Available online at www.sciencedirect.com
ScienceDirect
Organic Geochemistry 39 (2008) 992–999
Organic Geochemistry
www.elsevier.com/locate/orggeochem

Intact polar lipids of anaerobic methanotrophic archaea and associated bacteria ☆

Pamela E. Rossel [a], Julius S. Lipp [a], Helen F. Fredricks [b], Julia Arnds [c], Antje Boetius [c], Marcus Elvert [a], Kai-Uwe Hinrichs [a,*]

[a] *Organic Geochemistry Group, Department of Geosciences, University of Bremen, 28334 Bremen, Germany*
[b] *Woods Hole Oceanographic Institution, Department of Marine Chemistry and Geochemistry, Woods Hole, MA 02543, USA*
[c] *Max-Planck-Institute for Marine Microbiology, 28359 Bremen, Germany*

Received 8 January 2008; received in revised form 25 February 2008; accepted 27 February 2008
Available online 8 March 2008

Abstract

Previous biomarker studies of microbes involved in anaerobic oxidation of methane (AOM) have targeted non-polar lipids. We have extended the biomarker approach to include intact polar lipids (IPLs) and show here that the major community types involved in AOM at marine methane seeps can be clearly distinguished by these compounds. The lipid profile of methanotrophic communities with dominant ANME-1 archaea mainly comprises diglycosidic GDGT derivatives. IPL distributions of microbial communities dominated by ANME-2 or ANME-3 are consistent with their phylogenetic affiliation with the euryarchaeal order *Methanosarcinales*, i.e., the lipids are dominated by phosphate-based polar derivatives of archaeol and hydroxyarchaeol. IPLs of associated bacteria strongly differed among the three community types analyzed here; these differences testify to the diversity of bacteria in AOM environments. Generally, the bacterial members of methanotrophic communities are dominated by phosphatidylethanolamine and phosphatidyl-(*N*,*N*)-dimethylethanolamine species; polar dialkylglycerolethers are dominant in the ANME-1 community while in ANME-2 and ANME-3 communities mixed acyl/ether glycerol derivatives are most abundant. The relative concentration of bacterial lipids associated with ANME-1 dominated communities appears significantly lower than in ANME-2 and ANME-3 dominated communities. Our results demonstrate that IPL analysis provides valuable molecular fingerprints of biomass composition in natural microbial communities and enables taxonomic differentiation at the rank of families to orders.

Abbreviations: ANME, anaerobic methanotrophic archaea; AR, archaeol; AOM, anaerobic oxidation of methane; CARD–FISH, catalyzed reporter deposition–fluorescence in situ hybridization; GDGT, glyceroldialkylglyceroltetraether; IPL, intact polar lipid; SRB, sulfate-reducing bacteria; OH-AR, hydroxyarchaeol; PC, phosphatidylcholine; PDME, phosphatidyl-(*N*,*N*)-dimethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PME, phosphatidyl-(*N*)-methylethanolamine; PS, phosphatidylserine; 2-Gly, diglycosyl; 2OH-AR, dihydroxyarchaeol.

☆ Presented as poster at 23rd IMOG, Torquay 2007.

* Corresponding author. Tel.: +49 421 21865700; fax: +49 421 21865715.
E-mail address: khinrichs@uni-bremen.de (K.-U. Hinrichs).

0146-6380/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.orggeochem.2008.02.021

1. Introduction

Anaerobic oxidation of methane (AOM) in the marine environment is mediated by three phylogenetically distinct clusters of *Euryarchaeota* called ANME-1, -2 or -3 (cf. Hinrichs et al., 1999; Boetius et al., 2000; Hinrichs and Boetius, 2002; Niemann et al., 2006) that form consortia with sulfate-reducing bacteria (SRB) (Boetius et al., 2000; Orphan et al., 2001a, 2002; Lösekann et al., 2007). ANME-2 are phylogenetically affiliated with the order *Methanosarcinales* and are typically observed in physical association with SRB of the *Desulfosarcina/Desulfococcus* group (Boetius et al., 2000; Orphan et al., 2001b, "ANME-2/DSS aggregates"). ANME-3 are closely related to the genera *Methanococcoides* and *Methanolobus* and have been found in association with SRB related to *Desulfobulbus* spp. (Lösekann et al., 2007, "ANME-3/DBB aggregates"). ANME-1 are not directly affiliated with any of the major orders of methanogens (Hinrichs et al., 1999; Orphan et al., 2001b; Knittel et al., 2005). These archaea have been observed in physical association with SRB of the *Desulfosarcina/Desulfococcus* group in microbial mats (Michaelis et al., 2002) but also frequently as monospecific aggregates or as single cells without a clear bacterial partner (Orphan et al., 2002).

