; Kuypers et al., 2004). However, other bacteria have also been found to synthesise C-2 methylated BHPs [i.e. anoxicogenic phototroph Rhodopseudomonas palustris TIE-1 (Rashby et al., 2007), Methylobacterium spp. (Bisseret et al., 1985; Knani et al., 1994) and Bradyrhizobium spp. (Bravo et al., 2001)]. Furthermore, the gene coding for the enzyme responsible for C-2 methylation in BHPs was found to occur across the bacterial domain (Welander et al., 2010). Thus, using the C-2 methylation in hopanoids as a biomarker for cyanobacteria input and the onset of atmospheric oxygen is complicated.

A more appropriate BHP biomarker for marine N₂-fixation may be BHT cyclitol ether (BHT-CE; Fig. 2) with an unsaturation at the C-6 or C-11 position. Unsaturated BHT-CE is synthesised by Trichodesmium sp. (Talbot et al., 2008), one of the most prolific and biogeochemically-important marine cyanobacteria. However, this lipid has also been found in acetic acid bacterial cultures (Simonin et al., 1994; Herrmann et al., 1996; Talbot et al., 2007), and several strains of Burkholderia cepacia isolated from acetic grasslands (Cvejic et al., 2000). Thus, care must be taken when applying unsaturated BHT-CE as a biomarker for Trichodesmium. As it is unlikely that either of these other types of bacteria proliferate in open marine settings, unsaturated BHT-CE was successfully applied as a biomarker for Trichodesmium in sediments from the Congo deep sea-fan to interpret the past marine nitrogen cycle (Handley et al., 2010). Unsaturated BHT-CE was present in unprecedented abundance at a specific sedimentary horizon (480–610 ka). Here, the abundance of unsaturated BHT-CE was interpreted to be caused by a decrease in nutrient supply, which resulted in increased N₂-fixation by diazotrophic cyanobacteria.

Heterocyst glycolipids

A large group of diazotrophic cyanobacteria contain heterocysts, specialised cells where diazotrophy occurs under induced low oxygen conditions. To maintain anoxia, heterocystous cyanobacteria surround these cells with a thick envelope limiting the diffusion of gases (Walsby, 1985; Murry and Wolk, 1989), which is made up of glycolipids known as heterocyst glycolipids (Nichols and Wood, 1968; Bryce et al., 1972; Lambein and Wolk, 1973). Heterocyst glycolipids consist of sugar
Fig. 2. Biomarkers of microbial processes in the marine nitrogen cycle. Diazotrophy biomarkers: unsaturated bacteriohopanetetrol cyclitol ether; 2-methyl-bacteriohopanepoyols are diagenetically altered in sediment burial to 2-methyl-geohopanoids; heterocyst glycolipids with C₆ and C₅ sugar head groups. Nitrification biomarkers: crenarchaeol and GDGT-0-4; 11-methyl-hexadecanoic acid. Anammox biomarkers: ladderane fatty acids undergo oxic degradation into short chain ladderane fatty acids; bacteriohopanetetrol stereoisomer; anammox bacterial biomass is thermally altered to produce branched long chain alkanes. Nitrite-reducing methanotrophy biomarkers: bacteriohopanethexol; 3-methyl-bacteriohopanethexol.
functions glycosidically bound to long-chain \((C_{26}-C_{32})\) diols, triols, keto-ols, or keto-diols (Fig. 2). The distribution of heterocyst glycolipids in cyanobacteria was screened using high-performance liquid chromatography coupled to electrospray ionisation tandem mass spectrometry, and was found to vary according to taxonomic groups (Bauersachs et al., 2009a,b). For example, species belonging to the family *Nostocaceae* synthesise the glycolipids 1-(O-hexose)–3,25-hexacosanediol and 1-(O-hexose)–3-keto-25-hexacosanol, whereas members of the *Calothrix* genus primarily synthesise 1-(O-hexose)–3,25,27-octacosanetriol and 1-(O-hexose)–3-keto-25,27-octacosanediol.

In marine systems, heterocystous cyanobacteria are also commonly found in symbiosis with diatoms (Villareal, 1992). Schouten et al. (2013a) found that these endosymbiotic marine cyanobacteria species synthesise heterocyst glycolipids with distinct C-5 sugar moieties (Fig. 2). These have been found in suspended particulate matter and surface sediments from the marine Amazon plume region, and proposed to be specific biomarkers for marine endosymbiotic heterocystous cyanobacteria (Bale et al., 2015).

**Converting waste into nutrients: Nitrification**

Most fixed organic nitrogen is remineralised back into \(\text{NH}_4^+\) by heterotrophic bacteria. This pool of reduced nitrogen is then oxidised by nitrifying microorganisms (Fig. 1). Nitrification is the primary source of oxidised nitrogen species in the ocean, converting reduced \(\text{NH}_4^+\) to oxidised \(\text{NO}_2^-\) and \(\text{NO}_3^-\). Traditionally, nitrification is divided into two distinct steps, performed by different groups of microorganisms: (1) ammonia oxidation to nitrite [initially thought to be performed by ammonia-oxidising members of Proteobacteria (AOB), and since expanded to include ammonia-oxidising archaea (AOA)], and (2) nitrite oxidation to nitrate (nitrite-oxidising bacteria; NOB) (Ward, 2013a). Recently, specific species of the bacterial genus *Nitrospira* have been identified performing both of the nitrification steps completely [complete ammonia oxidation (comammonox); Daims et al., 2015; van Kessel et al., 2015; Pinto et al., 2016].

**Lipids of ammonium-oxidising bacteria (AOB)**

The most common AOBs in natural environments belong to the genus *Nitrosomonas*. However, the lipids of *Nitrosomonas* spp. are undiagnostic, including generic BHPs (aminobacteriohopanetriol, diploptene) and fatty acids (unsaturated-, cis-9- and trans–9-hexadecanoic acids) (Sakata et al., 2008).
clusters of AOA Thaumarchaeota (e.g. Francis et al., 2005; Hallam et al., 2006; Hu et al., 2011). This trend was also observed recently in the production of GDGTs within the water column by evaluating a key gene involved in the lipid biosynthetic pathway coding for GDGT precursor geranylgeranylglyceryl phosphate (GGGP) synthase in Thaumarchaeota (Villanueva et al., 2015). These authors observed a positive correlation between an increase of GGGP synthase and the relative proportion of GDGT-2 with water depth, suggesting a niche differentiation in GDGT distributions. Interestingly, this depth speciation was not observed in 16S gene-based phylogeny (Schouten et al., 2015). These authors observed a positive correlation between an increase of GGGP synthase and the relative proportion of GDGT-2 with water depth, suggesting a niche differentiation in GDGT distributions. Interestingly, this depth speciation was not observed in 16S gene-based phylogeny (Schouten et al., 2015). Ammonia availability was suggested to be responsible for the niche partitioning of thaumarchaeota in the Arabian Sea (Villanueva et al., 2015). The ‘deep’ cluster of AOA on the Portuguese continental margin was found to contribute significantly to the GDGTs present in sediments at this depth speciation was not observed in 16S gene-based phylogeny (Schouten et al., 2015). Ammonia availability was suggested to be responsible for the niche partitioning of thaumarchaeota in the Arabian Sea (Villanueva et al., 2015). The ‘deep’ cluster of AOA on the Portuguese continental margin was found to contribute significantly to the GDGTs present in sediments at greater water depth, implying this non-surface population does have an influence on TEX86 (Kim et al., 2016). Hurley et al. (2016) recently suggested that the distribution of GDGTs of thaumarchaeota may be dependant on the nitrification rate. These studies merely highlight the further work required to understand the signal that TEX86 records.

