Organic Geochemistry 39 (2008) 1572–1588

Contents lists available at ScienceDirect

Organic Geochemistry

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

Vertical distribution of microbial lipids and functional genes in chemically distinct layers of a highly polluted meromictic lake

Tobias F. Ertefai [a,*], Meredith C. Fisher [b], Helen F. Fredricks [d], Julius S. Lipp [a], Ann Pearson [c], Daniel Birgel [a], Kai M. Udert [e], Colleen M. Cavanaugh [b], Philip M. Gschwend [e], Kai-Uwe Hinrichs [a]

[a] Organic Geochemistry Group, Department of Geosciences and MARUM – Center for Marine Environmental Sciences, Leobener Str, 28359 Bremen, Germany
[b] Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA
[c] Department of Earth and Planetary Sciences, Harvard University, Cambridge, MA, USA
[d] Woods Hole Oceanographic Institution, Department of Marine Chemistry and Geochemistry, Woods Hole, MA 02543, USA
[e] Department of Civil and Environmental Engineering, Massachusetts Institute of Technology (MIT), Cambridge, MA, USA

ARTICLE INFO

Article history:
Received 29 August 2007
Received in revised form 4 April 2008
Accepted 22 July 2008
Available online 31 July 2008

ABSTRACT

We examined vertical changes in microbial lipid composition and functional genes in the 4.5 m deep water column of a highly contaminated, meromictic lake in eastern Massachusetts. Lipid based techniques and compound specific isotopic analysis indicate marked differences in community structure between the sulfate and methane laden hypolimnion and the aerated epiliminon. The major intact polar lipids (IPLs) detected throughout the water column include diacyl phosphatidylethanolamine, its methylated derivatives phosphatidylmethylethanolamine and phosphatidyldimethylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, and the glycolipids monoglycosyldiacylglycerol and glycuronic acid dialkylglycerol. These compounds are attributed mainly to bacteria. The predominance of betaine lipids in the epilimnion is consistent with the dominant role of eukaryotic photoautotrophs in the oxic surface. Bacteriohopanepolyols and slight negative inflections of $\delta^{13}C$ values of bacterial phospholipid-bound fatty acids (PLFAs) at the metalimnion indicate a low contribution of methanotrophic bacteria to the pool of metalimnic bacteria. In the hypolimnion, dialkylglycerol-phosphatidylethanolamine, C_{17}-PLFAs and *dsrAB* gene sequence data suggest the presence of sulfate reducing bacteria (SRB). Five distinct groups of sulfate reducers and methanogens were detected in the hypolimnion. While SRB appear to inhabit the hypolimnion, the methanogens most likely entered the lake through the inflow of methane laden groundwater. We did not detect either of the methyl-coenzyme M reductase genes or characteristic lipids related to known anaerobic methane oxidizing Archaea although the hypolimnion exhibited conditions conducive to anaerobic methanotrophy. The large amount of dissolved organic carbon appears to be the major pool of carbon for the lake's microbial biomass.

1. Introduction

Meromictic lakes are widespread on earth and represent an excellent opportunity to study microbial community structure and metabolism throughout chemically stratified ecosystems. We have studied one such lake, the *Halls Brook Holding Area* (HBHA) (eastern Massachusetts, USA), which continuously receives inflow of highly polluted, salty groundwater rich in methane, sulfate, and a wide variety of organic compounds into its hypolimnion (Wick and Gschwend, 1998; Wick et al., 2000). This situation makes this lake a unique microbial habitat allowing study of processes like methane oxidation and aromatic compound degradation. In particular, we sought molecular

* Corresponding author. Tel.: +49 421 218 65709; fax: +49 421 218 65715.
E-mail address: tobias.ertefai@uni-bremen.de (T.F. Ertefai).

0146-6380/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.orggeochem.2008.07.009

indicators for the presence or absence of microbial communities responsible for the chemical cycling in this freshwater lake system.

In principle, the chemical conditions were permissive of aerobic methanotrophy at the oxycline and anaerobic methane oxidation (AOM) in the anoxic bottom waters. AOM, well known from marine sediments (e.g. Martens and Berner, 1977; Hinrichs et al., 1999; Boetius et al., 2000; Elvert et al., 2000) is not common in freshwater lakes. Only a few lakes with geochemical conditions similar to the HBHA show the occurrence of AOM (Joye et al., 1999; Eller et al., 2005). Extreme conditions, such as high alkalinity or hypersalinity characterize most continental settings exhibiting AOM, e.g., Big Soda Lake, Nevada (Iversen et al., 1987), hypersaline Solar Lake, Sinai, Egypt (Cytryn et al., 2000) or mud volcanoes in the Carpathian Mountains, Romania (Alain et al., 2006).

Molecular studies of AOM and sulfate reduction in terrestrial and aquatic environments target 16S rRNA as well as the functional genes, *mcrA* and *dsrAB*, which are both highly conserved and universal to methanogenic Archaea and sulfate reducing bacteria (SRB), respectively (Chang et al., 2001; Castro et al., 2002; Luton et al., 2002; Dhillon et al., 2003; Conrad et al., 2006). The gene *mcrA* encodes the α-subunit of the key enzyme, methyl-coenzyme M reductase (MCMR), and *dsrAB* encodes the α- and β-subunits of dissimilatory sulfite reductase (DSR). MCMR, which may also be involved in AOM, catalyzes the reduction of methyl-coenzyme M leading to the release of methane in methanogenesis and DSR catalyzes the reduction of sulfite to sulfide in sulfate reduction (Scholten et al., 2005).

In this study of the water column and surface sediment of the HBHA, we combined the analysis of intact polar lipids (IPLs) and bacteriohopanepolyols (BHPs) with geochemical analyses of dissolved chemical species and the analysis of functional genes for sulfate reduction and methanogenesis. IPLs are indicative of live biomass, since they rapidly decompose after cell death (e.g. White et al., 1979; Rütters et al., 2002; Sturt et al., 2004). Their taxonomic specificity is higher compared to phospholipid fatty acids (PLFAs) and, more importantly, IPL analysis does not discriminate against Archaea and is useful for identifying the dominant groups of microorganisms (e.g. Fang et al., 2000a,b; Rütters et al., 2001, 2002; Sturt et al., 2004; Zink and Mangelsdorf, 2004). Complementary isotopic analyses of microbial lipids sets constraints on the carbon metabolism of prokaryotes (e.g. Hinrichs et al., 1999; Londry and Des Marais, 2003; Elvert et al., 2003; Zhang et al., 2003, 2004; Bühring et al., 2005; Biddle et al., 2006).

BHPs are membrane lipids found in numerous cultured bacteria, including aerobic methanotrophs, cyanobacteria and nitrogen fixing bacteria (e.g. Rohmer et al., 1984). Only recently, BHPs were described as excellent recorders of various bacterial populations in modern environments, including aerobic and anaerobic bacteria (see Talbot and Farrimond (2007) for a review). However, BHPs are currently known to be synthesized only by a limited number of bacteria (Pearson et al., 2007). This makes them even more interesting as source specific biomarkers in environmental samples.

The vertical stratification of microorganisms and their metabolic pathways of carbon utilization in the ecosystem of HBHA are the main focus of this work. Specifically, we addressed the following research questions. (I) What is the distribution of microbial lipids in chemically distinct layers of the lake? (II) Is the chemical zonation of the lake reflected in the composition of functional microbial groups?

2. Materials and methods

2.1. Setting

The *Halls Brook Holding Area* (HBHA) is a shallow lake (~4.5 m) in the Aberjona Watershed in Woburn, Massachusetts, several miles northwest of Boston (USA). It is an elongated, artificial lake, created in the early 1970s in place of the former Lake Mishawum for flood control purposes. At the northern end, the eutrophic lake receives contaminated groundwater from an adjacent Superfund Site with a long history of chemical manufacturing and waste disposal. This site has previously been investigated for its hydrologic and mixing properties as well as for the persistence of specific organic contaminants like benzene (Wick and Gschwend, 1998; Wick et al., 2000), resulting in a well studied freshwater ecosystem. Seepage of contaminated saline groundwater at HBHA causes a density stratification of the lake's water column which stays stratified year round (meromictic) (Wick et al., 2000). Episodic storms only mix the upper layer of the lake which is oxygenated, but generally do not affect most of the hypolimnion which remains permanently oxygen free. At the metalimnion, organic compounds, such as benzene and methane, and inorganic substances like ferrous iron (Diez et al., 2007) are oxidized in a distinct chemocline rather than exiting via the outflow channel (Wick and Gschwend, 1998).