Previous biomarker studies of AOM communities have focused on non-polar lipids, such as hydrocarbons of archaeal origin, bacterial fatty acids, and archaeal and bacterial glycerol-based ether lipids (e.g., Elvert et al., 1999, 2005; Hinrichs et al., 1999, 2000; Pancost et al., 2000; Blumenberg et al., 2004; Niemann et al., 2006). However, an interpretation of the lipid profiles with regard to the distribution and composition of active methanotrophic communities is limited by their relatively low taxonomic specificity and the likelihood of incorporating signals from the past. The latter point is particularly crucial due to the temporally highly dynamic physical–chemical conditions encountered in many of the intensely studied AOM environments. By contrast, intact polar lipids (IPLs) offer a more detailed view of microbial communities due to their higher taxonomic specificity and property to select for live biomass (Rütters et al., 2002; Sturt et al., 2004; Biddle et al., 2006).

Here we report the composition of IPLs in environmental samples dominated by either one of the three major ANME groups and associated bacteria. We show that IPL profiles can serve as valuable community fingerprints and relative indicators of biomass of ANME archaea and associated bacteria in natural systems.

2. Materials and Methods

2.1. IPL analysis

Samples from four different seep environments were analyzed, each dominated by one distinct ANME group (Table 1 and Fig. 1): two microbial mats from the northwestern Black Sea, one sediment sample from Hydrate Ridge and one sediment sample from Håkon Mosby Mud Volcano. Both surface sediment samples from Hydrate Ridge and Håkon Mosby Mud Volcano were covered by *Beggiatoa* mats.

IPL analysis was performed with a HPLC–ESI–MS^n system using protocols described previously by Sturt et al. (2004) and Biddle et al. (2006). Total lipid extracts from microbial mats from the Black Sea and the sediment from Håkon Mosby Mud Volcano were obtained with an automated microwave-assisted extraction system (MARS-X, CEM, USA) at a temperature of 70 °C, while the sediment from Hydrate Ridge was extracted via ultrasonication. The latter sample was analyzed after chromatographic separation as glyco- and phospholipids fraction (Sturt et al., 2004), while the former samples were analyzed as total lipid extracts. Structural assignments were based on mass spectral interpretation (cf. Sturt et al., 2004) and by comparison with IPL inventories of cultured archaea and bacteria (e.g., Koga et al., 1998; Koga and Morii, 2005; Hinrichs et al., unpublished data). Chain length assignment, degree of unsaturation, and determination of ether and ester bond linkages of bacterial IPLs were based on molecular masses and fragments according to Sturt et al. (2004). Due to the limited availability of commercial standards, we did not use response factors for IPL quantification. Based on calibration curves we observed response factors for various commercially available IPLs that can differ up to a factor of three. Thus, reported relative distributions are semi-quantitative. Only compounds with a signal-to-noise ratio higher than 6 were reported. The least concentrated reported compounds amounted to 0.12%, 0.06%, 5.0% and 5.2% of the total quantified IPLs in samples from Black Sea (two samples), Hydrate Ridge and Håkon Mosby Mud Volcano, respectively. Composite chromatograms of extracted quasi-molecular ions of

Table 1
Overview of AOM samples analyzed, relative distribution of both archaeal and bacterial cells and IPLs, and core lipid distribution of diglycosyl-GDGTs