Fatty acids of nitrite-oxidising bacteria (NOB)

Nitrospina spp. are the most abundant NOB in marine systems, and dominate microaerophilic zones of OMZs (Lücker et al., 2013). Fatty acids of four genera of NOB (Nitrobacter, Nitrooccus, Nitrospina, and Nitrospira) have been described, and each genus appears to have its own distinct profile (Auran and Schmidt, 1972; 1976; Lipski et al., 2001) (Fig. 2). Although fatty acids are typically not specific enough to be applied as biomarkers, these differences would enable differentiation between NOB bacteria in particular environments. A novel fatty acid, 11-methyl-hexadecanoic acid, was identified in Nitrospira moscoviensis (Lipski et al., 2001). Unfortunately, however, fatty acids are not recalcitrant enough to endure diagenesis in buried marine sediments, and hence, fatty acids can typically not be used in sediments older than ca. 1 Ma.

Given that the comammox bacteria described performing complete nitrification belong to the genus Nitrospira, it is possible that comammox species synthesise 11-methyl-hexadecanoic acid. However, non-comammox species of Nitrospira also synthesise 11-methyl-hexadecanoic acid (Lebedeva et al., 2011), showing that this fatty acid is not an appropriate biomarker for comammox. The Nitrospira genus is diverse. Nitrospira has at least six sub-lineages (Daims et al., 2001), and has been found in various natural environments (e.g. marine, Watson et al., 1986; fresh water, Daims et al., 2001; and terrestrial, Lebedeva et al., 2011; Pester et al., 2014). Lipski et al. (2001) found that the two species they studied belonging to Nitrospira already showed variations between their lipids. Thus, it is possible that comammox Nitrospira spp. have their own distinct fatty acid lipid profile, which has yet to be characterized.

Closing the circle: Denitrifying processes return nitrogen to the atmosphere

In anoxic environments, the system shifts towards favouring organisms that use reaction pathways resulting in net nitrogen loss through the production of N-containing gases, i.e. nitrous oxide (N₂O) and N₂ (Mulder et al., 1995; Zumft, 1997). There are two major pathways of nitrogen loss from the marine environment: denitrification and anaerobic ammonium oxidation (anammox) (Fig. 1). In modern marine environments, the production of N₂ by anammox and denitrification represents an important sink of bio-available nitrogen.

Before the discovery of anammox in a waste water treatment plant (Mulder et al., 1995), NH₄⁺ was thought to be unreactive in anoxic systems. However, in the following decades the bacteria that anaerobically oxidise NH₄⁺ using NO₂⁻ have been found to be prevalent in marine systems (Dalsgaard and Thamdrup, 2002; Kuypers et al., 2003; Capone, 2008; Gruber, 2008), especially within OMZs (Kuypers et al., 2005; Hammersley et al., 2007; Lam et al., 2009; Sollai et al., 2015). Anammox bacteria are slow-growing, taking weeks to divide (Strous et al., 1999), which might be to their advantage in certain systems as they may not have to respond to sudden changes in substrate concentration. For example, the anammox reaction was found consistently, though at low rates, within the Eastern Tropical South Pacific OMZ, whereas the presence of denitrification was sporadic (Dalsgaard et al., 2012). This could indicate that anammox represents a constant background loss of nitrogen from anoxic systems.

Ladderane and BHT lipids of anammox bacteria

One of the most challenging puzzles in membrane lipid composition of the last 15 years was the stereochemical structure of lipids found exclusively in anammox bacteria. Strained cyclobutyl moieties require such a high amount of energy to synthesise that they were not thought to exist in nature. However, isolated lipids from anammox bacterial enrichments proved to have three or five concatenated cyclobutyl moieties (Fig. 2; Sinninghe Damsté et al., 2002b), and were named ‘ladderane lipids’ for their 3-dimensional structure, which was later confirmed by total synthesis (Mascitti and Corey, 2004; Mercer et al., 2016). Ladderanes play an important role
within the anammox cells. These lipids create a dense membrane bilayer that prevents the diffusion of the toxic intermediate gas (hydrazine) of the anammox reaction into other cell functions. The anammox reaction is thus entirely contained within the organelle-like anammoxosome. Originally thought to be specific to the anammoxosome, ladderane lipids have subsequently been found in other anammox cell fractions (Neumann et al., 2014). These results seem to indicate that the anammox bacteria do not possess a mechanism to sort their lipids. Nevertheless, ladderanes are excellent biomarkers because they are uniquely synthesised by anammox bacteria.

Unfortunately, both the strain on the cyclobutyl moieties in addition to the carboxyl functionality of ladderane fatty acids means that these biomarkers do not tolerate diagenesis to a significant extent, and have not been found in mature marine sediments. Recently, a relatively rare stereoisomer of BHT (BHT isomer) was found to be synthesised in high abundance by marine anammox bacteria (Fig. 2; Rush et al., 2014a). This BHT was also found in sediments from an anoxic marine fjord. Considering BHPs have been found in sediments of 56 Ma old (Talbot et al., 2016), BHT isomer shows potential to fill the gap as a biomarker for anammox in sediments that are not yet thermally mature, but which are older than the limit of diagenetic alteration for ladderanes.

Nitrite-reducing methanotrophy biomarkers

Methylomirabilis oxyfera is an exceptional methanotroph that produces its own oxygen from NO2 via the production of NO by nitrite reductase (Ettwig et al., 2010). This O2 is subsequently used to oxidise methane. M. oxyfera was shown to synthesise characteristic BHP biomarkers: bacteriophepanehexol (BHP-hexol) and 3-Me-BHP-hexol (Fig. 2; Kool et al., 2014). Although the significance of this process within the marine nitrogen cycle is currently unknown, intra-aerobic methanotrophy has recently been detected in marine OMZs, suggesting it may play a role in the nitrogen cycle of these environments (Padilla et al., 2016). Using 3-Me-BHP-hexol, we may be able to understand past NO2 availability in dynamic OMZ systems, where up to 50% of marine bioavailable nitrogen loss occurs (Codispoti et al., 2001; Lam et al., 2009).

