Marine Chemistry 136–137 (2012) 7–13

Contents lists available at SciVerse ScienceDirect

Marine Chemistry

journal homepage: www.elsevier.com/locate/marchem

# Distribution of anaerobic ammonia-oxidizing bacteria in a subterranean estuary

James P. Sáenz [a,*], Ellen C. Hopmans [b], Dan Rogers [a,1], Paul B. Henderson [c], Matthew A. Charette [c], Stefan Schouten [b], Karen L. Casciotti [c,2], Jaap S. Sinninghe Damsté [b], Timothy I. Eglinton [c,3]

[a] MIT-WHOI Joint Program in Chemical Oceanography, Woods Hole, MA 02543, United States
[b] NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Organic Biogeochemistry, P.O. Box 59, 1790 AB Den Burg Texel, The Netherlands
[c] Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02453, United States

ARTICLE INFO

*Article history:*
Received 20 January 2012
Received in revised form 22 April 2012
Accepted 26 April 2012
Available online 4 May 2012

*Keywords:*
Anammox
Ladderanes
Subterranean estuary

ABSTRACT

The traditional paradigm that rivers and terrestrial run-off are the major contributors of nutrients to coastal waters has been challenged by observations that nutrient fluxes originating from coastal aquifer subterranean estuaries can equal or even exceed that of other terrestrial sources. Within a coastal aquifer where organic carbon is scarce and ammonium is abundant, bacteria capable of anaerobic ammonium oxidation (anammox bacteria) may play a role in the removal of fixed nitrogen. We investigated the presence of anammox bacteria in a coastal groundwater system (Waquoit Bay, MA USA) using lipid biomarkers. From the distribution of sediment-bound ladderane phospholipids, biomarkers for viable anammox bacteria, we demonstrate the presence of these organisms in association with aqueous chemical transition zones within the aquifer. The distribution of ladderane fatty acids in contrast, provided insight into the historical distribution of anammox bacteria and temporal stability of that distribution. The results suggest that anammox communities have been present over a broad range of depths, most likely determined by changes in the depths of the redox transition zones over time, but that they are more prevalent in the upper portion of the subterranean estuary where ammonium and nitrate coexist.



## 1. Introduction

Nitrogen is an essential element in the growth and survival of all living organisms. Since most organisms depend on the presence of fixed nitrogen species such as ammonium and nitrate for growth, understanding the processes controlling the availability of fixed nitrogen in the environment is critical to understanding biochemistry and ecology of the carbon cycle. In coastal marine environments, where nitrogen is often the limiting nutrient in primary productivity, anthropogenic activities can generate large fluxes of fixed nitrogen leading to the formation of toxic algal and cyanobacterial blooms (Howarth et al., 2000) and the emergence of hypoxic "dead zones" (Diaz and Rosenberg, 2008). Rivers and overland runoff have traditionally been viewed as the most important terrestrial sources of fixed nitrogen to coastal waters (Valiela et al., 2000), however it has recently been recognized that nutrient input from groundwater discharge to coastal waters can equal or in some cases exceed nutrient input from rivers (Taniguchi et al., 2002; Slomp and Van Cappellen, 2004; Kroeger et al., 2007; Kroeger and Charette, 2008).

Coastal regions with permeable sediments can host aquifers that are characterized by subsurface mixing of fresh terrestrial ground water and seawater. The interface between fresh groundwater and saltwater within coastal aquifers, termed a "subterranean estuary", is a biogeochemically active zone that can act as a major chemical sink or source for coastal waters (Charette and Sholkovitz, 2002; Charette et al., 2005; Windom et al., 2006; Beck et al., 2007; Hays and Ullman, 2007; Kroeger and Charette, 2008). In the past decade studies have shown that strong redox gradients can be generated by hydrographic mixing processes within such environments (Moore, 1999; Charette and Sholkovitz, 2002; Testa et al., 2002; Windom and Niencheski, 2003; Charette et al., 2005). The coexistence of high concentrations of ammonium, in close proximity to suitable oxidants such as manganese, nitrate, nitrite, and dissolved oxygen suggests the potential for removal of fixed nitrogen by microbially mediated ammonium oxidation pathways.

One such pathway is anaerobic ammonium oxidation (anammox) (Mulder et al., 1995; Strous et al., 1999). Bacteria capable of anammox catalyze the direct conversion of nitrite and ammonium to dinitrogen ($N_2$) gas to generate the energy needed for biosynthesis (Strous et al.,

* Corresponding author at: Max Planck Institute of Molecular Cell Biology and Genetics, 108 Pfotenhauestr., 01307 Dresden, Germany.
*E-mail address:* saenz@mpi-cbg.de (J.P. Sáenz).
[1] Present address: Department of Earth and Planetary Sciences, Harvard University, Cambridge, MA 02138, United States.
[2] Present address: Department of Environmental Earth System Science, Stanford University, Stanford, CA 94305, United States.
[3] Present Address: Geologisches Institut, Eidgenössische Technische Hochschule Zürich, 8092 Zurich, Switzerland.

0304-4203/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.marchem.2012.04.004

2006). The existence of an anammox pathway was first demonstrated in a wastewater treatment facility (Mulder et al., 1995) and has since been identified in a variety of natural marine systems including anoxic basins (Dalsgaard et al., 2003; Kuypers et al., 2003), oxygen minimum zones associated with high productivity upwelling regions (Kuypers et al., 2005; Thamdrup et al., 2006; Woebken et al., 2007), marine sediments (Thamdrup and Dalsgaard, 2002; Freitag and Prosser, 2003; Rysgaard et al., 2004) and estuarine sediments (Risgaard-Petersen et al., 2004). Removal of fixed nitrogen via anammox is now thought to represent one of the dominant terms in nitrogen removal from many marine upwelling systems, and by some estimates may represent a major component of nitrogen loss from the oceans (Gruber and Sarmiento, 1997; Codispoti et al., 2001; Brandes et al., 2007). Anammox is rarely accounted for in mass balance estimates of coastal nitrogen cycling and, in particular, subterranean estuaries (Kroeger and Charette, 2008; Spiteri et al., 2008). Given the low organic carbon concentrations in subterranean estuarine environments (Charette et al., 2005; Kroeger and Charette, 2008), it is possible that chemoautotrophic processes such as anammox would play an important role in $N_2$ production in these settings, as in other marine sediments (Thamdrup and Dalsgaard, 2002).