Location	Sample	*Archaea* vs. *bacteria* (% of total cells)[a]	ANME -1/-2/-3 (% of total archaeal cells)	Source	Archaeal glyco- and phospholipids (% of total archaeal IPLs)	Bacterial phospholipids (% of total bacterial IPLs)[b]	Core lipid ring distribution of 2-Gly-GDGTs
Northwestern Black Sea, Dniepr area	Mat 795, pink (189 m water depth)	33/16	100/0/0	This study	2-Gly-GDGT (>99), 2-Gly-AR (<1)	DEG-PE $C_{30:0}$ (31), DAG/AEG-PE $C_{33:2}$ (19), DEG-PE $C_{31:1}$ (18), DAG/AEG-PE $C_{35:2}$ (11), DAG/AEG-PE $C_{32:2}$ (11), other PEs (10)	$3 > 2 \gg 1 \gg 0 > 5$
Northwestern Black Sea, Dniepr area	Mat 822, reef top (190 m water depth)	31/43	35/65/0	This study	PG-AR (35), 2-Gly-GDGT (33), tentative phospho-AR (19), 2-Gly-AR (5), PS-OH-AR (2), 2-Gly-OH-AR (1), PE-OH-AR (1)	DAG/AEG-PE $C_{31:2}$ (32), DEG-PE $C_{32:1}$ (11), DAG/AEG-PE $C_{31:1}$ (10), DAG/AEG-PE $C_{32:2}$ (9), DEG-PE $C_{31:1}$ (8), other PEs and PCs (32)	$3 > 2 \gg 1 \gg 0$[c]
Hydrate Ridge	Sediment (station 19-2, 2–3 cm)	31/67	<2/97/0	Knittel et al. (2005)	PG-OH-AR (23), PE-OH-AR (15), PI-OH-AR (13), PS-OH-AR (14), PG-AR (12), 2-Gly-AR (11), 2-Gly-GDGT (6), PS-2OH-AR (2), tentative phospho-AR (2), PS-AR (1)	DAG/AEG-PE $C_{32:2}$ (16), DAG/AEG-PG $C_{34:2}$ (15), DAG/AEG-PE $C_{34:2}$ (11), DAG/AEG-PE $C_{32:1}$ (7), DAG/AEG-PG $C_{36:2}$ (7), other PEs, PCs, and PGs (44)	$3 > 2 > 1$[d]
Håkon Mosby Mud Volcano	Sediment (station 19, 1–2 cm)	77/10	0/0/99	Lösekann et al. (2007)	PG-OH-AR (53), PS-OH-AR (16), PI-OH-AR (9), PS-2OH-AR (5), PS-AR (2)	DAG/AEG-PDME $C_{32:2}$ (21), DAG/AEG-PE $C_{32:2}$ (18), DAG/AEG-PDME $C_{34:2}$ (11), other PEs, PCs, PGs, PMEs, and PDMEs (50)	No GDGTs

Relative amounts of IPLs are based on peak area in mass chromatograms of selected molecular ions. For bacterial IPLs, bond types between alkyl moieties and glycerol are distinguished (DEG = diether, DAG = diacyl, AEG = mixed), followed by head groups, the sum of carbon atoms in both alkyl chains and number of unsaturations. Bacterial IPL data from Hydrate Ridge were previously reported by Sturt et al. (2004).

[a] Percentage derived from CARD–FISH, expressed relative to DAPI counts (100%).

[b] Distinction between DAG and AEG not possible under HPLC–MS conditions applied, indicated alkyl chains provided for DAG.

[c] No GDGT with 5 rings detected.

[d] No GDGTs with 0 and 5 rings detected.