Anaerobic nitrogen cycle processes without specific biomarker lipids

The importance of dissimilatory nitrate reduction to ammonium (DNRA; Fig. 1) in marine systems is unresolved. DNRA was proposed to be an important source of NH4 in the Peruvian OMZ (Lam et al., 2009), but was not detected within the Chilean OMZ (De Brabandere et al., 2014). There are currently no biomarker lipids available to trace past shifts of DNRA.

Perhaps the most versatile anaerobic nitrogen cycle process is denitrification, where NO3 is reduced via NO2, NO, and N2O to N2 (Fig. 1). However, this process is not related to a specific taxonomic group (e.g. autotrophic and heterotrophic bacteria, archaea, and foraminifera can all perform denitrification; Zumft, 1997; Risgaard-Petersen et al., 2006), which severely complicates finding biomarkers that are specific to denitrification. It is thus more likely that if we are going to trace denitrification using lipids, it will be with a suite of biomarkers related to individual denitrifiers. Another possible avenue is using anammox biomarkers to infer denitrification. Ward (2013b) proposed that the proportion of anammox is thermodynamically linked to denitrification, where the latter accounts for 70% of N loss from the ocean. If this proves to hold true over nitrogen cycle history, we could reconstruct total nitrogen loss due to anammox and denitrification solely using anammox biomarkers.

Application of biomarkers to interpret nitrogen cycling in the geological past

Nitrogen cycle biomarkers can be applied to the modern ocean, complementing molecular, genetic, and isotope-labeling approaches (e.g. Kuyper et al., 2003; 2005; Hammersley et al., 2007; Pitcher et al., 2011). However, such biomarkers are especially beneficial for the geological past, where these other techniques can no longer be used. Below, we discuss four examples of nitrogen cycle biomarker application.

Ladderane lipids: The importance of anammox in the past Arabian Sea OMZ

The abundance of ladderane fatty acids was investigated in sediments underlying the Arabian Sea OMZ, spanning the last 140 ka (Fig. 3a; Jaeschke et al., 2009). During this time period, the maxima in ladderane lipid concentration correlated with events of high total organic carbon (TOC) content and elevated 15N. This would appear to indicate enhanced anammox activity coincides with periods of increased intensity in the Arabian Sea OMZ and large-scale changes in the nitrogen cycle (i.e. preferential removal of the lighter 14N isotope). Unfortunately, it has so far not been possible to assess anammox further back in time using ladderane lipids as they are not resistant to the degradative stresses of sediment burial. Ladderane fatty acids undergo β-oxidation in the presence of even very low oxygen concentrations to form short chain ladderane fatty acids, which have been found in sediments underlying OMZs (Rush et al., 2011; 2012). However, these too appear to undergo further
degradation, and these Arabian Sea sediments are currently the oldest known detection of past anammox activity (Jaeschke et al., 2009).

In order to potentially reveal degradation lipid products better-suited for past detection of the anammox reaction, conditions undergone during catagenesis of organic matter were simulated; anammox biomass was artificially thermally matured via hydrous pyrolysis (Jaeschke et al., 2008). Ladderane lipids were found to be completely structurally altered at relatively low levels of thermal stress. However, thermally stable products in which some of the cyclobutane rings had opened, could still be detected at 260°C (Jaeschke et al., 2008). Additionally, unusual branched long chain alkanes originating from anammox biomass were identified in the oils generated during these artificial maturation experiments (Fig. 2; Rush et al., 2014b). Investigating these alkanes alongside BHT isomer might be an appropriate approach to detect anammox in thermally more mature sediments such as those deposited in the Cretaceous during so called oceanic anoxic events (OAEs).

Heterocyst glycolipids: Expansion of cyanobacteria during arctic black shale deposition

Like the modern-day Black Sea, the Arctic Ocean during the middle Eocene (ca. 49 Ma) was a warm, stratified basin with fresh surface waters. Such conditions induced

---

Fig. 3. Biomarker evidence for paleo-nitrogen cycling processes from (a) ladderane fatty acid abundances (Jaeschke et al., 2009) of Arabian Sea sediments underlying the oxygen minimum zone (core MD04-2879) over the past 140 ka, spanning Marine Isotope Stages (MIS) 1 to 6; (b) heterocyst glycolipid abundances (Bauersachs et al., 2010) and Azolla massulae counts (Speelman et al., 2009) of Eocene Arctic Ocean sediments including black shale intervals (Integrated Ocean Drilling Project Expedition 302, ACEX); (c) 2-methyl-hopanoid indices (Kuypers et al., 2004) of organic-rich sediment from the last 200 Ma, including the Cenomanian ocean anoxic event (OAE 2) (Deep Sea Drilling Project Sites 144, 367, and 603B, proto North Atlantic) and Aptian OAE 1a (Cismon apticore, Southern Alps, Italy, Aptian Thetys; Deep Sea Drilling Project Site 463, Pacific Ocean). Values represent averages of 1-24 samples.; (d) crenarchaeol abundances and carbon isotopic values of tricyclic biphytanes (C40:3cy) derived from archaea (Kuypers et al., 2001) of Albian OAE 1b (Ocean Drilling Project site 1049C). Warm MIS and anoxic events are indicated as grey shaded areas in the graphs. TOC: total organic carbon; δ15N: bulk sediment nitrogen isotope value (in per mil vs. N2atm); 2-methyl-hopanoid index = weighted average of the dominant 2-methyl/2-desmethylhopanoid pairs; δ13C: carbon isotope values (in per mil vs. Vienna Pee Dee Belemnite) for bulk organic carbon (OC) and C40:3cy.
an intense bloom of the free-floating aquatic fern, *Azolla*. *Azolla* microspores occurred abundantly in the palynological record (Brinkhuis *et al*., 2006), and corresponded to increased TOC values. These *Azolla* ferns probably thrived in the fresh Arctic surface waters and high CO₂ atmosphere. The carbon fixed by *Azolla* subsequently represented a significant sink of CO₂ as their biomass was incorporated into buried sedimentary organic matter (Speelman *et al*., 2009). Bauersachs *et al.* (2010) found co-variance in the enhanced input of *Azolla* spores and the abundance of heterocyst glycolipids in these high TOC Eocene Arctic black shale sediments (Fig. 3b). Additionally, the averaged values of the bulk sedimentary nitrogen isotope ratio ($\delta^{15}$N) at the time were $-1\%$, which is consistent with a primarily diazotrophic signal. These results would seem to indicate that diazotrophs lived in symbiosis with *Azolla*, as seen in modern systems (Peters, 1991), and that they supplied fixed nitrogen to a phytoplankton community in the stratified Arctic Ocean. It is possible that N₂-fixation sustained photosynthetic anoxygenic green sulphide-containing waters occurred in the photic zone, providing a niche for photosynthetic anoxygenic green sulfur bacteria (Sinninghe Damsté and Köster, 1998). This resulted in widespread deposition of organic rich black shales (Arthur and Sageman, 1994). The extreme scenario of an OAE would involve a total reorganisation of the nitrogen cycle.