Anammox bacteria can be unambiguously identified by the presence of ladderane lipids (Fig. 1), which are unique in that they possess a linearly concatenated cyclobutane structure that has not been detected in any other organism (Sinninghe Damsté et al., 2002a, 2002b; Sinninghe Damsté et al., 2005). The ladderanes are thought to be the principal lipids in the membrane of an organelle called the anammoxosome in which the anammox reaction is carried out (van Niftrik et al., 2004). Since the biophysical properties of ladderanes are thought to be essential to the physiology of the anammox pathway (Sinninghe Damsté et al., 2002a, 2002b) their detection provides a robust means for establishing the presence of anaerobic ammonium oxidizers.

We investigated the distribution of two classes of ladderane lipids within a subterranean estuary in the northeastern United States. The first is an intact phospholipid (Fig. 1: IV), which is a membrane-bound lipid that degrades rapidly upon cell death (Boumann et al., 2006; Jaeschke et al., 2009a; Brandsma et al., 2011). The second class of ladderanes, which we refer to as ladderane fatty acids (Fig. 1: I–III), represents components of the intact ladderane phospholipids that are released when they are degraded. The presence of the ladderane phospholipid is thus thought to be diagnostic of viable or recently deceased cells (Jaeschke et al., 2009a), whereas ladderane fatty acids are likely to persist for thousands of years following cell death (Jaeschke et al., 2009b). Our results show, for the first time, that ladderanes are present in *permeable* sediments within a subterranean estuary and highlight the potential importance of anammox as a pathway for nitrogen removal in subterranean environments.

## 2. Materials and methods

### 2.1. Study site and sediment sampling

Waquoit Bay is a partially enclosed bay on the south shore of Cape Cod, Massachusetts (Fig. 2). Waquoit Bay itself is underlain by low permeability marine sediment, however, from the tidal zone inland there is sandy soil overlying a freshwater aquifer. Since the soil is permeable in this area, precipitation mostly penetrates the surface and flows to sea as groundwater, and as a result groundwater is the primary source of freshwater to the bay. Salt water from the bay also penetrates the aquifer beneath the bay, flows inland, and circulates back to the Bay beneath the fresh groundwater plume, discharging along a narrow band in the intertidal zone (Michael et al., 2003). Sediment samples were collected down to 7 m depth at core site PZ6 (Fig. 2) in July 2006 using a pulse auger. Sediments from a single core were sampled at 0.3 m resolution and kept frozen at −80 °C until analysis.

### 2.2. Pore water nutrient, pH, and Eh analysis

Groundwater profiles from PZ6 were obtained on the same day as sediment samples in July 2006 with a drive-point piezometer system called Retract-A-Tip (AMS, Inc.; Charette and Allen, 2006). The stainless steel piezometer was driven to the depth of interest, and groundwater samples were pumped through Teflon tubing using a peristaltic pump. Samples for nutrients were collected into 30 mL acid cleaned scintillation vials after passage through a Pall Aquaprep 0.2 μm capsule filter and stored frozen until analysis. Water properties including temperature, salinity, pH, dissolved oxygen, and redox potential (Eh) were recorded using a YSI 600XLM multiprobe and 650MDS handheld computer. Back in the laboratory combined $NO_3^-$ and $NO_2^-$, and $NH_4^+$ were quantified on a Lachat QuickChem 8000 Flow Injection Analyzer using standard colorimetric techniques (Solorzano, 1969; Braman and Hendrix, 1989).

### 2.3. Ladderane fatty acid analysis

Sediment samples were freeze-dried and extracted (~40 g dry weight) by ultrasonication three times each in methanol, methanol/dichloromethane (1:1, v/v), and dichloromethane. Sediment was