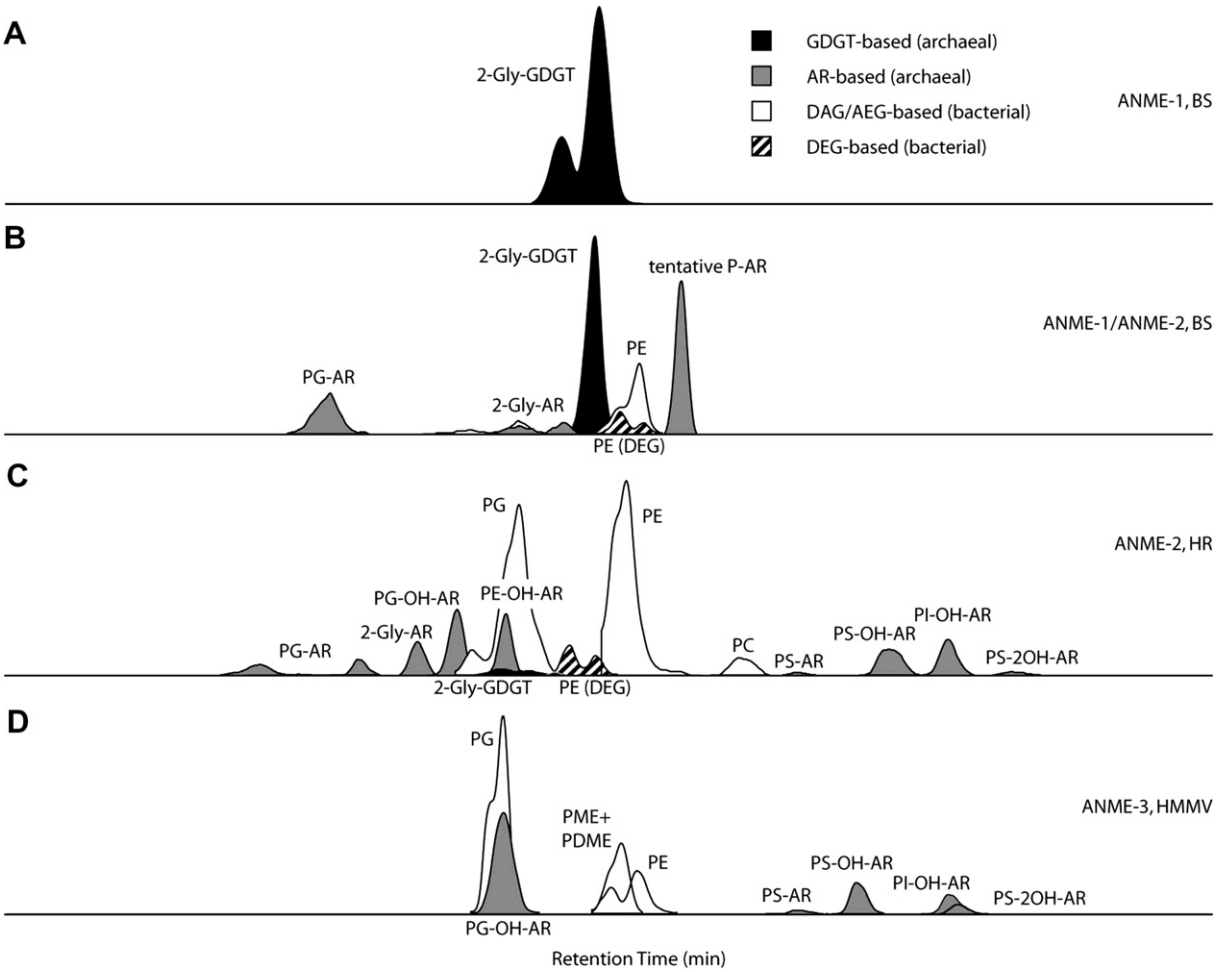

Fig. 1. Composite mass chromatograms of molecular ions of IPLs in samples dominated by ANME-1 (A), a mixed ANME-1/ANME-2 community (B), both from microbial mats collected in the Black Sea (BS), and sediments dominated by ANME-2 (C) and ANME-3 (D) from Hydrate Ridge (HR) and Håkon Mosby Mud Volcano (HMMV), respectively. Extracted *m/z* of quasi-molecular ions for archaeal IPLs are 1632-1645, 994, 807, 956, 823, 792, 820, 836, 852, 911 for the identified 2-Gly-GDGT, 2-Gly-AR, PG-AR, tentative P-AR, PG-OH-AR, PE-OH-AR, PS-AR, PS-OH-AR, PS-2OH-AR, PI-OH-AR, respectively. The major bacterial IPLs are represented by the following quasi-molecular ions: *m/z* 674 and 688 for DAG/AEG-PE, 662 and 660 for DEG-PE, 764 and 736 for DAG/AEG-PG, 760 for DAG/AEG PC, 716 for DAG/AEG-PDME and 702 for PME.

individual IPLs were obtained from the full scan (*m/z* 500–2000) from each sample (Fig. 1). Structural details of hexoses linked via glycosidic bonds to archaeal ether lipids are not resolved; hence hexoses are designated as glycolipids.

2.2. Catalyzed reporter deposition–fluorescence in situ hybridization (CARD–FISH) analyses

Microbial mats were fixed as described previously (Treude et al., 2007) and homogenized. *In situ* hybridizations with horseradish peroxidase (HRP)-conjugated probes followed by tyramide signal amplification were carried out as described by Pernthaler et al. (2002) with slight modifications: endogenous peroxidases were inhibited with methanol (30 min) and rigid archaeal cell walls were permeabilized with proteinase K (15 μg ml^{-1}, for 2 min at room temperature). Total cell counts were determined by 4′,6′-diamidino-2-phenylindole (DAPI)-staining.

Hybridized and DAPI-stained samples were examined with an epifluorescence microscope (Axiophot II microscope; Carl Zeiss, Jena, Germany). For each probe and sample 700 DAPI-stained cells in 70 independent microscopic fields were counted. Probe sequences and formamide concentrations required for specific hybridization were: ARCH915 (35% formamide), EUB338 I–III (35% formamide),

ANME-1–350 and EelMS932 (40 and 60% formamide) (Amann et al., 1990; Daims et al., 1999; Boetius et al., 2000).

3. Results and discussion

3.1. IPLs of ANME-1

In the microbial mat from the trunk of a microbial reef in the Black Sea, all archaeal cells were affiliated with ANME-1 (Table 1). In this sample, diglycosyl glyceroldialkylglyceroltetraethers (2-Gly-GDGTs) were the most abundant IPLs (Fig. 1A). Only small amounts of 2-Gly-archaeol (2-Gly-AR) were detected (Fig. 2A). The main GDGT core lipids in 2-Gly-GDGT were the di- and tri-cyclopentyl derivatives (Table 1). No polar derivative of hydroxyarchaeol (OH-AR) was detected. In terms of the IPL composition, ANME-1 are distinct from other methanogens (e.g., Koga and Morii, 2005), i.e., all major families of methanogens produce significant amounts of AR and multiple types of phosphate-based IPLs. In fact, ANME-1 is most similar to members of the hyperthermophilic *Archaeoglobales* that largely produce 2-Gly-GDGT, combined with lower amounts of both 1-Gly-GDGT and small quantities of Gly-AR (Hinrichs et al., unpublished data). Our results are consistent with evidence provided by Thiel et al. (2007) who applied molecular imaging techniques based on ToF-SIMS to mat sections obtained from the same reef system and dominated by cells of the ANME-1 morphology; these sections consisted mainly of free GDGTs and 2-Gly-GDGTs.