2.2. Sampling of lipids, DNA and bulk parameters

Water samples were taken at discrete depths (±10 cm) using a Masterflex E/S Portable Sampling Drive (Cole Parmer). Sampling of lipids and genes took place in October 2004, along with grab sampling of surface sediment for lipid analysis. For IPL and PLFA analysis, water volumes of 1.5 to 2.5 l from depths of 1.47 m, 2.38 m and 3.71 m were filtered onto glass fiber filters (1 μm; Pall Corp.). Two parallel filters from each lake horizon (epi-, meta- and hypolimnion) were extracted and mean values used for quantitative considerations.

For DNA collection, 1 l samples of water from depths 2.38 m and 3.71 m were filtered onto 0.22 μm nitrocellulose filters (Millipore cat. GSWP14250) using a 142 mm Millipore filter holder and KNF Neuberger pump. Filters were either immediately used for nucleic acid extraction or placed into *Whirlpak* bags and flash frozen in a bath of dry ice and ethanol and then stored at −80 °C until use.

During sampling in October 2004, chemical parameters, including temperature, dissolved oxygen (DO), pH, specific conductivity, oxidation-reduction potential (ORP), and

chlorophyll fluorescence, were measured with a Hydrolab MiniSonde water quality multiprobe. Moreover, water samples for methane and benzene concentration measurements were taken in 20 ml glass syringes at discrete depths (Fig. 1). Samples were filtered (0.2 μm) and 100 μl of 0.2 M sodium azide solution were added to inhibit microbial activity. Methane was measured with a gas chromatograph with flame ionization detector (GC-FID) (PE AutoSystem XL, column: J&W DB-624, length 60 m, ID 0.32 mm) connected to a purge and trap sampler (Tekmar LSC 2000). Purging time was 0.5 min and the GC run time was 4 min at 40 °C. Benzene was measured as previously described (Wick et al., 2000).

Two larger samples of water (10 l) were collected in July 2005 and particles for analysis of BHPs were concentrated by centrifugation (5300$g \times 15$ min), followed by filtration of the supernatant as below; pellets and filters were combined for subsequent extraction. Although these were obtained several months after the October sampling, water column parameters like O_2 and methane exhibited profiles that were similar to the previous fall. The chlorophyll profile in June showed a maximum at 1.5 m which was absent in the previous fall.

Water column isotopic data of dissolved methane, particulate organic carbon (POC), dissolved organic carbon (DOC), and dissolved inorganic carbon (DIC) were taken in July 2003. Water for isotopic analysis of methane was sampled into 100 ml serum vials poisoned with 10 μl of saturated $HgCl_2$ solution. Headspace was excluded and vials were capped with blue butyl rubber stoppers and stored at 4 °C in the dark; separation of methane and isotopic analysis was performed by Isotech Labs (http://www.isotechlabs.com/).

POC was filtered onto 15 mm quartz fiber filters (Whatman GFF, 0.7 μm), acidified with 1 N HCl, neutralized, dried, sealed in evacuated quartz tubes with 1 g CuO, and combusted for 5 h at 850 °C. All quartz tubes and filters had been pre-combusted under identical conditions.

For DOC, ~4 ml of lake water were acidified to pH < 3, bubbled with N_2, added to a quartz tube, and lyophilized. CuO was added, and the tube was evacuated and combusted. For DIC, samples were collected as for methane above, and CO_2 was extracted from each sample under vacuum using standard methods for DIC extraction (dry H_3PO_4) and water vapor removal. Measurements of $\delta^{13}C$ values for all samples (except methane) were performed using a dual inlet VG Optima isotope ratio mass spectrometer.

2.3. Lipid extraction and purification

Five to six grams of wet filtered material and 2.1 g of wet sediment were spiked with internal standards for liquid chromatographic analyses (1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine; platelet activation factor, PAF), and for gas chromatographic analyses (GC) (*n*-hexatricontane; 5α(H)-cholestane; behenic acid methyl ester; nonadecanol; nonadecanoic acid). For IPLs, a modified Bligh and Dyer extraction method with four steps (Sturt et al., 2004) was applied, followed by 10 min centrifugation at 800*g*. The combined supernatants were washed with water and evaporated to dryness. A similar extraction protocol was utilized for BHPs involving one extraction step. The total lipid extract (TLE) was separated chromatographically into three fractions on a glass column: a non-polar fraction (dichloromethane), glycolipid fraction (acetone), and a phospholipid fraction (methanol) using 3 g of silica gel (60 mesh). Both the glycolipid and phospholipid fractions were analyzed for IPLs. The TLEs obtained for BHP analyses were separated into non-polar (hexane:dichloromethane, 95:5) and polar (methanol)

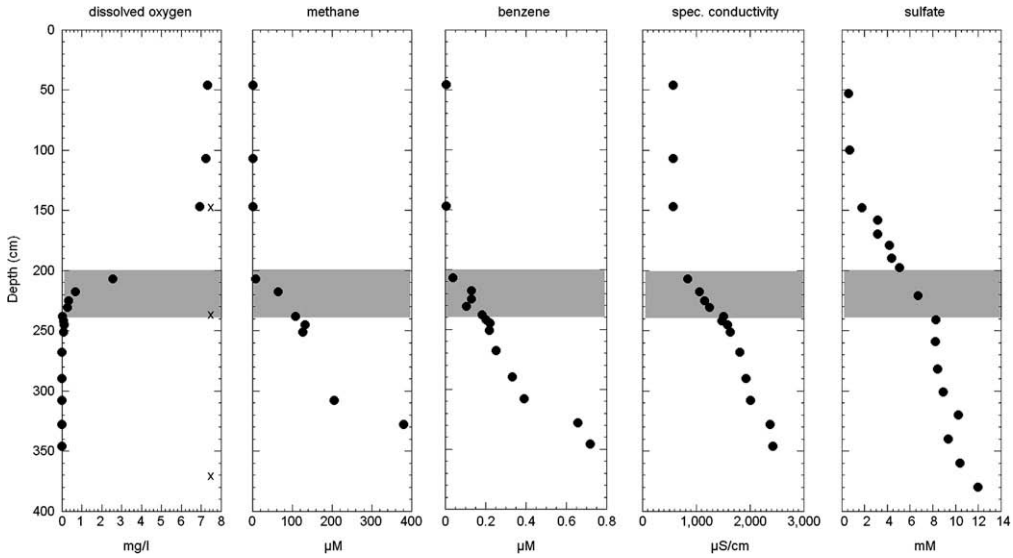

Fig. 1. Depth profile of geochemical parameters at HBHA (former Lake Mishawum, Woburn, MA, USA) measured in October 22, 2004, except sulfate (4th November 2004). Grey-shaded area indicates the location of the metalimnion with superimposed oxygenated epilimnion and underlying anoxic hypolimnion. Letter *x* on the *y*-axis of left panel designates sampling points.

fractions. The non-polar fractions were further separated for GC analyses following standard protocols for separation, derivatization and transesterification (e.g. Elvert et al., 2000, 2003). Polar fractions containing ether-bound IPLs were treated with HI and $LiAlH_4$ to cleave the hydrophobic alkyl chains from the glycerol backbones of IPLs, in order to measure compound specific stable carbon isotope ratios of released alkyl derivatives (e.g. Biddle et al., 2006).

2.4. High performance liquid chromatography–mass spectrometry

Analyses of IPLs and BHPs were performed at the University of Bremen according to Sturt et al. (2004) and Talbot et al. (2003), respectively. For IPLs, all samples were analyzed by high performance liquid chromatography/electrospray ionization-multiple stage mass spectrometry (HPLC/ESI-MS^n) in separate experiments for positive and negative ionization modes as this provided complementary structural information. A LiChrospher® Diol column (125 mm × 2.1 mm, 5 μm; Alltech Associates Inc., Deerfield, IL, USA) was fitted with a 7.5 mm × 4 mm guard column of the same packing material and was used at 30 °C in a column oven using a ThermoFinnigan Surveyor HPLC system. The following linear gradient of eluants was used with a flow rate of 0.2 ml min^{-1}: 100% A to 35% A : 65% B over 45 min, then back to 100% A for 1 h to re-equilibrate the column for the next run (A = mixture of hexane/2-propanol/formic acid/14.8 M NH_3(aq) in the portions of 79:20:0.12:0.04 v/v; and B = 2-propanol/water/formic acid/14.8 M NH_3(aq), 88:10:0.12:0.04 v/v). Multiple stage mass spectrometry experiments (MS^n) were performed using a ThermoFinnigan LCQ Deca XP plus ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) with an electrospray ionization interface (ESI). ESI settings derived from tuning with diester-C16-phosphatidylethanolamine have been described previously (Sturt et al., 2004). A typical mass range of 500–2000 m/z was scanned while fragmenting the base peaks up to MS^3. Compound identification is based on characteristic molecular masses of ionized IPLs shown in the mass spectra, and product ions formed by loss of the neutrally charged headgroups (Table 1), indicating ether or ester bonds between the hydrophilic headgroup and the core lipid with different numbers of carbon atoms attached (Rütters et al., 2002; Sturt et al., 2004). Relative abundances and total concentrations of IPL classes were obtained by integration of peak areas in mass chromatograms. Therefore, individual molecular ions of each IPL class were extracted from the full scan chromatograms and compared with the peak area of the internal standard. A similar response factor for the internal standard was assumed as for individual IPL classes, resulting in semi-quantitative data. The relative abundance of the acyl/alkyl moieties directly associated with the polar headgroups was determined by comparing the relative intensities of diagnostic ions in the corresponding mass spectra.