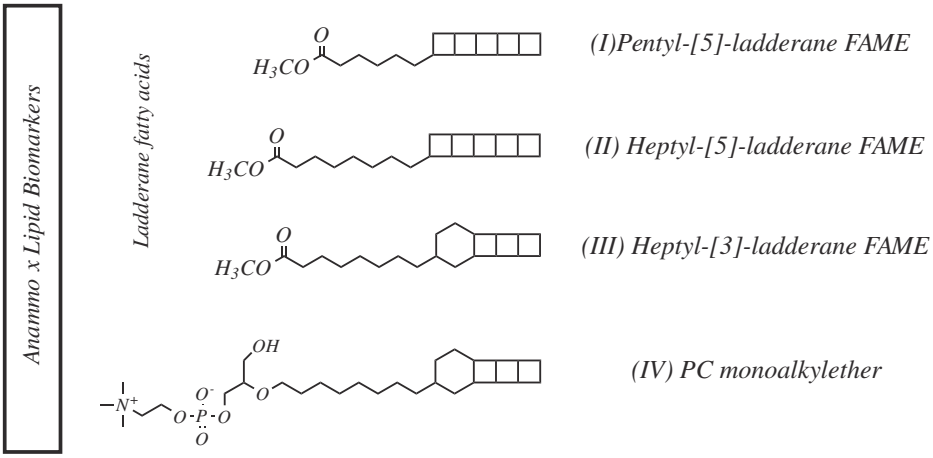


**Fig. 1.** Structures of lipid biomarkers detected in this study.

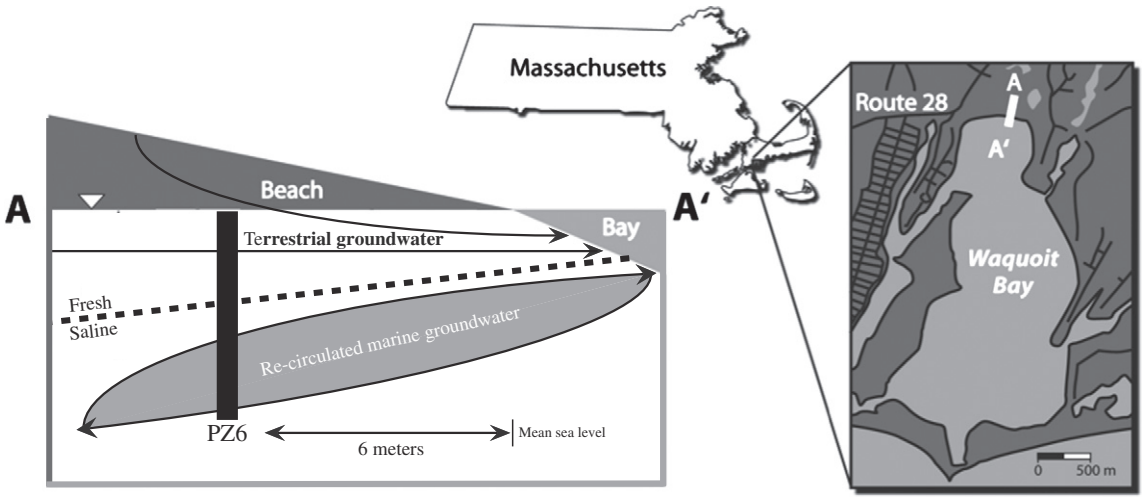


**Fig. 2.** Map showing the location of Waquoit Bay in Falmouth, Massachusetts, USA and location of the core site at the head of the bay. The cartoon on the left depicts the location of site PZ6 with respect to low tide.

removed by centrifugation and solvent was dried using rotary evaporation. Lipid extracts were combined to form a total lipid extract (TLE), and then saponified in 1 N sodium hydroxide in methanol. After addition of water, the saponified TLE was separated into a neutral fraction and a fatty acid fraction (containing ladderane fatty acids) by extraction into dichloromethane at pH 12 and pH 2, respectively. Fatty acids were derivatized with diazomethane to produce fatty acid methyl esters (FAMEs). Polyunsaturated fatty acids were removed by eluting the fatty acid fraction using dichloromethane over a column packed with silver nitrate-impregnated alumina oxide. Ladderane fatty acids were detected and quantified according to methods described in Hopmans et al. (2006) using high performance liquid chromatography coupled to positive ion atmospheric pressure chemical ionization mass spectrometry (HPLC/APCI-$MS^2$) in Selected Reaction Monitoring (SRM) mode. Quantification of the ladderane lipids was achieved using an external standard curve with two authentic standards heptyl-[3]-ladderane FAME and heptyl-[5]-ladderane FAME. Concentrations for the pentyl-[5]-ladderane FAME detected in samples were estimated using the heptyl-[5]-ladderane FAME standard curve. The analytical reproducibility of concentration was less than 10%, based on duplicate analysis of the same extract. We performed duplicate extraction and analysis on samples from depths 1.1, 2, 2.3, 2.7, 2.9, 3.2, and 4.4 m. Average error between duplicate extractions from the same depth was 42% of the average concentration, and ranged from 25 to 72%. Ladderane fatty acid (Fig. 1: I–III) concentrations are presented as the sum of all three measured fatty acids and are reported relative to sediment dry mass.

### 2.4. Ladderane monoether–phosphocholine analysis

The intact ladderane phosphocholine (PC) monoethers were extracted and analyzed by methods described in Boumann et al. (2006) and Jaeschke et al. (2009a, 2009b). Briefly, freeze dried sediments (~40 g dry weight) were extracted using a modified Bligh and Dyer protocol (Sturt et al., 2004) with phosphate buffer. The resulting extracts were dried by rotary evaporation and kept frozen at −40 °C until analysis. Samples were analyzed using HPLC coupled to positive ion Electrospray ionization mass spectrometry (HPLC/ESI-$MS^2$) in Selected Reaction Monitoring (SRM) mode (Jaeschke et al., 2009a). Quantification was achieved by comparison of peak areas with an external standard curve of an authentic PC-monoether standard. The concentrations of the ladderane PC monoether were normalized to sediment dry mass. The analytical reproducibility was better than 12% based on duplicate measurements (Jaeschke et al., 2009a).

## 3. Results

### 3.1. Pore water profiles

Pore water profiles of nitrate + nitrite, ammonium, dissolved oxygen, redox potential, and salinity from July 2006 at site PZ6 are shown in Fig. 3. Salinity data is only shown below 5 m in order to capture the transition from fresh ground water to saltwater. In the upper 5 m salinity remained below 1. Down core pore water chemistry is characterized by (in order with increasing depth from the top of the water table): 1) an oxic and nitrate-rich surface layer (~0–2 m), 2) an anoxic and ammonium-rich plume (~2–3 m), 3) a mid-depth oxic nitrate + nitrite-rich zone, (~3–6.5 m) and 4) a deep salinity transition zone (~6.5–7 m), with increasing ammonium concentrations. These features are delineated by three gradients in redox potential and in nitrate + nitrite and ammonium concentrations. We have qualitatively demarcated these transition zones by visual inspection of the pore water profiles of Eh, nitrate + nitrite, and ammonium. At the time of our sampling, the three redox transition zones occurred roughly between 1–2 m, 2.75–3.5 m, and 6.25–7 m. In the discussion that follows we will refer to these as upper redox transition zone (URTZ), middle redox transition zone (MRTZ) and deep redox transition zone (DRTZ), respectively.