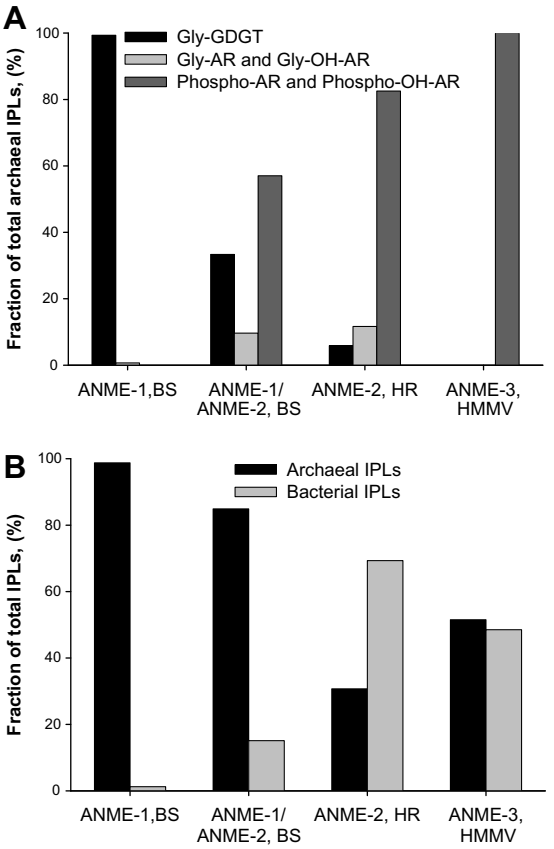

Fig. 2. Compositional variation of IPL groups in the four AOM communities. (A) Distribution of archaeal IPLs (Gly-GDGT, Gly-AR and Gly-OH-AR, P-AR and P-OH-AR [P = phospho]), (B) relative amounts of archaeal and bacterial IPLs.

The absence or extremely low relative abundance of OH-AR in ANME-1 archaea was not apparent in earlier studies that focused on its non-polar derivatives (Hinrichs et al., 1999; Blumenberg et al., 2004). However, in the ANME-1 dominated mat, the concentration of non-polar OH-AR of 15 μg/g mat was very low compared to the mat dominated by ANME-2 (436 μg/g mat). Low ratios of OH-AR/AR have been used as indicator signatures of active ANME-1 (Blumenberg et al., 2004; Niemann and Elvert, in press), but probably have to be interpreted with caution. We suggest that in ANME-1-dominated environments lacking polar OH-AR, non-polar OH-AR is a relict from the past, when environmental conditions selected for ANME-2.

3.2. IPLs of ANME-2

The mat from the top part of a reef structures in the Black Sea was characterized by a mixture of ANME-1 and ANME-2 (Table 1, 35% and 65% of total archaeal cells, respectively), while the surface sediment sample from Hydrate Ridge was dominated by ANME-2 (Table 1, 97% of total archaeal cells, Knittel et al., 2005). Archaeal IPLs of ANME-2 were largely based on AR and OH-AR with either glycosidic or phosphate-based headgroups. Specifically, these included 2-Gly-AR, 2-Gly-OH-AR, phosphatidylglycerol- (PG-) OH-AR, PG-AR, phosphatidylinositol- (PI-) AR, PI-OH-AR, phosphatidylserine- (PS-) AR, PS-OH-AR, PS-2-OH-AR and a tentatively identified AR with a phosphate-based headgroup of unknown structure (Fig. 1B and C). This phospho-AR, present in both samples containing ANME-2, but not in the ANME-1 and ANME-3 dominated communities,

was tentatively assigned based on information obtained in negative ionization mode, which yielded an intense fragment of 433.5 Da (interpreted as dehydrated *lyso* fragment with one phytanyl chain and without head group). The unknown compound is formed by two masses 956.0 and 939.3 that probably correspond to the ammonium adduct and the protonated lipid, respectively. None of the corresponding ions yielded intense, interpretable fragments during MS^2 experiments in positive ionization mode. 2-Gly-GDGT was also detected in the two ANME-2 dominated communities from Black Sea and Hydrate Ridge; relative concentrations of this compound are consistent with the relative amounts of ANME-1 cells in these samples (Table 1 and Fig. 1).