For the analyses of BHPs, the HPLC-MS^n system (as above) was coupled to an atmospheric pressure chemical ionization source (APCI) operated in positive ionization mode according to Talbot et al. (2003) and equipped with an Alltech-Grace Prevail C_{18} column (150 × 2.1 mm, 3 μm), applying a flow rate of 0.2 ml min^{-1} at 30 °C in the column oven and the following gradient profile: hold for 3 min at 90% A and 10% B; ramp to 59% A, 1% B and 40% C at 25 min, then isocratic for 32 min; re-equilibration of column after each run with 90% A and 10% B for 30 min (where A = MeOH, B = water and C = propan-2-ol; A and C—HPLC grade, B—de-ionized, filtered at 0.22 μm). For analysis of BHPs, aliquots of the polar fraction were measured as their acetate derivatives according to Innes et al. (1997). The resulting acetylated samples were injected in MeOH/propan-2-ol (60:40 v/v). LC/MS settings were as follows: capillary temperature 150 °C, APCI vaporizer temperature 400 °C, discharge current 5 μA, sheath gas flow 40 (arbitrary units). MS instrument parameters were optimized using the automated tune program while infusing an acetylated standard of BHT. MS^n data for this standard were recorded following its direct introduction into the MS by infusion and identified according to Talbot et al. (2001).

2.5. Gas chromatography

PLFAs were analyzed by coupled gas chromatography–mass spectrometry (GC–MS). A 30 m polar HP silica capillary column (0.32 mm i.d.; DB-5MS) coated with a cross-linked methyl silicone phase (film thickness 0.25 μm; Hewlett Packard) was employed in a ThermoFinnigan Trace GC interfaced to a Finnigan Trace MS+. The GC–MS was operated in electron ionization (EI) mode at 70 eV with a full scan mass range of m/z 40–900 (1.5 scans per second). The detector was set at 350 V, the interface temperature was 300 °C, and helium was used as carrier gas (constant flow: 1.4 ml min^{-1}). The samples were injected in splitless mode using an autosampler with the injector set to 310 °C. Initial oven temperature was 60 °C, held for 1 min, subsequently increased to 150 °C at a rate of 10 °C min^{-1}, then raised to 310 °C at a rate of 4 °C min^{-1} and held for 25 min at 310 °C.

2.6. Isotopic analyses of PLFAs

Carbon isotope compositions of fatty acid methyl esters (FAMEs) obtained from the phospholipid fractions of TLEs were determined by GC-irMS. The mass spectrometer (MAT 252) was connected via a Finnigan combustion interface III to a HP 5890 Series GC equipped with a 60 m HP DB-5 column (0.32 mm internal diameter, 0.25 μm film thickness). The carrier gas was He at a constant flow rate of 1.5 ml min^{-1}. On column injection of 1–2 μl out of 20–50 μl was applied (injector temperature: 310 °C). Initial oven temperature was 60 °C, held for 2 min, subsequently increased to 150 °C at a rate of 15 °C min^{-1}, then raised to 320 °C at a rate of 4 °C min^{-1} and held at 320 °C for 20 min. Carbon isotope ratios are reported in the δ notation as per mil (‰) deviation from Vienna Pee Dee Belemnite standard (VPDB). $\delta^{13}C$ values reported have an analytical error of less than ±1.0‰. Isotopic values of fatty acid methyl esters (FAMES) were corrected for the introduction of an additional carbon atom during transesterification with BF_3/MeOH.

Table 1
Mass spectral data for identified IPLs and BHPs at HBHA and their putative biogeochemical origins

	Major molecular species $[M+H]^{+*}$ (Da)	Diagnostic neutral losses* (Da)	MS^2 diagnostic ions$[M+H]^{+*}$ (Da)	Major acyl/ alkyl chains	Retention time (min)	MS-reference	Putative origin	Reasoning	Reference
HPLC–ESI-MS^n IPL									
Betaines: DGTS/A	736.9; 764.9	264; 282	236; 500	C16:1 C18:1*	25–27	This study	Photosynthetic eukarya	Eutrophic conditions	4, 5
1-Gly-DG	744.7; 746.7 $[M+NH_4^+]^+$	180	311	C16:0 C16:1*	12–14	This study	Photosynthetic prokaryotes	Di-acyl groups of IPLs: C14 - C18, no further glycolipids	6, 7
1-GlyA-DG	758.4	194		C16:1*	30	This study	Bacteria	Di-acyl groups of IPL: C16:1	8
PE	688.7; 690.7	141		C16:1**	27–29	1, 2		Di-acyl groups of IPLs: C14 - C18,	9, 10, 11, 12, 13, 14, 15, 16
PME	758.7	155		C18:1**	27–29	This study	(Gram-negative) bacteria, SRB, methanotrophic bacteria	First major occurence in sub-oxic water	
PDME	718.7; 744.5	169		C16:1 C18:1**	27-29	This study			
Diether-PE	676.9; 688.9; 692.2	43		C16:1 C17:0 C18:1**	26	1	SRB	Di-alkyl IPLs & C_{17} -PLFAs	13
PG	736.5 $[M+NH_4^+]^+$	189		C16:0 C16:1**	25–27	1, 2	Methanotrophic bacteria	IPL-abundance, PLFA-pattern & δ^{13}[PLFA]	16
DPG	1345.9 $[M+NH_4^+]^+$	n.d.***	n.d.***	C16:0 C16:1**	26–28	1	Bacteria, green sulfur bacteria	Di-acyl groups of IPLs: C16 - C18	10, 11, 27
PC	706.5; 758.7; 786.7		184	C16:0 C16:1 C18:1**	29–31	1, 2	Bacteria	Di-acyl range of IPLs: C14 - C18	10, 11, 15
Ornithines	623.7; 651.7		333; 351; 377	C16:1 C18:1*	29	28	Gram-negative (positive) bacteria, anoxygenic phototrophs, SRB	First occurence in sub-oxic water	17, 18, 19, 20, 21
HPLC-APCI-MS^n BHP									
BHT	655 $[M+H-AcOH]^+$	60	595; 535		31.6	3	*Geobacter*, methanotrophs,		22, 23, 24, 25, 26
Aminotriol	714 $[M+H]^+$	60	654; 594; 534		30.6	3	*Thiobacillus*, *Desulfovibrio*		25, 26

(1) Sturt et al., 2004, (2) Rütters et al., 2002, (3) Talbot et al., 2001, (4) Kato et al., 1996, (5) Sanina et al., 2004, (6) Awai et al., 2001, (7) Joyard, 1996, (8) Batrakov et al., 1996, (9) Christie, 2003, (10) Fang et al., 2000a, (11) Goldfine, 1984, (12) Makula, 1978, (13) Rütters et al., 2001, (14) Wilkinson, 1988, (15) Zink and Mangelsdorf, 2004, (16) Fang et al., 2000b, (17) López-Lara et al., 2003, (18) Aygun-Sunar et al., 2006, (19) Imhoff et al., 1982, (20) Gorchein, 1968, (21) Makula and Finnerty, 1975, (22) Pearson et al., 2007, (23) Kannenberg and Poralla, 1999, (24) Fischer et al., 2005, (25) Blumenberg et al., 2006, (26) Talbot and Farrimond, 2007, (27) Immhoff and Immhoff, 1995, (28) Aygun-Sunar et al., 2006.

* Identified in positive ionization mode.
** Identified in negative ionization mode.
*** Ions diagnostic for 3 $C_{16:1}$ and 1 $C_{16:0}$ FAs detected.

2.7. DNA extraction

Simultaneous RNA and DNA extraction from the HBHA filters was performed using the Qiagen RNA/DNA Max kit as per the instructions with minor adjustments. Briefly, filters were broken into small pieces, thawed on ice and then digested with lysozyme (15 mg ml^{-1} in 3 ml TE buffer, pH 8.0) for 10 min at room temperature in 50 ml conical tubes. Qiagen buffer QRL1 (3 ml) was added and samples were then sonified with a Branson 450 sonifier for 30 s and vortexed. The resulting suspensions were then transferred away from the original filters to a clean tube to complete the extractions. Extracted DNA was quantified on a NanoDrop ND-1000 spectrophotometer and stored at −20 °C until use in PCR experiments.