A comparison of summer pore water profiles for the previous three years at site PZ6 indicates that the ammonium and nitrate + nitrite distributions have remained persistent, although the three redox transition zones have migrated vertically by up to 1 m between Fall 2005 and Summer 2006 (Fig. 4).

### 3.2. Ladderane lipid profiles

Ladderane fatty acids, and the ladderane PC monoether were detected in all of the samples we measured, and within the URTZ and MRTZ, both ladderane profiles revealed elevated concentrations at similar depths (Fig. 3). Ladderane fatty acids II and III were most abundant (average 39% and 43%, respectively; Fig. 5) while ladderane fatty acid I was in lowest relative abundance (average abundance 18%). These relative abundances are similar to those found for ladderane fatty acids in cultured anammox bacteria (Fig. 5) (Rattray et al., 2008). Summed ladderane fatty acid concentrations ranged from roughly 3 pg $g^{-1}$ deep in the sediment column up to nearly

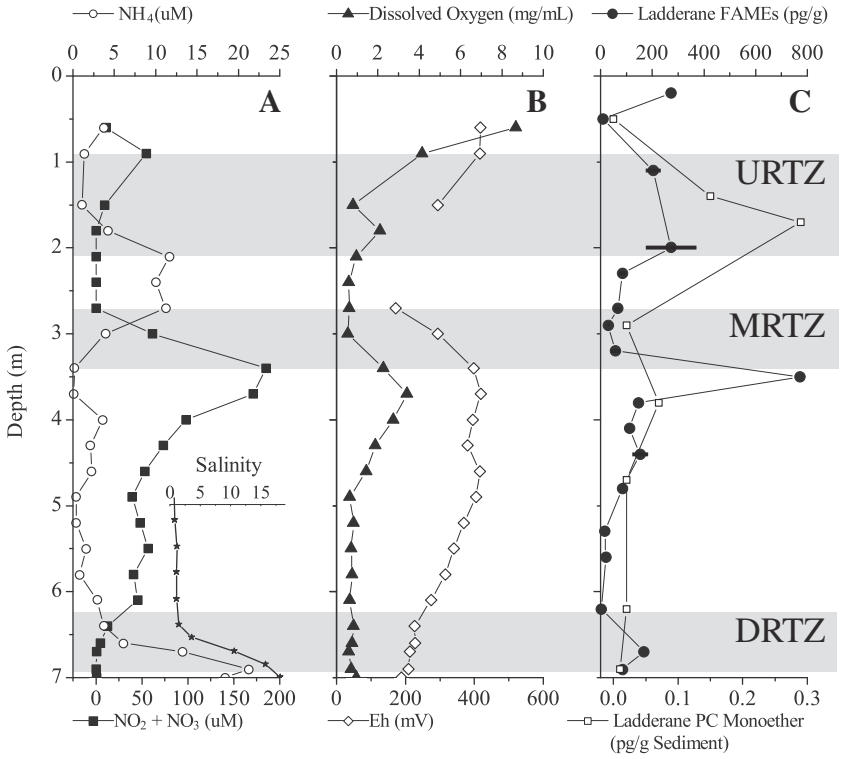


**Fig. 3.** Pore water chemistry and lipid concentrations for PZ6. Going from left to right, panels show A) ammonium and nitrate + nitrite concentrations and salinity, B) dissolved oxygen and Eh, and C) ladderane fatty acid methyl ester (FAME) and ladderane PC-monoether concentration reported as ng $g^{-1}$ sediment. For depths where duplicate extractions and analysis of fatty acids were preformed, reported values are the mean of duplicates and error bars are shown to indicate the range of error between duplicates. Three gray bars are shown to indicate the position of the redox transition zones (URTZ, MRTZ and DRTZ).

800 pg $g^{-1}$ within the nitrate plume just below the MRTZ. Maximum ladderane fatty acid concentrations occurred at 3.5 m, with secondary peaks at 0.2 m, 1–2 m, and 6.7 m. While the upper and lower peaks in fatty acid concentration coincide with the depths of the URTZ and the DRTZ, respectively, the peak value observed at 3.5 m appeared to occur just below the depth of the MRTZ when this core was sampled in 2006. However, as we will elaborate on in the discussion, this may be due to depth variability in the pore water redox profiles.

We also analyzed an intact ladderane polar lipid as a marker for living anammox bacteria (cf Jaeschke et al., 2009a). However, the

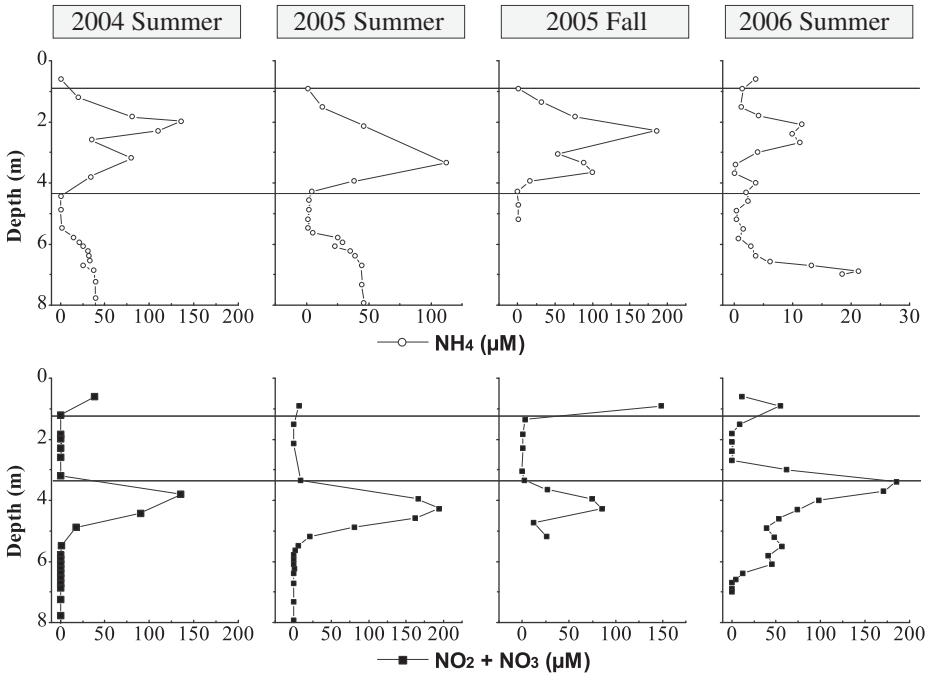


**Fig. 4.** Pore water concentrations of ammonium (top) and nitrate + nitrite (bottom) sampled at four time points over three years at site PZ6. Horizontal lines indicate the position of the transition from nitrate + nitrite-rich to ammonium-rich water at the URTZ and MRTZ.