3.3. IPLs of ANME-3

The sediments from Håkon Mosby Mud Volcano were dominated by ANME-3 (Table 1, 99% total archaeal cells, Lösekann et al., 2007). The sample contained the most diverse distribution of archaeal and bacterial IPLs (Fig. 1D). The main archaeal IPLs were various phospholipids of AR and OH-AR, similar to those observed in the ANME-2 system of Hydrate Ridge. In contrast to the ANME-1 and ANME-2 dominated communities, neither GDGT-based IPLs nor glycosidic archaeol derivatives were present. Likewise, the tentatively identified phospho-AR from the ANME-2 community (Fig. 1B and C) was not detected.

3.4. Bacterial IPLs

Compositional differences of bacterial IPLs reflect differences in the phylogenetic affiliation of the bacterial members of AOM communities such as SRB (Fig. 1A–D). Bacterial IPLs vary in both structural diversity and relative abundance; ANME-3 and ANME-2 dominated samples displayed both the highest abundance and highest diversity of bacterial IPLs (Table 1 and Fig. 2). The Black Sea ANME-1 system was dominated by phosphatidylethanolamine (PE) derivatives of dietherglycerol (DEG) lipid types (Table 1), while the ANME-2 systems contained mainly PE of mixed acyl/ether glycerol (AEG) lipids or diacyl glycerol (DAG) lipids, although the corresponding DEG types were also present. The high relative amounts of DAG/AEG lipids in combination with PE and PG is consistent with the IPL composition of *Desulfosarcina variabilis* (Rütters et al., 2001), a close relative of the sulfate reducers in ANME-2 communities, although the chain length distribution and degree of unsaturation differ (Table 1; cf. Rütters et al., 2001; Sturt et al., 2004). In ANME-2 dominated communities, we also observed phosphatidylcholine (PC) and PG (the latter observed in the Hydrate Ridge sample only). With respect to the bacterial IPLs, the ANME-3 community is distinguished from the ANME-2 community by a higher abundance of phosphatidyl-(*N*)-methylethanolamine (PME) and phosphatidyl-(*N*,*N*)-dimethylethanolamine (PDME).

In all samples, PE was a major bacterial IPL and contributes between ~1 and 15% to total IPLs in Black Sea mat samples, and between 15% and 40% in the sediments from Håkon Mosby Mud Volcano and Hydrate Ridge, respectively (cf. Sturt et al., 2004, for detailed discussion of bacterial IPLs in Hydrate Ridge sample). The total number of carbon atoms in glycerol-bound acyl and/or alkyl moieties further distinguished the three ANME community types. In the ANME-1 dominated sample, the dominant bacterial IPL was a $C_{30:0}$ DEG-PE; in samples from Hydrate Ridge and Håkon Mosby Mud Volcano, $C_{32:2}$ DAG/AEG-PE and $C_{32:2}$ DAG/AEG-PDME, respectively, were more abundant (Table 1).

3.5. Lipid taxonomy of uncultured AOM archaea

The presence of AR and OH-AR based core lipids in ANME-2 and ANME-3 archaea is consistent with their affiliation with the methanogenic orders *Methanococcales* and *Methanosarcinales* (cf. Kates, 1997). When considering the presence and/or absence of polar headgroups in ANME-2 and ANME-3 communities, the taxonomic relationship is narrowed down to the *Methanosarcinales*: PI and PG derivatives are abundant in the *Methanosarcinales* and but are absent in the *Methanococcales* (Koga and Morii, 2005). Notably, no clear chemotaxonomic relationship exists between the phylogenetically distinctive ANME-1 and any of the cultured methanogens (cf. Koga et al., 1998; Koga and Morii, 2005). Closest relatives of ANME-1 in terms of IPL composition are the *Archaeoglobales* (Hinrichs et al., unpublished data). Environmental IPL fingerprints that resemble those from ANME-1 are those related to uncultured marine sedimentary archaea (Biddle et al., 2006).