It is probable that like the Eocene Arctic, surface waters of Cretaceous OAEs 1a and 2 were characterized by increased cyanobacterial activity. The extraordinary abundance of, albeit less source-specific, 2-Methyl-hopanoids during these OAEs (Fig. 3c) indicates a shift towards diazotrophy as the primary source of nitrogen (Kuyper *et al*., 2004). Increased N₂-fixation could have been induced by limited vertical mixing of deep-water nitrogen species due to a stratification of the water column. It has been proposed that increased denitrification and anammox activity in the expanded anoxic waters of OAEs would have led to a substantial decrease in nitrogen availability (Kuyper *et al*., 2004). In order sustain oceanic primary productivity, this loss of nitrogen would have needed to be compensated by increased cyanobacterial N₂-fixation. This is seemingly apparent by the increased levels of 2-Me hopanoids (Fig. 3c). However, this nitrogen loss hypothesis should still be tested by investigating BHT isomer and branched long chain alkanes in these OAE sediments.

### Crenarchaeol: Albion OAE bloom of thaumarchaeota

In disagreement with lipid evidence from OAEs 1a and 2, biomarkers for diazotrophs were not detected in Albion OAE 1b sediments from the proto North Atlantic and Tethys Ocean (Kuyper *et al*., 2001; Tsikos *et al*., 2004). In contrast, OAE 1b sediments contained an unprecedented contribution of crenarchaeol and organic material derived from nitrifying archaea (e.g. Fig. 3d; proto North Atlantic; Kuyper *et al*., 2001). This was evident from the $\delta^{13}$C isotopic values of TOC, which was substantially enriched ($\sim -20\%$) during OAE 1b, compared with non-OAE conditions ($\sim -25\%$) (Fig. 3d). Neither plant ($-28$ to $-29\%$ leaf-waxes), algal ($-27$ to $-29\%$; steroids), nor bacterial ($-24$ to $-27\%$; hopanoid) lipids showed the large change in $\delta^{13}$C observed for TOC, suggesting a temporary source of $^{13}$C-enriched organic matter during OAE 1b (Kuyper *et al*., 2002). Extant Thaumarchaeota use a modified hydroxypropionate-hydroxybutyrate cycle carbon fixation pathway (Berg *et al*., 2010; Köneke *et al*., 2014), which results in $^{13}$C-enriched AOA lipids in sediment of the present-day ocean (Schouten *et al*., 2013b). In agreement with this, the tricyclic biphytane (C₄₀:₃cy), a derived ether-cleaved component of crenarchaeol, is $^{13}$C-enriched during OAE 1b ($-17$ to $-18\%$; Fig. 3). The same holds for AOA-derived macromolecular matter (Kuyper *et al*., 2002). Thus, the $^{13}$C enrichment of bulk TOC in OAE 1b was likely due to enhanced archaeal contribution to the bulk organic carbon pool. These results would seem to indicate that AOA played a significant role in the onset and maintenance of OAE 1b.

The reason for the abundance of AOA during OAE 1b remains enigmatic. It seems that only a trigger independent of primary production (the source of organic nitrogen and ultimately ammonium in the present-day ocean) could effect this change. The mid-Cretaceous was a period of increased oceanic crust formation through enhanced volcanism (Larson, 1991). This volcanic activity would have introduced vast amounts of NH₄⁺ to the marine water column. As some nitrifiers can function at very low oxygen concentrations, they can thrive at the oxic-anoxic water boundary and provide oxidised nitrogen species to denitrifying organisms in anaerobic niches (Lam and Kuyper, 2011; Füssel *et al*., 2012). During OAE 1b, it is possible that AOA were oxidising volcanic NH₄⁺ to NO₂⁻, which was provided to denitrifying...
organisms. The consequent production of $\text{N}_2$ before this bio-available nitrogen could be exploited by photoautotrophs may have helped maintain water column anoxia during the Albian OAE. In comparison, injection of $\text{NH}_4^+$ from a modern submarine arc-volcano has been shown to support a nitrifying archaea community (closely related to *Nitrosopumilus maritimus*) in Fe-rich microbial mats (Killas *et al*., 2013). Here, AOA is potentially supplying oxidised nitrogen species to be used to chemical oxidise hydrothermal Fe$^{2+}$ into ferricyclides.

These results show that there was no universal mechanism for OAE formation and black shale deposition in the Cretaceous. This further underscores the need to better constrain the marine nitrogen cycle during diverse past biogeochemical events in order to predict future change.

**Conclusions**

Biomarker lipids have been used effectively to constrain past nitrogen cycling. However, available biomarkers are still limited, especially for anaerobic nitrogen cycling processes. Without biomarker observations in paleoenvironments, we cannot predict how the current expansion of marine oxygen minimum and anoxic zones will affect the removal of bio-available nitrogen, nor the feedback mechanisms that will take place within the nitrogen cycle. Accordingly, more robust lipids must be developed as biomarkers for (anaerobic) nitrogen cycle processes.

In order for a biomarker to be used appropriately it must first be rigorously identified. Regrettably, this is often the most time consuming step in the pathway to introducing a novel biomarker. Advances in instrumentation (e.g. two-dimensional nuclear magnetic resonance, X-ray crystallography, scanning probe microscopy, gas chromatography, liquid chromatography, mass spectrometry) have primarily shaped the direction and limitation of biomarker identification/application. Often the biomarker community must wait for the development of improved methods in order to move forward with biomarker innovation. However, it is clearly essential to the advancement of novel biomarker for marine nitrogen cycle processes to invest the time in undertaking these types of investigations.

**Acknowledgements**

The authors would like to thank Dr N. Bale for her invaluable discussions on diazotrophy and tropical nitrogen cycling. DR and JSSD were financially supported by the Netherlands Earth Systems Science Centre (NESSC), a Gravitation grant (024.002.001) from the Dutch Ministry of Education, Culture and Science. JSSD also receives funding from the Soehngen Institute for Anaerobic Microbiology (SIAM) though a gravitation grant from the Dutch ministry for Education, Culture and Science. This project has also received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (grant agreement n° 694569 – MICROLIPIDS).

**References**

Allieri, A.H., and Gedan, K.B. (2015) Climate change and dead zones. *Glob Change Biol* 21: 1395–1406.

Arrigo, K.R. (2005) Marine microorganisms and global nutrient cycles. *Nature* 437: 349–355.

Arthur, M.A., and Sageman, B.B. (1994) Marine black shales: depositional mechanisms and environments of ancient deposits. *Annu Rev Earth Planet Sci* 22: 499–551.

Auran, T.B., and Schmidt, E.L. (1972) Similarities between *Hyphomicrobium* and *Nitrobacter* with respect to fatty acids. *J Bacteriol* 109: 450–451.

Auran, T.B., and Schmidt, E.L. (1976) Lipids of *Nitrobacter* and effects of cultural conditions on fatty-acid composition. *Biochim Biophys Acta* 391: 390–398.