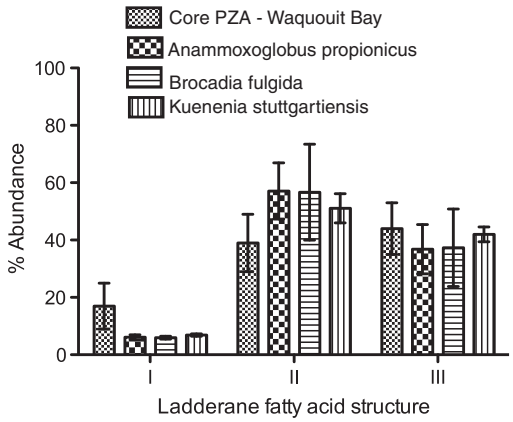


**Fig. 5.** The average relative abundance of individual ladderane fatty acids (Fig. 1: I–III) from core PZ6, and from enrichment cultures of three strains of anammox bacteria reported by Rattray et al. (2008). Values are the mean and error bars represent the standard deviation of relative abundance from all depths from core PZ6, or from triplicate extractions of enrichment culture material.

analytical methodology for detecting and quantifying the intact ladderane polar lipid did not become available until after the fatty acids were analyzed. Furthermore, owing to limited sample quantities it was not possible to re-analyze all of the original samples for PC-monoether abundance. Consequently, the sample depth resolution for the PC-monoether profile is substantially lower than for the ladderane fatty acid profile, and it appears to miss features that may be associated with the DRTZ and the MRTZ (Fig. 3). However, a concentration maximum is observed between 1.4 and 1.7 m, corresponding with the depth of the URTZ.

## 4. Discussion

Three redox transition zones occur in the pore waters of the Waquoit Bay subterranean estuary (URTZ, MRTZ, and DRTZ). They have been described previously for redox sensitive metals (Charette and Sholkovitz, 2006) and nutrients (Kroeger and Charette, 2008). In addition, repeat occupations of this subterranean estuary transect over the past decade have shown that the transition zones are a constant feature (Charette, M.A., unpublished data). The depth of these zones relative to the beach face may vary from year to year in response to changes in the water table and changes in the relative fluxes of ground water from different sources. The freshwater ammonium plume bounded by the URTZ and the MRTZ is thought to originate from groundwater recharge through a nearby wetland (Kroeger and Charette, 2008). The vertical distribution of nitrate + nitrite and ammonium pore water profiles and associated redox transition zones at PZ6 remained stable over two years, from 2004 to 2005, but in 2006 the URTZ and DRTZ appear to have deepened, whereas the MRTZ became shallower in concert with a vertical compression of the upper ammonium plume (Fig. 4).

The presence of viable anammox bacteria in the subterranean estuary is demonstrated by the detection of the ladderane PC-monoether at PZ6. The maximum concentrations observed are roughly an order of magnitude lower than concentrations observed in sediments from the Irish Sea, and Gullmarsfjorden, Sweden (Hopmans et al., 2006; Jaeschke et al., 2009a; Brandsma et al., 2011). However, the coarse-grained sands in the subterranean estuary have much lower surface area than the fine-grained marine sediments in the North Sea. It is therefore possible that although anammox bacterial biomass relative to sediment mass in the subterranean estuary may be much lower than in marine sediments, the surface area loading could be much closer in these two types of sedimentary environment.

Anammox bacteria are well-suited for a dynamic environment as they are reversibly inhibited by oxygen (Strous et al., 1997) and, therefore, can survive vertical migration in the position of the redox transitions zones. The ladderane PC-monoether profile shows that anammox bacteria are most abundant within the URTZ. The vertical extent of this peak in abundance appears to be roughly 0.5 m based on the sharp decrease in concentration from 1.7 to 1.4 m, suggesting that anammox is limited to a fairly narrow band with respect to the depth range over which the redox transition zone occurs.

We expected anammox bacteria to be the most abundant where ammonium and nitrate coexist and oxygen is suitably low to prevent inhibition of the anammox process. In support of this, the peak in ladderane abundance is positioned just below the oxycline and at the depth where ammonium concentration begins to increase. Anammox bacteria have relatively low abundance at depths corresponding to the MRTZ at the time pore water was sampled in the summer of 2006. A significant elevation in abundance concentration of the intact lipid at 4.0 m indicates an elevation in abundance of anammox at this depth, most likely due to active or living organisms. Taken together, these two observations suggest that these samples may have been collected shortly after the upward migration of the MRTZ, such that the anammox community had not yet relocated to the shallower depth of the redox transition zone. Due to the low depth resolution of the PC-monoether profile in the deeper portion of the core, we cannot determine from the PC-monoether data whether there was a peak in abundance of viable anammox bacteria within the DRTZ.

In contrast to the ladderane PC-monoether, ladderane fatty acids have the potential to be preserved in the environment long after the cells that produced them have died (Jaeschke et al., 2009b). This means that the depth profiles of these lipids reflect a time-integrated accumulation of biomass of anammox bacteria. We observed distinct peaks in abundance in the concentration profiles of ladderane fatty acids, indicating that, on average, the depth distribution of anammox has been stable over the time scale that ladderane fatty acids are preserved.