3.6. Archaeal vs. bacterial biomass

Relative amounts of archaeal vs. bacterial IPLs strongly varied in the samples dominated by a single ANME type (percentages of archaeal IPLs are ~99%, 85%, 31% and 52% for samples from the Black Sea, Hydrate Ridge, and Håkon Mosby Mud Volcano, respectively; Fig. 2B). These pronounced differences probably reflect similarly large differences in archaeal vs. bacterial biomass among active AOM community members, which in turn probably relate to ecophysiological characteristics of the three types of AOM communities sampled here. Notably, the IPLs partly provided a different picture of the relative abundance of archaeal vs. bacterial biomass than cell counts by CARD–FISH (Table 1). For example, for the Black Sea ANME-1 sample, IPL analysis suggests a lower bacterial contribution to the microbial community than CARD–FISH, while in the Håkon Mosby Mud Volcano ANME-3 sample, CARD–FISH detected more archaea than IPL analysis (Fig. 2B and Table 1). Possible causes include varying cellular IPL contents, e.g., due to differences in cell size and morphology, and/or differences in physiological status of the bulk community that in turn may affect both cellular IPL abundance and the ability to bind to CARD–FISH probes.

4. Conclusions

This study provides an unprecedented view of the lipid diversity of the three globally relevant anaerobic methanotrophic communities, that is, communities dominated by ANME-1, ANME-2, ANME-3 populations, and associated bacteria. The diversity and relative amounts of both archaeal and bacterial IPLs differ remarkably between the three community types. While lipid analysis is unable to capture the entire microbial diversity, our results demonstrate that quantitative differences in microbial community structure can be effectively resolved. Specifically, IPL analysis enables the differentiation of the major players in natural microbial communities at the rank of taxonomic orders or higher.

Acknowledgments

We thank the crew and shipboard scientist of R/V SONNE SO 148-1, R/V L'Atalante, and R/V Poseidon for support during sample collection. Tina Treude is gratefully acknowledged for providing microbial mat samples from the Black Sea, Katrin Knittel for helping with analysis of FISH data, and Philippe Schaeffer and Richard Pancost for their constructive reviews. This study was part of the program MUMM II (grant 03G0608C), funded by the *Bundesministerium für Bildung und Forschung* (BMBF, Germany) and the *Deutsche Forschungsgemeinschaft* (DFG, Germany). Further support was provided from the Center of Marine Environmental Sciences (MARUM) at the University of Bremen funded by the DFG. This is publication GEOTECH-316 of the R&D program GEOTECHNOLOGIEN and MARUM-publication 0573.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.orggeochem.2008.02.021.

Associate Editor—**P.D. Pancost**

References

Amann, R.I., Binder, B.J., Olson, R.J., Chisholm, S.W., Devereux, R., Stahl, D.A., 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Applied and Environmental Microbiology 56, 1919–1925.

Biddle, J.F., Lipp, J.S., Lever, M.A., Lloyd, K.G., Sørensen, K.B., Anderson, R., Fredricks, H.F., Elvert, M., Kelly, T.J., Schrag, D.P., Sogin, M.L., Brenchley, J.E., Teske, A., House, C.H., Hinrichs, K.-U., 2006. Heterotrophic archaea dominate sedimentary subsurface ecosystems off Peru. Proceedings of the National Academy of Sciences of the USA 103, 3846–3851.

Blumenberg, M., Seifert, R., Reitner, J., Pape, T., Michaelis, W., 2004. Membrane lipid patterns typify distinct anaerobic methanotrophic consortia. Proceedings of the National Academy of Sciences of the USA 101, 11111–11116.

Boetius, A., Ravenschlag, K., Schubert, C.J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B.B., Witte, U., Pfannkuche, K., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407, 623–626.

Daims, H., Bruhl, A., Amann, R., Schleifer, K.H., Wagner, M., 1999. The domain-specific probe EUB338 is insufficient for the detection of all *Bacteria*: development and evaluation of a more comprehensive probe set. Systematic and Applied Microbiology 22, 434–444.

Elvert, M., Suess, E., Whiticar, M.J., 1999. Anaerobic methane oxidation associated with marine gas hydrates: superlight C-isotopes from saturated and unsaturated C_{20} and C_{25} irregular isoprenoids. Naturwissenschaften 86, 295–300.

Elvert, M., Hopmans, E.C., Treude, T., Boetius, A., Suess, E., 2005. Spatial variations of methanotrophic consortia at cold

methane seeps: implications from a high-resolution molecular and isotopic approach. Geobiology 3, 195–209.

Hinrichs, K.-U., Boetius, A., 2002. The anaerobic oxidation of methane: new insights in microbial ecology and biogeochemistry. In: Wefer, G., Billett, D., Hebbeln, D., Jørgensen, B.B., Schlüter, M., van Weering, T. (Eds.), Ocean Margin Systems. Springer-Verlag, Berlin Heidelberg, pp. 57–477.

Hinrichs, K.-U., Hayes, J.M., Sylva, S.P., Brewer, P.G., DeLong, E.F., 1999. Methane-consuming archaebacteria in marine sediments. Nature 398, 802–805.