Bale, N.J., Hopmans, E.C., Zell, C., de Lima Sobrinho, R., Kim, J.H., Sinninghe Damsté, J.S., *et al* (2015) Long chain glycolipids with pentose head groups as biomarkers for marine endosymbiotic heterocystous cyanobacteria. *Org Geochem* 81: 1–7.

Bauersachs, T., Compaoré, J., Hopmans, E.C., Stal, L.J., Schouten, S., and Sinninghe Damsté, J.S. (2009a) Distribution of heterocyst glycolipids in cyanobacteria. *Phytochemistry* 70: 2034–2039.

Bauersachs, T., Hopmans, E.C., Compaoré, J., Stal, L.J., Schouten, S., and Sinninghe Damsté, J.S. (2009b) Rapid analysis of long-chain glycolipids in heterocystous cyanobacteria using high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry. *Rapid Commun Mass Spectrom* 23: 1387–1394.

Bauersachs, T., Speelman, E.N., Hopmans, E.C., Reichart, G.J., Schouten, S., and Sinninghe Damsté, J.S. (2010) Fossilized glycolipids reveal past oceanic $\text{N}_2$ fixation by heterocystous cyanobacteria. *Proc Natl Acad Sci U S A* 107: 19190–19194.

Bauersachs, T., Mudimu, O., Schulz, S., and Schwark, L. (2014) Distribution of long-chain heterocyst glycolipids in $\text{N}_2$-fixing cyanobacteria of the order Stigonematales. *Phytochemistry* 98: 145–150.

Berg, I.A., Koekelkorn, D., Ramos-Vera, W.H., Say, R.F., Zarzycki, J., Hügler, M., *et al* (2010) Autotrophic carbon fixation in archaea. *Nat Rev Microbiol* 8: 447–460.

Bisseret, P., Zundel, M., and Rohmer, M. (1985) Prokaryotic Triterpenoids. 2. 2-$\alpha$-methylhopanoids from *Methylobacterium organophilum* and *Nostoc muscorum*, a new series of prokaryotic triterpenoids. *Eur J Biochem* 150: 29–34.

Bravo, J.M., Perzl, M., Härnter, T., Kannenberg, E.L., and Rohmer, M. (2001) Novel methylated triterpenoids of the gammacerane series from the nitrogen-fixing bacterium *Bradyrhizobium japonicum* USDA 110. *Eur J Biochem* 268: 1323–1331.

Brinkhuis, H., Schouten, S., Collinson, M.E., Sluijs, A., Sinninghe Damsté, J.S., Dickens, G.R., *et al* (2006) Episodic fresh surface waters in the Eocene Arctic Ocean. *Nature* 441: 606–609.
Brochier-Armanet, C., Boussau, B., Gribaldo, S., and Forterre, P. (2008) Mesophilic crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. *Nature Rev Microbiol* **6**: 245–252.

Bryce, T.A., Welli, D., Walsby, A.E., and Nichols, B.W. (1972) Monohexoside derivatives of long-chain polyhydroxy alcohols: a novel class of glycolipid specific to heterocystous algae. *Phytochemistry* **11**: 295.

Capone, D.G., Bronk, D.A., Mulholland, M.R., and Carpenter, E.J. (1997) *Trichodesmium*, a globally significant marine cyanobacterium. *Science* **276**: 1221–1229.

Capone, D.G. (2001) Marine nitrogen fixation: what’s the fuss? *Curr Opin Microbiol* **4**: 341–348.

Capone, D.G. (2008) The marine nitrogen cycle. *Microbe* **3**: 186–192.

Carpenter, E.J., and Romans, K. (1991) Major role of the cyanobacterium *Trichodesmium* in nutrient cycling in the North-Atlantic Ocean. *Science* **254**: 1356–1358.

Carpenter, E.J., Montoya, J.P., Burns, J., Mulholland, M.R., Subramaniam, A., and Capone, D.G. (1999) Extensive bloom of a N₂-fixing diatom/cyanobacterial association in the tropical Atlantic Ocean. *Mar Ecol Prog Ser* **185**: 273–283.

Codispoti, L.A., Brandes, J.A., Christensen, J.P., Devol, A.H., Naqui, S.W.A., Paerl, H.W., and Yoshinari, T. (2001) The oceanic fixed nitrogen and nitrous oxide budgets: moving targets as we enter the Anthropocene? *Sci Mar* **65**: 85–105.

Cvejic, J.H., Putra, S.R., El-Beltagy, A., Hattori, R., Hattori, T., and Rohmer, M. (2000) Bacterial triterpenoids of the hopane series as biomarkers for the chemotaxonomy of *Burlholderia, Pseudomonas* and *Ralstonia* sp. *FEMS Microbiol Lett* **183**: 295–299.

Daims, H., Nielsen, J.L., Nielsen, P.H., Schleifer, K.H., and Wagner, M. (2001) In situ characterization of *Nitrosospira*-like nitrite-oxidizing bacteria active in wastewater treatment plants. *Appl Environ Microbiol* **67**: 5273–5284.

Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., et al. (2015) Complete nitrification by *Nitrosospira* bacteria. *Nature* **528**: 504–509.

Dalsgaard, T., and Thamdrup, B. (2002) Factors controlling anaerobic ammonium oxidation with nitrite in marine sediments. *Appl Environ Microbiol* **68**: 3802–3808.

Dalsgaard, T., Thamdrup, B., Farias, L., and Revsbech, N.P. (2012) Anammox and denitrification in the oxygen minimum zone of the eastern South Pacific. *Limnol Oceanogr* **57**: 1331–1346.

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

Dore, J.E., Brum, J.R., Tupas, L.M., and Karl, D.M. (2002) Seasonal and interannual variability in sources of nitrogen supporting export in the oligotrophic subtropical North Pacific Ocean. *Limnol Oceanogr* **47**: 1595–1607.

Ettwig, K.F., Butler, M.K., Le Paslier, D., Pelletier, E., Mangenot, S., Kuyper, M.M.M., et al. (2010) Nitrite-driven anaerobic methane oxidation by oxyogenic bacteria. *Nature* **464**: 543. U94.

Farnelid, H., Andersson, A.F., Bertilsson, S., Al-Soud, W.A., Hansen, L.H., Serensen, S., et al. (2011) Nitrogenase gene amplicons from global marine surface waters are dominated by genes of non-cyanobacteria. *PLoS One* **6**: e19223.

Farrimond, P., Fox, P.A., Innes, H.E., Miskin, I.P., and Head, I.M. (1998) Bacterial source of hohanoids in Recent sediments: improving our understanding of ancient hohan biomarkers. *Anc Biomol* **2**: 147–166.

Francis, C.A., Roberts, K.J., Bernan, J.M., Santoro, A.E., and Oakley, B.B. (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proc Natl Acad Sci U S A* **102**: 14683–14688.