The ladderane fatty acid profile shows three regions of high concentration within the subterranean estuary at depths that correspond to the URTZ, MRTZ, and DRTZ, in addition to high concentration in a surface sample that had a soil-like consistency. The broad depth range over which ladderane fatty acids are detected most likely reflects the range over which the redox transition zones and region of maximum anammox activity have persisted over time. From the ladderane fatty acid profile, it appears that the spatial distribution of anammox populations near the DRTZ has not shifted or expanded considerably with respect to depth in comparison with anammox populations associated with the URTZ and MRTZ, which have spanned nearly 2 m depth. The large peak in ladderane fatty acid concentration at 3.5 m could be indicative of abundant anammox bacteria at this depth. There is, however, an apparent depth offset between this peak in ladderane fatty acid concentration and the MRTZ centered at ~2.8 m (Fig. 3). As we argued in the case of the PC-monoether, this offset could be because of a recent change in the depth of the redox transition zone. It is possible, for instance, that a shoaling of the MRTZ occurred shortly prior to sampling and that anammox bacteria had not yet had sufficient time to re-locate to a new depth with more favorable growth conditions. This is supported by the absence of a local concentration maximum for either the ladderane fatty acids or PC-monoether (Fig. 3) at the depth of the MRTZ in 2006, and by elevated concentrations of both lipid classes at depths corresponding to a deeper MRTZ prior to 2006, which appears to represent the average depth for this transition zone in previous years (Fig. 4).

Ultimately, further work in this environment will need to quantitatively assess the distribution of anammox in terms of absolute rates of nitrogen removal and relative to other processes including denitrification, dissimilatory nitrate reduction to ammonium

(DNRA) and aerobic ammonia oxidation by bacterial and archaeal ammonia oxidizers (AOB and AOA, respectively). The co-occurrence of both aerobic ammonium oxidizing bacteria (AOB) and AOA with anammox bacteria has been observed in the Namibian Upwelling system (Woebken et al., 2008) and in the Black Sea (Lam et al., 2007; Coolen et al., 2007). The potential for syntrophy between aerobic ammonium oxidation and anammox was recognized soon after the discovery of anammox bacteria with the invention of a novel sewage treatment process known as the Completely Autotrophic Nitrogen removal Over Nitrate (CANON) process. In this process, anammox and aerobic ammonium oxidizing bacteria (AOB) are maintained in co-culture, with AOB providing nitrite and removing oxygen, and anammox bacteria converting ammonium and nitrite to $N_2$ gas (Sliekers et al., 2002). Within the redox transition zones of the subterranean estuary AOA/AOB could increase the ecological range of anammox and enhance anammox activity, by simultaneously drawing down oxygen levels in the oxic end of the chemocline and supplying nitrite towards the anoxic end of the chemocline, thereby extending the conditions favorable for anammox activity. For example, the presence of anammox in the well-aerated surface soil sample implies that anammox is thriving in microenvironments that are possibly facilitated by syntrophic interactions with AOA/AOB present there (Rogers and Casciotti, 2010).

## 5. Conclusions

We demonstrate the presence of anammox bacteria in the Waquoit Bay subterranean estuary through the detection of ladderane PC-monoether and ladderane fatty acids. Our results suggest that anammox bacteria are associated with three redox transition zones within the subterranean estuary, and could potentially play an important role in the nitrogen cycle of subterranean estuaries. The distribution of ladderane fatty acids – presumably vestiges of fossil biomass – indicates that the vertical distribution of anammox bacteria has been fairly constant over the time period that these lipids have accumulated. Additional work in this environment will need to address the quantitative importance of anammox in terms of absolute rates of nitrogen removal.

## Acknowledgments

We acknowledge funding from NSF OCE (#05-24994), an NSF Graduate Research Fellowship (JPS), WHOI Coastal Ocean Institute Student Research Fund, and a travel grant from European Association for Organic Geochemistry. We thank Daniel Montlucon, Matt McIlvin, and Andrea Jaeschke for help with laboratory and field work, and Erin Banning for helpful discussions.

## References

Beck, A.J., Tsukamoto, Y., Tovar-Sanchez, A., Huerta-Diaz, M., Bokuniewicz, H.J., Sanudo-Wilhelmy, S.A., 2007. Importance of geochemical transformations in determining submarine groundwater discharge-derived trace metal and nutrient fluxes. Appl. Geochem. 22 (2), 477–490.

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

Braman, R.S., Hendrix, S.A., 1989. Nanogram nitrite and nitrate nanogram nitrite and nitrate determination in environmental and biological materials by vanadium (III) reduction with chemiluminescence detection. Anal. Chem. 61 (24), 2715–2718.

Brandes, J.A., Devol, A.H., Deutsch, C., 2007. New developments in the marine nitrogen cycle. Chem. Rev. 107 (2), 577–589.

Brandsma, J., van de Vossenberg, J., Risgaard-Petersen, N., Schmid, M.C., Engstrom, P., Eurenius, K., Hulth, S., Jaeschke, A., Abbas, B., Hopmans, E.C., Strous, M., Schouten, S., Jetten, M.S.M., Damste, J.S.S., 2011. A multi-proxy study of anaerobic ammonium oxidation in marine sediments of the Gullmar Fjord, Sweden. Environ. Microbiol. Rep. 3, 360–366.

Charette, M.A., Allen, M.C., 2006. Precision ground water sampling in coastal aquifers using a direct-push, shielded-screen well-point system. Ground Water Monit. R. 26 (2), 87–93.

Charette, M.A., Sholkovitz, E.R., 2002. Oxidative precipitation of groundwater-derived ferrous iron in the subterranean estuary of a coastal bay. Geophys. Res. Lett. 29 (10).