2.8. Polymerase chain reactions

Polymerase chain reactions (PCR) for diagnostic enzymes of sulfate reduction and methanogenesis were performed using previously characterized primers. The primers DSR1F and DSR4R were used to target the dissimilatory sulfite reductase gene *dsrAB* (Perez-Jimenez and Kerkhof, 2005). The alpha subunit of methyl-coenzyme M reductase was targeted with universal *mcrA* primers ME1 and ME2 for the *mcrA* group a and c–e (Hales et al., 1996) and the *mcrA* group b was targeted with specific primer pair AOM39_F and AOM40_R (Hallam et al., 2003). PCR protocols were identical for all primer sets. Amplifications were performed in 30 μl volumes containing a 200 μM concentration of each dNTP, 1× PCR buffer, 5 mM $MgCl_2$, 50 ng of each primer, 2.5 U of *Taq* polymerase (Qiagen), and 50 ng of sample DNA. PCR conditions were: denaturation for 5 min at 94 °C followed by 30 cycles of 55 °C for 30 s, 72 °C for 90 s, and 94 °C for 30 s with a final elongation step at 72 °C for 10 min.

PCRs specifically targeting the 16S rRNA gene from anaerobic methane oxidizing Archaea groups ANME1 and ANME2 and the *Desulfosarcina–Desulfococcus* ANME associated SRB were performed (October 2004 nitrocellulose filters) using previously published primers. The universal Archaea primer A20_F was used in combination with ANME1–862 or ANME2 targeted EelMSMX932 and the universal bacterial primer 27F in combination with DSS658 (Orphan et al., 2002). The PCR protocol was identical to that listed above for all primer combinations.

2.9. Cloning, sequencing and phylogenetic analysis

Positive PCR products from the above reactions were used to generate clone libraries using Qiagen PCR cloning kit (#231124) as per the manufacturer's instructions. Positive clone cultures were selected and 2 ml of each were used to purify plasmid DNA using QIAprep Spin Miniprep kit (#27106) and sequenced by the Dana-Farber/Harvard Cancer Center DNA Resource Core using the M13 sequencing primer. Sequences were then compared to known sequences for each gene using BLAST (National Center for Biotechnology Information) and aligned with similar sequences using Genetics Computer Group (GCG). Phylogenetic trees were constructed in PAUP* 4.0 using maximum parsimony analysis with 100 bootstrap replicates.

2.10. Direct cell counts

Direct cell counts of water samples from October 2004 nitrocellulose filters were made using the acridine orange protocol (Francisco et al., 1973). Samples were treated for 2 min with acridine orange (final concentration, 5 mg l^{-1}) and were filtered onto black Nuclepore polycarbonate 0.2 μm filters. Filters were washed twice with 3 ml of sterilized Milli-Q water, mounted on microscope slides for oil immersion, and analyzed using epifluorescence microscopy on a Zeiss Axioskop. For each slide, 10 microscope fields were observed and counted with an average of 200 cells counted per filter.

3. Results

3.1. Geochemical conditions

Measurements of geochemical parameters revealed a clear vertical zonation of the HBHA water column. In September 2004 (not shown) and at the time of IPL/RNA sampling (October 2004), the epilimnion was well oxygenated to a water depth of 2.07 m (Fig. 1), whereas on November 22, 2004, the epilimnion was only 1.50 m thick (data not shown). Dissolved oxygen (DO) was significantly depleted but present below 2.07 m water depth. The metalimnion between 2.07 and 2.51 m was indicated by a sudden drop in DO and the increasing specific conductivity with depth (Fig. 1). The anoxic bottom water formed the hypolimnion below 2.51 m with highest specific conductivity values (Fig. 1). Benzene and methane concentrations in the hypolimnion were as high as 720 nM and 380 μM, respectively (Fig. 1). These hydrocarbons declined steeply across the metalimnion and were nearly absent in the epilimnion (methane at ∼2 μM and benzene at ∼6 nM, Fig. 1).

The sulfate concentration in the hypolimnion was 11 mM in October 2004. A vertical sulfate profile was measured on November 22, 2004 revealing an increase from 0.62 mM to 12.9 mM from the epilimnion towards the anoxic hypolimnion (Fig. 1).

3.2. Diversity of intact polar lipids

The relative and absolute abundances of individual IPLs and IPL classes at HBHA varied with water depth (Figs. 2 and 3). In the epilimnion, the most abundant IPLs were betaine ether linked lipids (Fig. 2A, cf. Appendix A for structures). Betaine lipids are glycerol based complex lipids. Their polar headgroups do not contain phosphate, but instead they have a positively charged trimethyl ammonium group and the amino acids alanine or serine (Kato et al., 1996). Two of three known betaine lipids elute together as a cluster of 1,2-diacylglycerol-*O*-4′-(*N*,*N*,*N*-trimethyl)homoserine (DGTS) and 1,2-diacylglyceryl-*O*-2′-(hydroxymethyl)-(*N*,*N*,*N*-trimethyl)-β-alanine (DGTA) that both show the characteristic headgroup ion of 236 Da

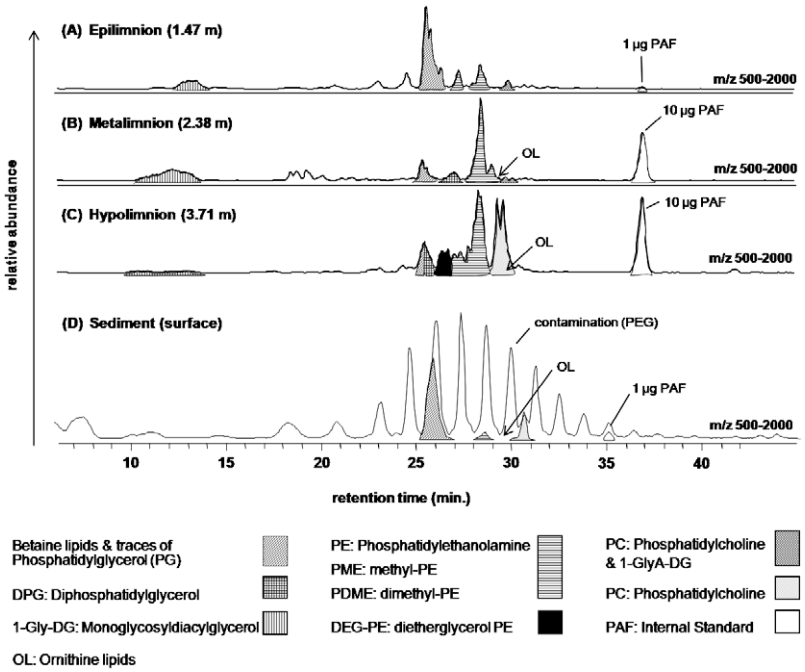

Fig. 2. Positive ion base peak chromatograms of phospholipid fractions of HBHA samples showing the variation of IPLs through the water column and surface sediment. The internal standard was added in different amounts prior to extraction (1 or 10 µg). The sediment sample showed contamination of polyethyleneglycol (PEG) that caused a symmetric pattern of peaks. To visualize the IPL content of the surface sediment, mass chromatograms of individual lipid protonated molecules (in Daltons: betaine lipids: 708.8; 732.9; 734.9; 736.9; 738.9; 732.8; 760.8; 764.8; PE: 688.6; 690.2; PC: 786.9; 788.6) were integrated.

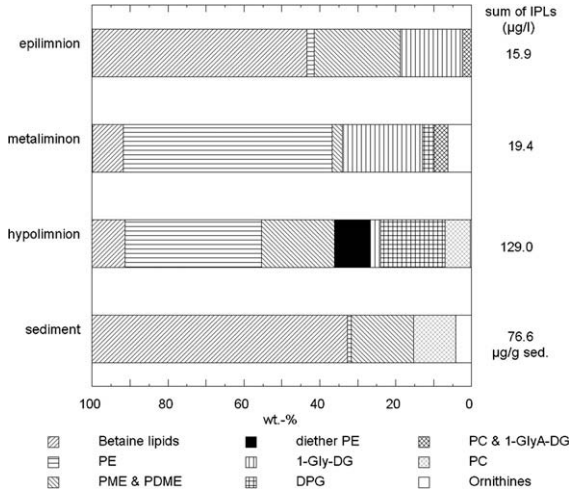

Fig. 3. Relative and absolute concentrations of IPLs at HBHA.

and lyso fragments of 500 and 482 Da representing $C_{18:1}$ fatty acids attached to the glycerol backbone (Table 1).