Füssel, J., Lam, P., Lavik, G., Jensen, M.M., Holtappels, M., Günter, M., and Kuyper, M.M.M. (2012) Nitrite oxidation in the Namibian oxygen minimum zone. *ISME J* **6**: 1200–1209.

Gallon, J.R. (2001) N₂ fixation in phototrophs: adaptation to a specialized way of life. *Plant Soil* **230**: 39–48.

Gruber, N. (2004) The dynamics of the marine nitrogen cycle and its influence on atmospheric CO₂ variations. In *Carbon Climate Interactions*. Oguz, T. (ed). Hoboken, NY: John Wiley, pp. 97–148.

Gruber, N. (2008) The marine nitrogen cycle: overview and challenges. In *Nitrogen in the marine environment*. Capone, D.G., Bronk, D.A., Mulholland, M.R., and Carpenter, E.J. (eds). Burlington, MA: Elsevier, pp. 1–50.

Hallam, S.J., Mincer, T.J., Schieper, C., Preston, C.M., Roberts, K., Richardson, P.M., and DeLong, E.F. (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. *Plos Biol* **4**: 520–536.

Hamersley, M.R., Lavik, G., Woebken, D., Rattray, J.E., Lam, P., Hopmans, E.C., et al. (2007) Anaerobic ammonium oxidation in the Peruvian oxygen minimum zone. *Limnol Oceanogr* **52**: 923–933.

Handley, L., Talbot, H.M., Cooke, M.P., Anderson, K.E., and Wagner, T. (2010) Bacteriohopanepolysols as tracers for continental and marine organic matter supply and phases of enhanced nitrogen cycling on the late Quaternary Congo deep sea fan. *Org Geochem* **41**: 910–914.

Herrmann, D., Bisseret, P., Connan, J., and Rohmer, M. (1996) A non-extractable triterpenoid of the hopane series in *Acetobacter xylanum*. *FEMS Microbiol Lett* **135**: 323–326.

Ho, S.L., and Laappe, T. (2016) Flat meridional temperature gradient in the early Eocene in the subsurface rather than surface ocean. *Nat Geosci* **9**: 606–610.

Hofreiter, M., Serre, D., Poinar, H.N., Kuch, M., and Pääbo, S. (2001) Ancient DNA. *Nat Rev Genet* **2**: 353–359.

Hu, A., Jiao, N., Zhang, R., and Yang, Z. (2011) Niche partitioning of Marine Group I Crenarchaeota in the euphotic and upper mesopelagic zones of the East China Sea. *Appl Environ Microbiol* **77**: 7469–7478.

Hurley, S.J., Elling, F.J., Könneke, M., Buchwald, C., Wankel, S.D., Santoro, A.E., et al. (2016) Influence of ammonia oxidation rate on thaumarchaeal lipid composition and the TEX₈₆ temperature proxy. *Proc Natl Acad Sci U S A* **113**: 7762–7767.
a mid-Cretaceous oceanic anoxic event. Science 293: 92–94.

Kuyppers, M.M.M., Blokker, P., Hopmans, E.C., Kinkel, H., Pancost, R.D., Schouten, S., and Sinninghe Damsté, J.S. (2002) Archaeal remains dominate marine organic matter from the early Albian oceanic anoxic event 1b. Palaeo-geogr Palaeoclimatol Palaeoecol 185: 211–234.

Kuypers, M.M.M., Sliekers, A.O., Lavik, G., Schmid, M., Jørgensen, B.B., Kuenen, J.G., et al. (2003) Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. Nature 422: 608–611.

Kuypers, M.M.M., van Breugel, Y., Schouten, S., Erba, E., and Sinninghe Damsté, J.S. (2004) N2-fixing cyanobacteria supplied nutrient N for Cretaceous oceanic anoxic events. Geology 32: 853–856.

Kuypers, M.M.M., Lavik, G., Woebken, D., Schmid, M., Fuchs, B.M., Amann, R., et al. (2005) Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation. Proc Natl Acad Sci U S A 102: 6478–6483.

Lam, P., Lavik, G., Jensen, M.M., van de Vossenstegen, J., Schmid, M., Woebken, D., et al. (2009) Revising the nitrogen cycle in the Peruvian oxygen minimum zone. Proc Natl Acad Sci U S A 106: 4752–4757.

Lam, P., and Kuyper, M.M.M. (2011) Microbial nitrogen cycling processes in oxygen minimum zones. Ann Rev Mar Sci 3: 317–345.

Lambein, F., and Wolk, C.P. (1973) Structural studies on glycolipids from envelope of heterocyst of Anabaena cylindrica. Biochemistry (Mosc.) 12: 791–798.

Larson, R.L. (1991) Latest pulse of Earth: evidence for a mid-Cretaceous superplume. Geology 19: 547–550.

Lavin, M.F., Fiedler, P.C., Amador, J.A., Ballance, L.T., Farber-Lorda, J., and Mestas-Nunez, A.M. (2006) A review of eastern tropical Pacific oceanography: summary. Prog Oceanogr 69: 391–398.

Lebedeva, E.V., Off, S., Zumbrägel, S., Kruse, M., Shagzhina, A., Lücker, S., et al. (2011) Isolation and characterization of a moderately thermophilic nitrite-oxidizing bacterium from a geothermal spring. FEMS Microbiol Ecol 75: 195–204.

Lincoln, S.A., Wai, B., Epplle, J.M., Church, M.J., Summons, R.E., and Delong, E.F. (2014) Planktonic Euryarchaeota are a significant source of archaeal tetraether lipids in the ocean. Proc Natl Acad Sci U S A 111: 9858–9863.

Lipski, A., Spieck, E., Makolla, A., and Altenhof, K. (2001) Fatty acid profiles of nitrite-oxidizing bacteria reflect their phylogenetic heterogeneity. Syst Appl Microbiol 24: 377–384.

Lücker, S., Nowka, B., Rattei, T., Spieck, E., and Daims, H. (2013) The genome of Nitrospina gracilis illuminates the metabolism and evolution of the major marine nitrite oxidizer. Front Microbiol 4: 1–19.

Mahaffey, C., Michaels, A.F., and Capone, D.G. (2005) The conundrum of marine N2 fixation. Am J Sci 305: 546–595.

Martinez-Pérez, C., Mohr, W., Löschner, C.R., Dekaezemacker, J., Littmann, S., Yilmaz, P., et al. (2016) The small unicellular diazotrophic symbiont, UCYN-A, is a key player in the marine nitrogen cycle. Nat Microbiol 1: 16163.
Mascitti, V., and Corey, E.J. (2004) Total synthesis of (−/+)pentacycloanammox acid. J Am Chem Soc 126: 15664–15665.

Mercer, J.A., Cohen, C.M., Shuken, S.R., Wagner, A.M., Smith, M.W., Moss, F.R., III., et al. (2016) Chemical Synthesis and Self-Assembly of a Ladderane Phospholipid. J Am Chem Soc 138: 15845–15848.