Charette, M.A., Sholkovitz, E.R., Hansel, C.M., 2005. Trace element cycling in a subterranean estuary: part 1. Geochemistry of the permeable sediments. Geochim. Cosmochim. Acta 69 (8), 2095–2109.

Charette, M.A., Sholkovitz, E.R., 2006. Trace element cycling in a subterranean estuary: part 2. Geochemistry of the pore water. Geochim. Cosmochim. Acta 70, 811–826.

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

Coolen, M.J.L., Abbas, B., van Bleijswijk, J., Hopmans, E.C., Kuypers, M.M.M., Wakeham, S.G., Sinninghe Damsté, J.S.S., 2007. Putative ammonia-oxidizing Crenarchaeota in suboxic waters of the black sea: a basin-wide ecological study using 16s ribosomal and functional genes and membrane lipids. Environ. Microbiol. 9 (4), 1001–1016.

Dalsgaard, T., Canfield, D.E., Petersen, J., Thamdrup, B., Acuna-Gonzalez, J., 2003. N-2 production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. Nature 422 (6932), 606–608.

Diaz, R.J., Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems. Science 321, 926–929.

Freitag, T.E., Prosser, J.I., 2003. Community structure of ammonia-oxidizing bacteria within anoxic marine sediments. Appl. Environ. Microbiol. 69 (3), 1359–1371.

Gruber, N., Sarmiento, J.L., 1997. Global patterns of marine nitrogen fixation and denitrification. Global Biogeochem. Cycles 11 (2), 235–266.

Hays, R.L., Ullman, W.J., 2007. Dissolved nutrient fluxes through a sandy estuarine beachface (Cape Henlopen, Delaware, USA): contributions from fresh groundwater discharge, seawater recycling, and diagenesis. Estuaries Coasts 30 (4), 710–724.

Hopmans, E.C., Kienhuis, M.V.M., Rattray, J.E., Jaeschke, A., Schouten, S., Sinninghe Damsté, J.S.S., 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 Sp. 20 (14), 2099–2103.

Howarth, R., Anderson, D., Cloern, J., Elfrin, C., Hopkinson, C., Lapointe, B., Malone, T., Marcus, N., McGlathery, K., Sharpley, A., Walker, D., 2000. Nutrient pollution of coastal rivers, bays, and seas. Issues in Ecology 7, 1–15.

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

Jaeschke, A., Ziegler, M., Hopmans, E.C., Reichart, G.J., Lourens, L.J., Schouten, S., Damste, J.S.S., 2009b. Molecular fossil evidence for anaerobic ammonium oxidation in the Arabian Sea over the last glacial cycle. Paleoceanography 24.

Kroeger, K.D., Charette, M.A., 2008. Nitrogen biogeochemistry of submarine groundwater discharge. Limnol. Oceanogr. 53 (3), 1025–1039.

Kroeger, K.D., Swarzenski, P.W., Greenwood, W.J., Reich, C., 2007. Submarine groundwater discharge to Tampa bay: nutrient fluxes and biogeochemistry of the coastal aquifer. Mar. Chem. 104 (1–2), 85–97.

Kuypers, M.M.M., Sliekers, A.O., Lavik, G., Schmid, M., Jorgensen, B.B., Kuenen, J.G., Sinninghe Damsté, J.S.S., Strous, M., Jetten, M.S.M., 2003. Anaerobic ammonium oxidation by anammox bacteria in the black sea. Nature 422 (6932), 608–611.

Kuypers, M.M.M., Lavik, G., Woebken, D., Schmid, M., Fuchs, B.M., Amann, R., Jorgensen, B.B., Jetten, M.S.M., 2005. Massive nitrogen loss from the benguela upwelling system through anaerobic ammonium oxidation. PNAS 102 (18), 6478–6483.

Lam, P., Jensen, M.M., Lavik, G., McGinnis, D.F., Muller, B., Schubert, C.J., Amann, R., Thamdrup, B., Kuypers, M.M.M., 2007. Linking crenarchaeal and bacterial nitrification to anammox in the black sea. Proc. Natl. Acad. Sci. U. S. A. 104 (17), 7104–7109.

Michael, H.A., Lubetsky, J.S., Harvey, C.F., 2003. Characterizing submarine groundwater discharge: a seepage meter study in Waquoit bay, Massachusetts. Geophys. Res. Lett. 30 (6).

Moore, W.S., 1999. The subterranean estuary: a reaction zone of ground water and sea water. Mar. Chem. 65 (1–2), 111–125.

Mulder, A., Vandegraaf, A.A., Robertson, L.A., Kuenen, J.G., 1995. Anaerobic ammonium oxidation discovered in a denitrifying fluidized-bed reactor. FEMS Microbiol. Ecol. 16 (3), 177–183.

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

Risgaard-Petersen, N., Meyer, R.L., Schmid, M., Jetten, M.S.M., Enrich-Prast, A., Rysgaard, S., Revsbech, N.P., 2004. Anaerobic ammonium oxidation in an estuarine sediment. Aquat. Microb. Ecol. 36 (3), 293–304.

Rogers, D.R., Casciotti, K.L., 2010. Abundance and diversity of archaeal ammonia oxidizers in a coastal groundwater system. Appl. Environ. Microbiol. 76, 7938–7948.

Rysgaard, S., Glud, R.N., Risgaard-Petersen, N., Dalsgaard, T., 2004. Denitrification and anammox activity in arctic marine sediments. Limnol. Oceanogr. 49 (5), 1493–1502.

Sinninghe Damsté, J.S.S., Schouten, S., Hopmans, E.C., van Duin, A.C.T., Geenevasen, J.A.J., 2002a. Crenarchaeol: the characteristic core glycerol dibiphytanyl glycerol tetraether membrane lipid of cosmopolitan pelagic crenarchaeota. J. Lipid Res. 43 (10), 1641–1651.