The relative abundance of betaine lipids was 57% of a total of 15.9 µg IPLs per liter (Fig. 3). Phosphatidylethanolamine (PE) was the second most abundant IPL group in the epilimnion (23%). Its methylated derivatives, phosphatidyl(*N*-methyl)ethanolamine (PME) and phosphatidyl(*N*,*N*-dimethyl)ethanolamine (PDME), accounted for 2% (Fig. 3). The glycolipid monoglycosyldiacylglycerol (1-Gly-DG or MGDG) amounted to 16% of epilimnic IPLs. A further glycolipid, glycuronic acid dialkylglycerol (1-GlyA-DG), carrying an additional carboxyl moiety in the headgroup structure (Batrakov et al., 1996; Hölzl and Dörmann, 2007), co-eluted with phosphatidylcholine (PC); both represented a minor group of total IPLs in the oxygenated epilimnion (2%) (Fig. 3).

The concentration of IPLs increased slightly to 19.4 µg l^{-1} in the metalimnion (Fig. 3). The relative abundance of betaine lipids decreased significantly with depth to 8%, whereas PE, PME and PDME increased considerably (58%). Ornithine lipids (OL), a phosphorus-free membrane lipid (e.g. Gorchein, 1968; Lopez-Lara et al., 2003) and diphosphatidylglycerol (DPG) were not detected in the oxic surface layer but were found in the metalimnion (6% and 3%, respectively) (Figs. 2 and 3). DPG signals indicate four C_{16} acyl side chains attached to the headgroups (Table 1). 1-Gly-DG and 1-GlyA-DG were similarly abundant as in the epilimnion, whereas the PC signal was stronger. Phosphatidylglycerol (PG) was recorded in trace amounts.

In the anoxic hypolimnion, the diversity and absolute abundance of IPLs was largest (Figs. 2 and 3). Total concentrations of IPLs were almost an order of magnitude higher than values within the epi- and metalimnion (129 µg l^{-1}; Fig. 3). Diacyl IPLs, such as PE, DPG and PDME were most abundant (36%, 17% and 14%, respectively), followed by PC (7%) and 1-Gly-DG (3%) (Fig. 3). The occurrence of PE based dialkylglycerolethers (9%) is exclusive to the anoxic bottom waters. Ornithines were present at low concentration (0.5 µg l^{-1}).

IPL diversity within the surface sediment sample was less complex than in the water column with total IPL concentration of 73.4 μg per g dry sediment (Figs. 2 and 3). Betaine lipids were dominant (70%). PME/PDME accounted for 17%. PC, ornithines and PE were as low as 12%, 3% and 1%, respectively (Fig. 3).

At HBHA, no Archaeal IPLs such as archaeol, hydroxy-archaeol, glyceroldialkylglyceroltetraethers (GDGTs) or glyceroldialkylnonitoltetraethers (GDNTs) with one or more sugar moieties and ring structures (e.g. Sturt et al., 2004; Rossel et al., 2008) were detected in either the glyco- or phospholipid fractions of all depth horizons. A certain loss of Archaeal cells during sampling with a 1 μm filter and expected low diameters of Archaeal cells (e.g. Könneke et al., 2005) cannot be excluded.

3.3. Acyl and alkyl chains of IPLs

Throughout the water column and in surface sediment at HBHA, the chain lengths of acyl and alkyl moieties attached to the glycerol backbones of each IPL ranged between 14 and 21 carbon atoms. In general, monounsaturated acyl chains were more common than saturated ones. The most abundant acyl substituents were $C_{16:1}$ and $C_{18:1}$. Polyunsaturated acyl substituents were rare.

3.4. BHPs

Trace amounts of BHPs were identified only in surface sediments and in the metalimnion (Table 2). Bacteriohopane-32,33,34,35-tetrol (BHT) was identified in both samples and 35-amino-bacteriohopane-32,33,34-triol (aminotriol) was abundant in the metalimnion. Both compounds were identified by their base peaks and characteristic product ions in MS^n (Talbot et al., 2001). Samples from the epilimnion and hypolimnion did not contain detectable levels of BHPs under the given analytical conditions (Table 2).

Table 2
Vertical distribution of BHPs

Water depth	Aminotriol	BHT
Epilimnion (1.25 m)	n.d.	n.d.
Metalimnion (2.30 m)	√	√
Hypolimnion (2.75 m)	n.d.	n.d.
Hypolimnion (3.75 m)	n.d.	n.d.
Sediment surface	√	n.d.

3.5. PLFAs

HPLC results of core lipids were in good agreement with GC–MS data of PLFAs. The epilimnion showed the lowest range and the lowest concentrations of +PLFAs (2.5 μg l^{-1}) (Fig. 4). All deeper horizons indicated the same range of PLFAs with significant differences in absolute abundances of individual compounds (Fig. 4A). PLFAs were six times more concentrated in the metalimnion (15.3 μg l^{-1}) than the epilimnion, but they were only slightly higher than in the hypolimnion (11.3 μg l^{-1}). The sediment revealed PLFAs at 38.6 μg g^{-1} dry sediment. Certain PLFAs increased significantly from the metalimnion to the hypolimnion, such as *iso*-$C_{17:0}$ and *anteiso*-$C_{17:0}$ whose concentrations increased by a factor of ~30 to 50 to 260 and 280 ng l^{-1}, respectively, in the hypolimnion. Other PLFAs were most abundant in the metalimnion (e.g. $C_{16:1\omega7}$: 2900 ng l^{-1}) (Fig. 4A).

3.6. $\delta^{13}C$ values of PLFAs and other carbon species

PLFAs of the epilimnion were least depleted in ^{13}C (−23.3‰; reported as weighted means of C_{14} to C_{18} PLFAs) compared to the metalimnion (−27.6‰), hypolimnion (−26.1‰) and surface sediment (−24.9‰) (Fig. 4B). For specific fatty acids, the epilimnion contained the most ^{13}C-enriched PLFAs ($C_{16:0}$: −22.4‰; $C_{18:1\omega9}$: −20.4‰), while the metalimnion exhibited the most depleted PLFAs ($C_{16:1\omega9}$: −36.1‰; $C_{16:1\omega7}$: −32.5‰), indicative of the utilization of isotopically distinct carbon substrates at the oxy-

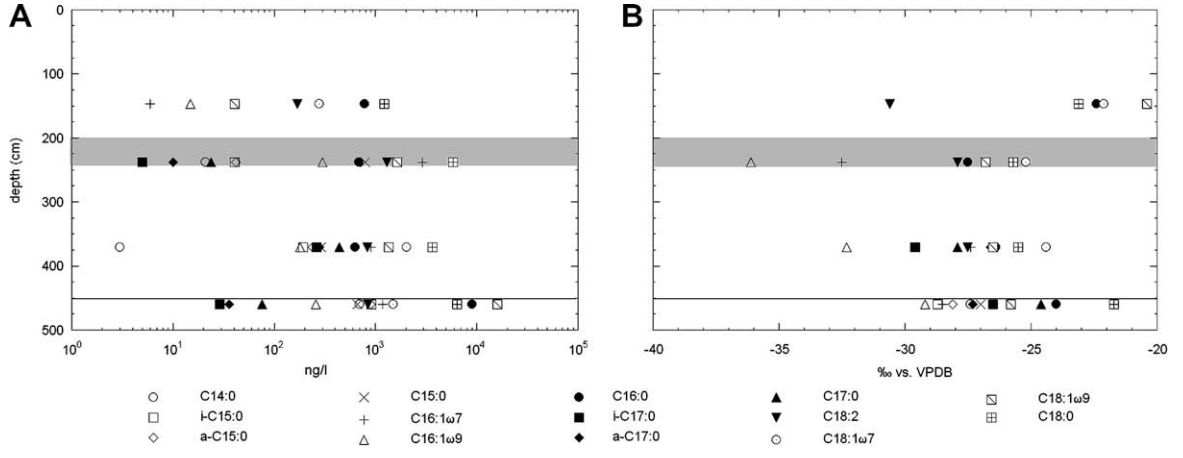

Fig. 4. (A) Absolute abundance and (B) $\delta^{13}C$ values of C_{14} to C_{18} PLFAs in the water column and surface sediments of HBHA (horizontal line at ca. 450 cm represents the sediment water interface).

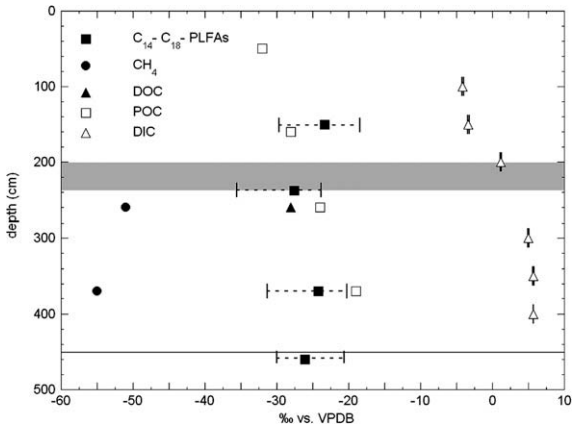

Fig. 5. Stable carbon isotopic signatures of various carbon species in the epi-, meta- and hypolimnion and sediment of HBHA. δ values of PLFAs are weighted averages of C_{14} to C_{18} PLFAs of each individual lake horizon (error bars denote range of values of individual compounds; horizontal line at ca. 450 cm represents the sediment water interface).

cline. In contrast, the latter compounds were enriched in ^{13}C in deeper layers (Fig. 4B).