Monteiro, F.M., Pancost, R.D., Ridgwell, A., and Donnadieu, Y. (2012) Nutrients as the dominant control on the spread of anoxia and euxinia across the Cenomanian-Turonian oceanic anoxic event (OA2): model-data comparison. Paleoceanography 27: PA4209.

Mulder, A., van de Graaf, A.A., Robertson, L.A., and Schouten, S., and Corey, E.J. (2004) Total synthesis of (−/+)/C19 pentacycloanammoxic acid. J Am Chem Soc 126: 15871–15876.

Murry, M.A., and Wolk, C.P. (1989) Evidence that the barrier to the penetration of oxygen into heterocysts depends upon two layers of the cell envelope. Arch Microbiol 151: 469–474.

Neumann, S., Wessels, H.J.C.T., Rijpstra, W.I.C., Sinninghe Damsté, J.S., and Hatzenpichler, R., Richter, A., et al. (2011) Thaumarchaeotes abundant in refinery nitrifying sludges express amoA but are not obligate autotrophic ammonia oxidizers. Proc Natl Acad Sci U S A 108: 16771–16776.

Nichols, B.W., and Wood, B.J.B. (1968) New glycolipid specific to nitrogen-fixing blue-green algae. Nature 217: 767.

Orissions, G., and Albrecht, P. (1995) Geohopanoids: the most abundant natural products on earth? Acc Chem Res 28: 398–402.

Padilla, C.C., Bristow, L.A., Sarode, N., Garcia-Robledo, E., Gomez Ramirez, E., Benson, C.R., et al. (2016) NC10 bacteria in marine oxygen minimum zones. ISME J 10: 2067–2071.

Paulmier, A., and Ruiz-Pino, D. (2009) Oxygen minimum zones (OMZs) in the modern ocean. Prog Oceanogr 80: 113–128.

Pester, M., Maixner, F., Berry, D., Rattei, T., Koch, H., Lücker, S., et al. (2014) NxrB encoding the beta subunit of nitrite oxidoreductase as functional and phylogenetic marker for nitrite-oxidizing Nitrosira. Environ Microbiol 16: 3055–3071.

Peters, G.A. (1991) Azolla and other plant-cyanobacteria symbioses: aspects of form and function. Plant Soil 137: 25–36.

Peters, K.E., Walters, C.C., and Moldowan, J.M. (2005) The Biomarker Guide: Biomarkers and Isotopes in Petroleum Exploration and Earth History, 2nd ed. New York, Cambridge University Press.

Pinto, A.J., Marcus, D.N., Ijaz, U.Z., Santos, Q.M.B., Lose, D., Dick, G.J., and Raskin, L. (2016) Metagenomic evidence for the presence of comammox Nitrosira-like bacteria in a drinking water system. mSphere 1: e00054-15.

Pitcher, A., Villanueva, L., Hopkins, E.C., Schouten, S., and Sinninghe Damsté, J.S. (2011) Niche segregation of ammonia-oxidizing archaea and anammox bacteria in the Arabian Sea oxygen minimum zone. ISME J 5: 1896–1904.

Rabotyagov, S.S., King, C.L., Gassman, P.W., Rabalais, N.N., and Turner, R.E. (2014) The economics of dead zones: causes, impacts, policy challenges, and a model of the Gulf of Mexico hypoxic zone. Rev Environ Econ Policy 8: 58–79.

Rashby, S.E., Sessions, A.L., Summons, R.E., and Newman, D.K. (2007) Biosynthesis of 2-methylbacteriohopanepolysols by an anoxygenic phototroph. Proc Natl Acad Sci 104: 15099–15104.

Rigaard-Petersen, N., Langezaal, A.M., Ingvarsden, S., Schmid, M.C., Jetten, M.S.M., Op den Camp, H.J.M., et al. (2006) Evidence for complete denitrification in a benthic foraminifer. Nature 443: 93–96.

Rush, D., Jaeschke, A., Hopmans, E.C., Geenevesen, J.A.J., Schouten, S., and Sinninghe Damsté, J.S. (2011) Short chain ladderanes: oxic biodegradation products of anammox lipids. Geochim Cosmochim Acta 75: 1662–1671.

Rush, D., Hopmans, E.C., Wakeham, S.G., Schouten, S., and Sinninghe Damsté, J.S. (2012) Occurrence and distribution of ladderane oxidation products in different oceanic regimes. Biogeosciences 9: 2407–2418.

Rush, D., Sinninghe Damsté, J.S., Poulton, S.W., Thamdrup, B., Garside, A., Gonzalez, J.A., et al. (2014a) Anaerobic ammonium-oxidising bacteria: a biological source of the bacteriohopanetetrol stereoisomer in marine sediments. Geochim Cosmochim Acta 140: 50–64.

Rush, D., Jaeschke, A., Geenevesen, J.A., Pureveen, J., Lewan, M.D., et al. (2014b) Generation of unusual branched long chain alkanes from hydrous pyrolysis of anammox bacterial biomass. Org Geochem 76: 136–145.

Sakata, S., Hayes, J.M., Rohmer, M., Hooper, A.B., and Seemann, M. (2008) Stable carbon-isotopic compositions of lipids isolated from the ammonia-oxidizing chemotrophic Nitrosomonas europaea. Org Geochem 39: 1725–1734.

Schouten, S., Hopmans, E.C., Schefuß, E., and Sinninghe Damsté, J.S. (2002) Distributional variations in marine cyanarchaeal membrane lipids: a new tool for reconstructing ancient sea water temperatures? Earth Planet Sci Lett 204: 265–274.

Schouten, S., Pitcher, A., Hopmans, E.C., Villanueva, L., van Bleijswijk, J., and Sinninghe Damsté, J.S. (2012) Intact polar and core glycerol dibiphytanyl glycerol tetraether lipids in the Arabian Sea oxygen minimum zone I. Selective preservation and degradation in the water column and consequences for the TEX86. Geochim Cosmochim Acta 98: 228–243.

Schouten, S., Villareal, T.A., Hopmans, E.C., Mets, A., Swanson, K.M., and Sinninghe Damsté, J.S. (2013a) Endosymbiotic heterocystous cyanobacteria synthesize different heterocyst glycolipids than free-living heterocystous cyanobacteria. Phytochemistry 85: 115–121.

Schouten, S., Hopmans, E.C., and Sinninghe Damsté, J.S. (2013b) The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: a review. Org Geochem 54: 19–61.

Schouten, S., Villanueva, L., Hopmans, E.C., van der Meer, M.T.J., and Damste, J.S.S. (2014) Are Marine Group II Lipids as paleomarkers 2131
Euryarchaeota significant contributors to tetraether lipids in the ocean?. *Proc Natl Acad Sci U S A* **111**: E4285–E4285.

Shah, S.R., Mollenhauer, G., Ohkouchi, N., Eglington, T.I., and Pearson, A. (2008) Origins of archaeal tetraether lipids in sediments: insights from radiocarbon analysis. *Geochim Cosmochim Acta* **72**: 4577–4594.