Sinninghe Damsté, J.S.S., Strous, M., Rijpstra, W.I.C., Hopmans, E.C., Geenevasen, J.A.J., van Duin, A.C.T., van Niftrik, L.A., Jetten, M.S.M., 2002b. Linearly concatenated cyclobutane lipids form a dense bacterial membrane. Nature 419 (6908), 708–712.

Sinninghe Damsté, J.S.S., Rijpstra, W.I.C., Geenevasen, J.A.J., Strous, M., Jetten, M.S.M., 2005. Structural identification of ladderane and other membrane lipids of planctomycetes capable of anaerobic ammonium oxidation (anammox). FEBS J. 272 (16), 4270–4283.

Sliekers, A.O., Derwort, N., Gomez, J.L.C., Strous, M., Kuenen, J.G., Jetten, M.S.M., 2002. Completely autotrophic nitrogen removal over nitrite in one single reactor. Water Res. 36 (10), 2475–2482.

Slomp, C.P., Van Cappellen, P., 2004. Nutrient inputs to the coastal ocean through submarine groundwater discharge: controls and potential impact. J. Hydrol. 295 (1–4), 64–86.
Solorzano, L., 1969. Determination of ammonia in natural waters by phenolhypochlorite method. Limnol. Oceanogr. 14, 799–801.
Spiteri, C., Slomp, C.P., Charette, M.A., Tuncay, K., Meile, C., 2008. Flow and nutrient dynamics in a subterranean estuary (Waquoit Bay, MA, USA): field data and reactive transport modeling. Geochim. Cosmochim. Acta 72 (14), 3398–3412.
Strous, M., vanGerven, E., Kuenen, J.G., Jetten, M., 1997. Effects of aerobic and microaerobic conditions on anaerobic ammonium-oxidizing (anammox) sludge. Appl. Environ. Microbiol. 63 (6), 2446–2448.
Strous, M., Fuerst, J.A., Kramer, E.H.M., Logemann, S., Muyzer, G., van de Pas-Schoonen, K.T., Webb, R., Kuenen, J.G., Jetten, M.S.M., 1999. Missing lithotroph identified as new planctomycete. Nature 400 (6743), 446–449.
Strous, M., Pelletier, E., Mangenot, S., Rattei, T., Lehner, A., Taylor, M.W., Horn, M., Daims, H., Bartol-Mavel, D., Wincker, P., Barbe, V., Fonknechten, N., Vallenet, D., Segurens, B., Schenowitz-Truong, C., Medigue, C., Collingro, A., Snel, B., Dutilh, B.E., Op den Camp, H.J.M., van der Drift, C., Cirpus, I., van de Pas-Schoonen, K.T., Harhangi, H.R., van Niftrik, L., Schmid, M., Keltjens, J., van de Vossenberg, J., Kartal, B., Meier, H., Frishman, D., Huynen, M.A., Mewes, H.W., Weissenbach, J., Jetten, M.S.M., Wagner, M., Le Paslier, D., 2006. Deciphering the evolution and metabolism of an anammox bacterium from a community genome. Nature 440, 790–794.
Sturt, H.F., Summons, R.E., Smith, K., Elvert, M., Hinrichs, K.-U., 2004. Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry — new biomarkers for biogeochemistry and microbial ecology. Rapid Commun. Mass Sp. 18 (6), 617–628.
Taniguchi, M., Burnett, W.C., Cable, J.E., Turner, J.V., 2002. Investigation of submarine groundwater discharge. Hydrol. Process. 16 (11), 2115–2129.
Testa, J.M., Charette, M.A., Sholkovitz, E.R., Allen, M.C., Rago, A., Herbold, C.W., 2002. Dissolved iron cycling in the subterranean estuary of a coastal bay: Waquoit Bay, Massachusetts. Biol. Bull. 203 (2), 255–256.
Thamdrup, B., Dalsgaard, T., 2002. Production of n-2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. Appl. Environ. Microbiol. 68 (3), 1312–1318.
Thamdrup, B., Dalsgaard, T., Jensen, M.M., Ulloa, O., Farias, L., Escribano, R., 2006. Anaerobic ammonium oxidation in the oxygen-deficient waters off Northern Chile. Limnol. Oceanogr. 51 (5), 2145–2156.
Valiela, I., Geist, M., McClelland, J., Tomasky, G., 2000. Nitrogen loading from watersheds to estuaries: VERIFICATION of the Waquoit Bay nitrogen loading model. Biogeochemistry 49 (3), 277–293.
van Niftrik, L.A., Fuerst, J.A., Sinninghe Damsté, J.S.S., Kuenen, J.G., Jetten, M.S.M., Strous, M., 2004. The anammoxosome: an intracytoplasmic compartment in anammox bacteria. FEMS Microbiol. Lett. 233 (1), 7–13.
Windom, H., Niencheski, F., 2003. Biogeochemical processes in a freshwater–seawater mixing zone in permeable sediments along the coast of Southern Brazil. Mar. Chem. 83 (3–4), 121–130.
Windom, H.L., Moore, W.S., Niencheski, L.F.H., Jahrike, R.A., 2006. Submarine groundwater discharge: a large, previously unrecognized source of dissolved iron to the South Atlantic Ocean. Mar. Chem. 102 (3–4), 252–266.
Woebken, D., Fuchs, B.A., Kuypers, M.A.A., Amann, R., 2007. Potential interactions of particle-associated anammox bacteria with bacterial and archaeal partners in the Namibian upwelling system. Appl. Environ. Microbiol. 73 (14), 4648–4657.
Woebken, D., Lam, P., Kuypers, M.M.M., Naqvi, S.W.A., Kartal, B., Strous, M., Jetten, M.S.M., Fuchs, B.M., Amann, R., 2008. A microdiversity study of anammox bacteria reveals a novel *Candidatus scalindua* phylotype in marine oxygen minimum zones. Environ. Microbiol. 10 (11), 3106–3119.