Dissolved methane was slightly enriched in ^{13}C in the metalimnion compared to the hypolimnion (−50.7‰; −55.1‰, respectively) (Fig. 5). $\delta^{13}C$ values of particulate organic carbon (POC) increased steadily from top to bottom by about 13‰ from −32 to −18‰. DIC was as low as −4.7‰ in the oxic waters, showing a trend towards positive values at the metalimnion (−3.3‰ and +1.2‰ before reaching constant values of +5.7‰ in the hypolimnion (Fig. 5).

3.7. Molecular microbial ecology

PCRs using DNA from the October 2004 sampling of the hypolimnion were performed with the primer set DSR1F and DSR4R as a proxy for SRBs. A product of the expected size (~1.9 kb) of the *dsrAB* gene was purified and used for cloning and sequencing. Twenty positive clones were selected for sequencing and five unique clone sequences of ~500 bp of the *dsrAB* gene were found. These sequences were translated to corresponding amino acids and aligned with closely related sequences identified by BLAST searches of the GenBank database. The five retrieved sequences appeared to represent a diverse set of SRB phylotypes which fell into four distinct clades (DSR Clusters I–IV) (Fig. 6). The first phylotype (DSR Cluster I) was most closely related to environmental *dsrAB* sequences from an acidic fen and a leachate polluted aquifer (Wu and Yang, unpublished data, GenBank). The second phylotype (DSR Cluster II) was found to be closely related to other sequences from these same two environments as well as *Desulfobacter acetoxidans*. The third phylotype (DSR Cluster III) clustered within the genus *Desulfomicrobium* and the fourth and fifth phylotypes (DSR Cluster IV) had no known cultured relatives but grouped with another set of *dsrAB* sequences from a polluted aquifer. No sequences from the *Desulfosarcina–Desulfococcus* group, the group associated with AOM (e.g. Hinrichs and Boetius, 2002) were retrieved.

To evaluate the diversity of methanogenic Archaea and the potential for AOM in the HBHA, PCR experiments were also run using with the universal *mcrA* primer set ME1 and ME2 as well as the ANME-specific AOM39_F and AOM40_R primer pair. Additionally, subsequent PCRs were run with varying combinations of universal and specific primers. Only the universal primer combination, ME1 and ME2, produced a product of the correct size. This product was purified and cloned; 20 positive clones were selected for sequencing. Five unique clone sequences of ~800 bp were identified and used for BLAST searches, sequence alignments, and phylogenetic analysis of the corresponding amino acid sequences (Fig. 7). Five phylotypes were amplified which cluster (HBHA *mcrA* Cluster) within the resultant *mcrA* tree and appeared to be most closely related to a set of environmental sequences presumed to originate from previously uncultured methanogens from a hypereutrophic lake (Earl et al., 2003). No sequences related to the ANME1 or ANME2 groups – which are most commonly credited with AOM – were found.

PCR experiments with primer combinations specifically targeting the 16S rRNA of both of SRB and methanogenic Archaea associated with AOM failed to produce any product from the October 2004 HBHA hypolimnion sample. Subsequently, no clone libraries were constructed for these experiments.

3.8. Bacterial abundance

At HBHA, direct counts of bacterial cells and the distribution of IPLs reflect significant differences of microbial biomass in the distinct lake horizons (Table 3). Bacterial cell numbers increased with water depth. Cell concentration was lowest in the epilimnion (3.6×10^6 cells ml^{-1}), increased slightly at the metalimnion (4.3×10^6 cells ml^{-1}) and reached the highest value in the hypolimnion (37.0×10^6 cells ml^{-1}) (Table 3). IPL concentrations showed the same trend throughout the water column (Table 3).

4. Discussion

Geochemical and molecular microbial methods were used to address the vertical stratification of microbial lipids and metabolisms throughout the ecosystem of HBHA. Comparison of isotopic compositions of various carbon pools, including biomass, suggest a complex network of reactions and carbon assimilation pathways within distinct layers of the meromictic lake.

4.1. Geochemical zonation at HBHA

The geochemical parameters indicated an epi- and metalimnion that varied in depth and thickness year round (cf. Wick et al., 2000). At the time of sampling (October 2004), the oxic-anoxic interface was ~40 cm thick and located between 2.07 and 2.51 m water depth with minor amounts of oxygen present. Vertical mixing and microbial degradation at the metalimnion were reported to be the major sinks for benzene (Wick et al., 2000), as suggested

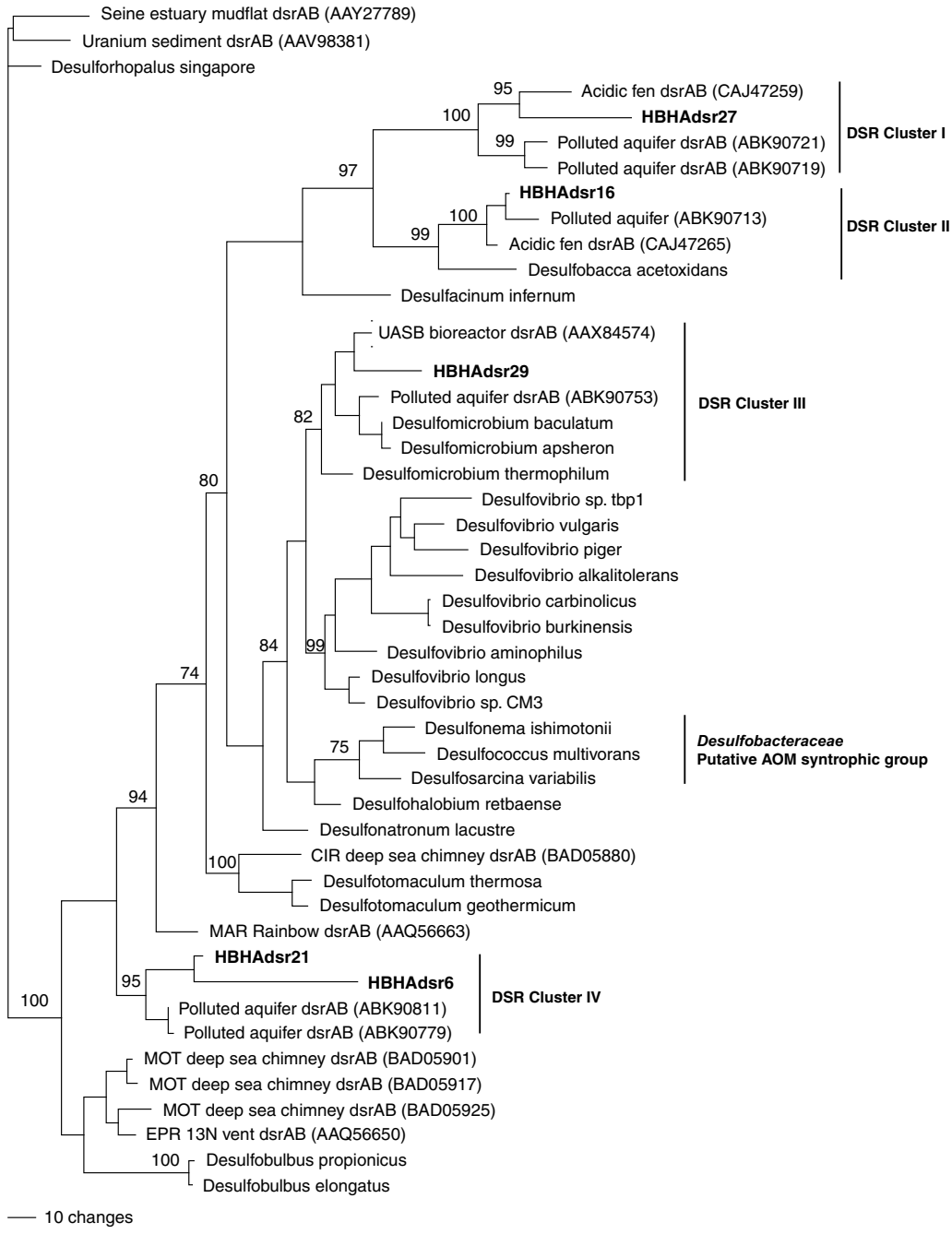

Fig. 6. Phylogenetic tree based on amino acid sequences of the dissimilatory sulfite reductase gene *dsrAB* using maximum likelihood analysis with 100 bootstrap replicates. Bootstrap values >75 are reported. DSR Cluster I-IV and *Desulfobacteracea*, i.e., the genera syntrophically involved in AOM, are shown. The GenBank accession numbers for *dsrAB* sequences are EF210715–EF210719.

by the geochemical profiles (Fig. 1). Anoxicity and high concentrations of methane and sulfate in the hypolimnion suggest conditions conducive for AOM in the HBHA.