Hinrichs, K.-U., Summons, R.E., Orphan, V., Sylva, S.P., Hayes, J.M., 2000. Molecular and isotopic analysis of anaerobic methane-oxidizing communities in marine sediments. Organic Geochemistry 31, 1685–1701.

Kates, M., 1997. Diether and tetraether phospholipids and glycolipids as molecular markers for archaeabacteria (archaea). In: Eganhause, R.P. (Ed.), Molecular Markers in Environmental Geochemistry. Oxford University Press, pp. 35–48.

Knittel, K., Lösekann, T., Boetius, A., Kort, R., Amann, R., 2005. Diversity and distribution of methanotrophic archaea at cold seeps. Applied and Environmental Microbiology 71, 467–479.

Koga, Y., Morii, H., 2005. Recent advances in structural research on ether lipids from archaea including comparative and physiological aspects. Bioscience Biotechnology and Biochemistry 69, 2019–2034.

Koga, Y., Morii, H., Akagawa-Matsushita, M., Ohga, I., 1998. Correlation of polar lipid composition with 16S rRNA phylogeny in methanogens. Further analysis of lipid component parts. Bioscience Biotechnology and Biochemistry 62, 230–236.

Lösekann, T., Knittel, K., Nadalig, T., Fuchs, B., Niemann, H., Boetius, A., Amann, R., 2007. Diversity and abundance of aerobic and anaerobic methane oxidizers at the Haakon Mosby Mud Volcano, Barents Sea. Applied and Environmental Microbiology 73, 3348–3362.

Michaelis, W., Seifert, R., Nauhaus, K., Treude, T., Thiel, V., Blumenberg, M., Knittel, K., Gieseke, A., Peterknecht, K., Pape, T., Boetius, A., Amann, R., Jorgensen, B.B., Widdel, F., Peckmann, J.R., Pimenov, N.V., Gulin, M.B., 2002. Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. Science 297, 1013–1015.

Niemann, H., Elvert, M., in press. Diagnostic lipid biomarker and stable carbon isotope signatures of microbial communities mediating the anaerobic oxidation of methane with sulphate. Organic Geochemistry, doi: 10.1016/j.orggeochem.2007.11.006.

Niemann, H., Lösekann, T., Beer, D., Elvert, M., Nadalig, T., Knittel, K., Amann, R., Sauter, E.J., Schlüter, M., Klages, M., Foucher, J.P., Boetius, A., 2006. Novel microbial communities of the Håkon Mosby mud volcano and their role as a methane sink. Nature 443, 854–858.

Orphan, V.J., Hinrichs, K.U., Ussler, W., Paull, C.K., Taylor, L.T., Sylva, S.P., Hayes, J.M., Delong, E.F., 2001a. Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. Applied and Environmental Microbiology 67, 1922–1934.

Orphan, V.J., House, C.H., Hinrichs, K.-U., McKeegan, K.D., DeLong, E.F., 2001b. Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. Science 293, 484–487.

Orphan, V., House, C.H., Hinrichs, K.-U., McKeegan, K.D., DeLong, E.F., 2002. Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. Proceedings of the National Academy of Sciences of the USA 99, 7663–7668.

Pancost, R.D., Sinninghe Damsté, J.S., de Lint, S., van der Maarel, M.J.E.C., Gottschal, J.C.and the Medinaut Shipboard Scientific Party, 2000. Biomarker evidence for widespread anaerobic methane oxidation on mediterranean sediments by a consortium of methanogenic archaea and bacteria. Applied and Environmental Microbiology 66, 1126–1132.

Pernthaler, A., Pernthaler, J., Amann, R., 2002. Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Applied and Environmental Microbiology 68, 3094–3101.

Rütters, H., Sass, H., Cypionka, H., Rullkötter, J., 2001. Monoalkylether phospholipids in the sulfate-reducing bacteria *Desulfosarcina variabilis* and *Desulforhabdus amnigenus*. Archives of Microbiology 176, 435–442.

Rütters, H., Sass, H., Cypionka, H., Rullkötter, J., 2002. Phospholipid analysis as a tool to study complex microbial communities in marine sediments. Journal of Microbiological Methods 48, 149–160.

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 Communications in Mass Spectrometry 18, 617–628.

Thiel, V., Heim, C., Arp, G., Hahmann, U., Sjövall, P., Lausmaa, J., 2007. Biomarkers at the microscopic range: ToF-SIMS molecular imaging of Archaea-derived lipids in a microbial mat. Geobiology 5, 413–421.

Treude, T., Orphan, V., Knittel, K., Gieseke, A., House, C.H., Boetius, A., 2007. Consumption of methane and CO_2 by methanotrophic microbial mats from gas seeps of the anoxic Black Sea. Applied and Environmental Microbiology 73, 2271–2283.