Simonin, P., Tindall, B., and Rohmer, M. (1994) Structure elucidation and biosynthesis of 31-methylhopanoids from *Acetobacter europeus*. *Eur J Biochem* **225**: 765–771.

Sinninghe Damsté, J.S., and Köster, J. (1998) A euxinic southern North Atlantic Ocean during the Cenomanian/Turonian oceanic anoxic event. *Earth Planet. Sci Lett* **158**: 165–173.

Sinninghe Damsté, J.S., Schouten, S., Hopmans, E.C., van Duin, A.C.T., and Geenevasen, J.A.J. (2002a) Crenarchaeol: the characteristic core glycerol dibiphytanyl glycerol tetraether membrane lipid of cosmopolitan pelagic Crenarchaeota. *J Lipid Res* **43**: 1641–1651.

Sinninghe Damsté, J.S., Strous, M., Ripistra, W.I.C., Hopmans, E.C., Geenevasen, J.A.J., van Duin, A.C.T., et al. (2002b) Linearly concatenated cyclobutane lipids form a dense bacterial membrane. *Nature* **419**: 708–712.

Sollai, M., Hopmans, E.C., Schouten, S., Keil, R.G., and Sinninghe Damsté, J.S. (2015) Intact polar lipids of Thaumarchaeota and am-anoxm bacteria as indicators of N cycling in the eastern tropical North Pacific oxygen-deficient zone. *Biogeosciences* **12**: 4725–4737.

Spang, A., Hatzenpichler, R., Brochier-Armanet, C., Rattei, T., Tischler, P., Speck, E., et al. (2010) Distinct gene set in two different lineages of ammonia-oxidizing archaea supports the phylum Thaumarchaeota. *Trends Microbiol* **18**: 331–340.

Speelman, E.N., van Kempen, M.M.L., Barke, J., Brinkhuis, H., Reichart, G.J., Smolders, A.J.P., et al. (2009) The Eocene Arctic *Azolla* bloom: environmental conditions, productivity and carbon drawdown. *Geobiology* **7**: 155–170.

Strous, M., Fuerst, J.A., Kramer, E.H.M., Logemann, S., Muyzer, G., van de Pas-Schoonen, K.T., et al. (1999) Missing lithotroph identified as new Planctomycete. *Nature* **400**: 446–449.

Summons, R.E., Jahnke, L.L., Hope, J.M., and Logan, G.A. (1999) 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature* **400**: 554–557.

Talbot, H.M., and Farrimond, P. (2007) Bacterial populations recorded in diverse sedimentary biohopanoid distributions. *Org Geochem* **38**: 1212–1225.

Talbot, H.M., Rohmer, M., and Farrimond, P. (2007) Structural characterisation of unsaturated bacterial hopanoids by atmospheric pressure chemical ionisation liquid chromatography/ion trap mass spectrometry. *Rapid Commun Mass Spectrom* **21**: 1613–1622.

Talbot, H.M., Summons, R.E., Jahnke, L.L., Cockell, C.S., Rohmer, M., and Farrimond, P. (2008) Cyanobacterial bacteriohopanepolyol signatures from cultures and natural environmental settings. *Org Geochem* **39**: 232–263.

Talbot, H.M., Bischoff, U., Inglis, G.N., Collinson, M.E., and Pancost, R.D. (2016) Polyfunctionalised bio- and geohopanoids in the Eocene Cobham Lignite. *Org Geochem* **96**: 77–92.

Tsikos, H., Karakitsios, V., van Breugel, Y., Walsworth-Bell, B., Bombardiere, L., Petrizzo, M.R., et al. (2004) Organic-carbon deposition in the Cretaceous of the Ionian basin, NW Greece: the Paquier Event (OAE 1b) revisited. *Geol Mag* **141**: 401–416.

Villanueva, L., Schouten, S., and Sinninghe Damsté, J.S. (2015) Depth-related distribution of a key gene of the tetraether lipid biosynthetic pathway in marine Thaumarchaeota. *Environ Microbiol* **17**: 3527–3539.

Villanueva, L., Schouten, S., and Damsté, J.S.S. (2017) Phylogenomic analysis of lipid biosynthetic genes of Archaea shed light on the “lipid divide”. *Environ Microbiol* **19**: 54–69.

Villareal, T.A. (1992) Marine nitrogen-fixing diatom-cyanobacteria symbioses. In *Marine Pelagic Cyanobacteria: Triodesmum and Other Diazotrophs*, Carpenter, E.J., Capone, D.G., and Rueter, J.G (eds). The Netherlands: Kluwer Academic, Dordrecht, pp. 163–175.

Voss, M., Bange, H.W., Dippner, J.W., Middelburg, J.J., Montoya, J.P., and Ward, B. (2013) The marine nitrogen cycle: recent discoveries, uncertainties and the potential relevance of climate change. *Philos Trans R Soc Lond B Biol Sci* **368**: 20130121–20130121.

Walsby, A.E. (1985) The permeability of heterocysts to the gases nitrogen and oxygen. *Proc R Soc Lond Ser B Biol Sci* **226**: 345–366.

Ward, B.B. (2013a) Nitrification. In *Reference Module in Earth Systems and Environmental Sciences*, Elsevier. Update of Encyclopedia of Ecology (2008), pp 2511–2518.

Ward, B.B. (2013b) How nitrogen is lost. *Science* **341**: 352–353.

Ward, B.B., and Jensen, M.M. (2014) The microbial nitrogen cycle. *Front Microbiol* **5**: 553.

Watson, S.W., Bock, E., Valois, F.W., Waterbury, J.B., and Schlosser, U. (1986) *Nitrosira marina* gen. nov. sp. nov.: a chemolithotrophic nitrite-oxidizing bacterium. *Arch Microbiol* **144**: 1–7.

Welander, P.V., Coleman, M.L., Sessions, A.L., Summons, R.E., and Newman, D.K. (2010) Identification of a methylase required for 2-methylhopanoid production and implications for the interpretation of sedimentary hopanes. *Proc Natl Acad Sci U S A* **107**: 8537–8542.

Wright, J.J., Konwar, K.M., and Hallam, S.J. (2012) Microbial ecology of expanding oxygen minimum zones. *Nat Rev Microbiol* **10**: 1–14.

Wuchter, C., Abbas, B., Coolen, M.J.L., Herfort, L., van Bleijswijk, J., Timmers, P., et al. (2006) Archaeal nitrification in the ocean. *Proc Natl Acad Sci* **103**: 12317–12322.

Zehr, J.P., and Kudela, R.M. (2011) Nitrogen cycle of the open ocean: from genes to ecosystems. *Annu Rev Mar Sci* **3**: 197–225.

Zumft, W.G. (1997) Cell biology and molecular basis of denitrification. *Microbiol Mol Biol Rev* **61**: 533–616.