4.2. Taxonomic significance of IPLs

The diversity of IPLs and their distribution at HBHA (Figs. 2 and 3) suggest both a metabolic and taxonomic diversity in the lake that varies between layers. The presence of betaine ether-linked glycolipids is linked to the activity of photoautotrophic algae (Table 1) in agreement with chlorophyll concentrations and fluorescence within the water column (Southworth, 1999, and this study). Betaine lipids are known as cell membrane components of eukaryotic marine algae and macrophytes (Kato et al., 1996; Sanina et al., 2004). More specifically, DGTS and

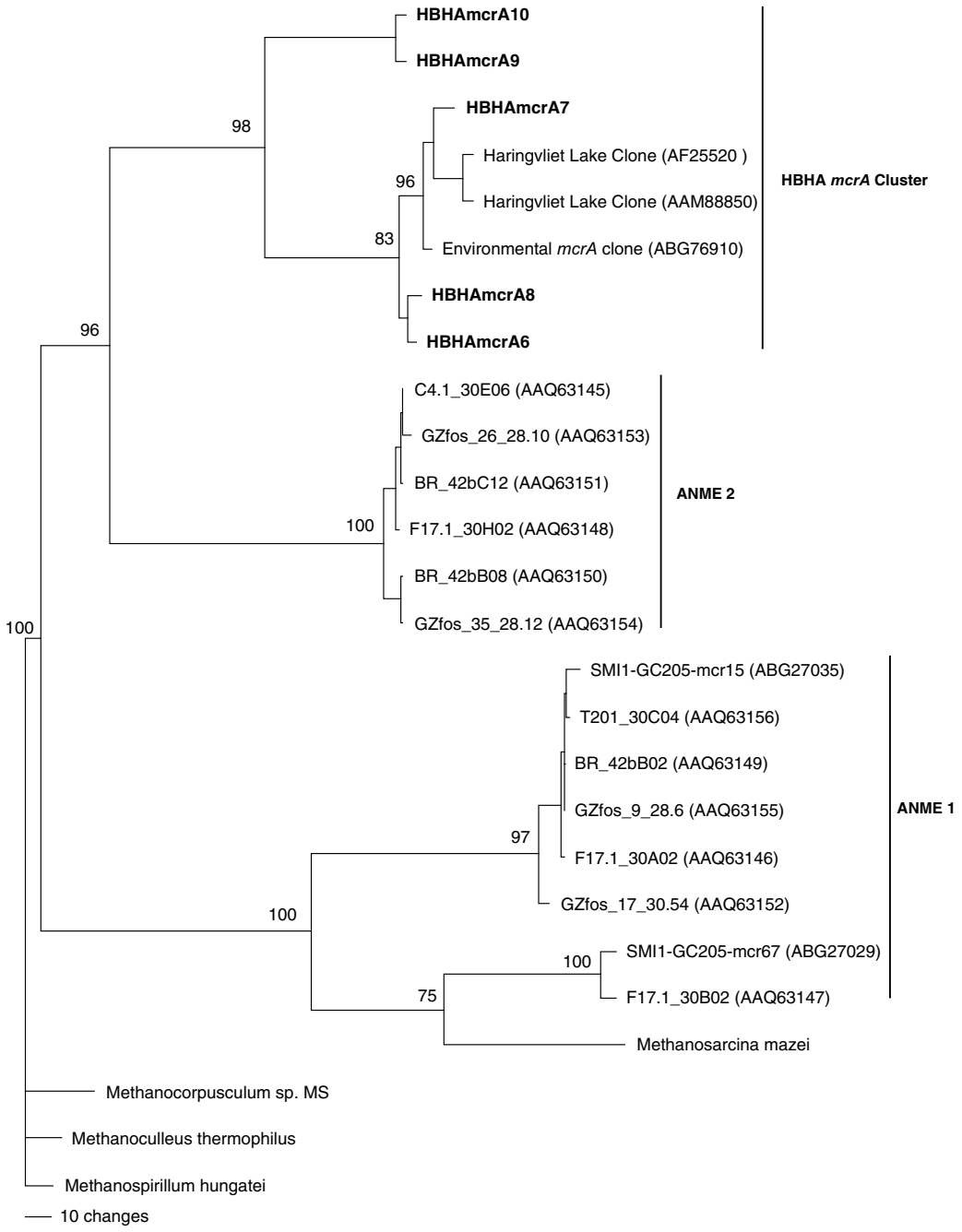

Fig. 7. Phylogenetic tree based on amino acid sequences of the methyl-coenzyme M reductase gene *mcrA* using maximum likelihood analysis with 100 Bootstrap replicates. Bootstrap values >75 are reported. The HBHA *mcrA* cluster is shown as well as the AOM associated Archaea ANME1 and ANME2 groups (October 2004 samples). The GenBank accession number for *mcrA* sequences is EF210720–EF210724.

DGCC were found in green algae and the haptophycea *Pavlova lutheri* (Kato et al., 1996). In bacteria, betaine lipids are rare. They have been found in the photosynthetic prokaryote *Rhodobacter sphaeroides* when cultured under phosphate-limiting conditions (Benning et al., 1995), in the firmicute *Desulfotomaculum putei* and in the α-proteobacterium *Agrobacterium tumefaciens* (Hinrichs et al., unpublished data). Similarly, the glycolipid 1-Gly-DG is attributed to photoautotrophic sources (Joyard et al., 1996) but unlike for the betaines, structural properties such as the presence of $C_{15:0}$ and $C_{16:1}$ acyl chains indicate bacterial sources for these IPLs (Awai et al., 2001). By contrast, eukaryotic 1-Gly-DG is characterized by two $C_{18:3}$ and $C_{16:3}$ acyl substituents, e.g., in peas or spinach, which are designated as 16:3 and 18:3 plants, respectively (e.g. Joyard et al., 1996; Awai et al., 2001). The lack of further

Table 3
Total concentrations of IPLs and directly counted prokaryotic cells

Depth (m)	Cell counts ($\times 10^6$ cells ml^{-1})	Total IPLs (μg l^{-1})	Bacterial IPLs	
			(μg l^{-1})	(%)
1.47 (oxic)	3.6 ± 2.5	15.9	6.8	43
2.38 (sub-oxic)	4.3 ± 1.2	18.2	16.6	92
3.71 (anoxic)	37 ± 11.0	128.5	105.4	91
4.50 (sediment)	–	73.4*	22.0*	33

Relative contributions of bacterial IPLs to the viable biomass at HBHA (October 2004) derived from total IPL concentrations excluding betaine lipids. IPLs were classified as bacterial IPLs based on the carbon number of hydrophobic side chains typical for bacteria and the eukaryotic origin of betaine lipids (see discussion for details).
* IPL concentration in μg g^{-1} dry sediment.

glycolipids, such as diglycosyldiacylglycerol (2-Gly-DG) or sulfoquinovosyldiacylglyceride (SQ-DG), as seen for green, brown and red algae with PUFA-dominated FA-patterns (Khotimchenko, 2002) add further support for a bacterial source of the lake's 1-Gly-DG (Table 1).

PG, DPG, PC and PE/PME/PDME were interpreted as being largely of bacterial origin, based on the structural information of their acyl side chains with carbon atom numbers ranging from 14 to 19 (Table 1). These IPLs also have been described from sediments of the Marianas Trench (Fang et al., 2000a), Lake Lienewitz (Brandenburg, Germany) and Lake Baikal (Zink and Mangelsdorf, 2004). The barophilic bacteria of the Marianas Trench contained the same range of IPLs, whereas PG was only present in trace amounts at HBHA but abundant in Lake Lienewitz and Lake Baikal sediments. The low concentrations of PG, one of the major lipid species in bacteria (present in 57 out of 74 environmentally relevant bacterial species cultured and profiled for its IPL content, Hinrichs et al., unpublished data) suggests that a few species that lack PG contribute strongly to the bacterial biomass pool. Assuming a bacterial origin of PE (Christie, 2003) in the meta- and hypolimnion, these dominant bacteria are likely producers of PE but not of PG. PE is a dominant cell constituent of gram-negative bacteria (Wilkinson, 1988), and the most abundant phospholipid type in many SRB (Rütters et al., 2001). In our own collection of environmental prokaryotes that have been systematically screened for their IPL inventory, PE is the second most prominent bacterial polar lipid type (present in 52 out of 74 bacterial cultures, Hinrichs et al., unpublished data). In this collection, only a few bacteria produce PE but not PG (eight of 52 bacterial PE producers, three of which are thermophiles; Hinrichs et al., unpublished data). Among these is an isolate of *Rhodococcus* sp., affiliated with some of the best-studied polychlorinated biphenyl and benzene degraders (Masai et al., 1995), and *Sporomusa silvacetica*, an acetogenic bacterium growing on wide variety of low-molecular-weight organic substrates (Kuhner et al., 1997). PC is a major eukaryotic IPL; it is found in membranes of only ~10% of bacterial species (Sohlenkamp et al., 2003). However, its vertical distribution and fatty acids distribution suggest a bacterial origin.

OL are widespread among gram-negative bacteria and described for some gram-positive bacteria (e.g. Lopez-Lara et al., 2003, and references therein). They can be good indicators for anoxygenic phototrophic bacteria. Photoautotrophic organisms capable of oxygenic and anoxygenic photosynthesis are known to synthesize OL, such as purple bacteria *Rhodobacter capsulatus* (Aygun-Sunar et al., 2006), or non-sulfur purple bacteria *Rhodospirillaceae* (Imhoff et al., 1982). OL are attributed to the anoxygenic photoautotrophic species *Rhodopseudomonas* (Gorchein, 1968), and to sulfate reducing *Desulfovibrio gigas* (Makula and Finnerty, 1975). Oxidizers of iron and inorganic sulfur compounds, such as *Thiobacillus* and chemolithotrophic/chemoorganotrophic *Achromobacter* species with the capability of anoxygenic denitrification contained OL (Thiele et al., 1984). Strains of green non-sulfur *Chloroflexi* and green sulfur *Chlorobium* in deeper layers of the lake were evident in 16S rRNA analysis of surface sediment (A. Pearson, unpublished data). Both organisms are known to fix carbon photoautotrophically under anoxic conditions (e.g. Sprague et al., 1981; Cork et al., 1985). *Chlorobium* is commonly found in anoxic zones of eutrophic lakes (Prescott et al., 2005). Furthermore, strains of *Thiobacillus* in lake sediment (data not shown) most likely settled from the water column, in accordance with an intense microbial iron oxidation at the metalimnion (cf. Diez et al., 2007). The first occurrence of DPG is consistent with green sulfur bacteria as likely sources (Imhoff and Bias-Imhoff, 1995).

4.3. Vertical cell distribution

Abundance of IPLs and bacterial cell counts increase with water depth (Table 3). The high relative proportions of bacterial IPLs to the total biomass (Table 3) are consistent with an ecosystem dominated by bacteria with a high number of cells. The distinction of bacterial vs. total IPLs is based on the eukaryotic origin of betaine lipids (algae) and the carbon number of hydrophobic side chains of IPLs typical for bacteria (compare discussion above). Other limnic environments, such as oligotrophic Gossenkoellesee (Tyrol, Austria) revealed stable densities of bacteria over time and depth with cell concentrations of 5.5×10^5 cells ml^{-1} (Wille et al., 1999), whereas bacteria in eutrophic Lake Lucerne and Lake Sempach were heterogeneously distributed over depth with cell numbers ranging from 0.7 to 5.2×10^6 cells ml^{-1} (Friedrich et al., 1999). Elevated microbial standing stocks and bacterial chemical turnover in redox transition zones have been documented for stratified marine water bodies of basinal environments, such as the Black Sea (e.g. Wakeham et al., 2003; Lin et al., 2006), and the Cariaco Basin, where within a depth interval of 240–450 m water depth, the steep gradient in redox potential is associated with significant new production of organic carbon (Taylor et al., 2001). Bacterial chemoautotrophy, fueled by reduced sulfur species, is proposed as primary energy source in those marine chemoclines. A similar scenario is conceivable in the metalimnion of HBHA as indicated by the sulfate profile (Fig. 1), the highest relative contribution of bacterial IPLs to total biomass (Table 3), and comparisons of isotopic relationships between PLFAs, DIC and POC (discussed in Section 4.4). The highest concentration of IPLs and cells in the hypolimnion (Table 3) is consistent with intense microbial metabolism and associated biomass production.

The observed similarity of the IPL profiles in the sediment and epilimnion (Figs. 2 and 3), and especially the betaine lipids, may be due to (a) their relatively high stabilities because their headgroups are linked via an ether bond to the glycerol backbone while the other IPLs are linked via glycosidic or phosphate ester bonds, or (b) the main flux of betaine lipids to the sediment takes place via rapidly settling particles.

4.4. Microbial carbon fixation at HBHA

The high concentrations of methane suggested the activity of methanogens in the hypolimnion, the underlying sediments and/or an input via groundwater flowing through the lake. The high $\delta^{13}C$ values of DIC in the hypolimnion may imply that methane was generated by CO_2 reduction. However, mass balance studies of methane cycling in the HBHA reveal that methane concentrations in the hypolimnion follow changes in specific conductance of that water, suggesting that methane is chiefly derived from the incoming groundwater (McNeill and Gschwend, unpublished data). Nonetheless, evidence for the presence of methanogenic Archaea in the HBHA hypolimnion was provided by five unique *mcrA* phylotypes (HBHA *mcrA* Cluster) (Fig. 7). These methanogens were most likely derived from methane-rich groundwater entering the lake from the subsurface since in situ activity of hydrogenotrophic and acetoclastic methanogenesis will be limited by competition with SRBs (Froelich et al., 1979; Hoehler et al., 2001). Based on the relative concentrations of IPLs and their less polar derivatives, Archaea contribute, at the most, a minor fraction to the standing stock of microbial biomass in the hypolimnion.

Three *mcrA* clones, HBHA*mcrA*6, HBHA*mcrA*7 and HBHA*mcrA*8, grouped with clones found in a hypereutrophic lake, Priest Pot (Earl et al., 2003) while HBHA*mcrA*9 and 10 were closely related to the other HBHA clones. None of the clones were found to be closely related to any named methanogens suggesting these *mcrA* sequences are unique lineages and it remains unknown if they represent methanogenic or methanotrophic Archaea. While the geochemical profiles (Figs. 1 and 5) do not rule out AOM as methane sink in the hypolimnion, both the carbon isotopic composition of DIC and the concentration profile of methane clearly point to the metalimnion as the major sink of methane.

Methanotrophy in the oxycline was indicated by the ^{13}C enrichment of methane at the metalimnion, the production of ^{13}C depleted CO_2 in the oxycline (Fig. 5), and the maximum isotopic depletion of certain PLFAs at the metalimnion (Fig 4). Although the molecular genetic data suggested a relatively constant population density of methanotrophic bacteria throughout the water column of HBHA, the concentration of 16S rRNA per methanotroph cell was highest at about 16 fg cell^{-1} in the metalimnion (Fisher, 2007). These molecular data implied that methanotrophs affiliated with the α- and γ-proteobacterial subdivisions were metabolically active in the water column of HBHA, and particularly at the metalimnion.

This may be consistent with the highest diversity of both δ- and γ-proteobacterial squalene-hopene cyclase gene (*sqh*C) sequences that was observed in a previous study (Pearson et al., 2007). The most common clone among the *sqh*C sequences was an apparent relative of *Methylococcus capsulatus* or other γ-proteobacterial aerobic methanotrophs. The second most abundant group of clones was a diverse cluster of *sqh*Cs related to iron- or sulfate-reducers within the δ-proteobacteria (*Geobacter* spp.) (Masai et al., 1995). The BHPs observed in the metalimnion (Table 2) were consistent with the *sqh*C genes found at that depth. Bacteriohopaneaminotriol is particularly abundant in type II methanotrophs (Talbot and Farrimond, 2007) and tetrafunctionalized BHPs, potentially including both BHT and aminotriol, have been described for *Geobacter* species (Härtner et al., 2005; Fischer et al., 2005).

IPLs detected in the metalimnion can be partly attributed to methanotrophic bacteria. For example, PG, PE and PDME with monounsaturated acyl side chains with 14–17 carbon atoms are common in all types of methanotrophs (Fang et al., 2000b). The most depleted PLFAs $C_{16:1\omega7}$ and $C_{16:1\omega9}$ (−32.5 and −36.1‰; Fig. 4) can be related to type I methanotrophs on the basis of environmental observations of aerobic methanotrophs in the Black Sea water column (Schubert et al., 2006) and PLFA investigations of methanotrophic bacterial species (Hanson and Hanson, 1996, and references therein), such as *Methylomonas methanica*, *Methylomonas rubra* and/or *Methylomicrobium album BG8* showing mostly $C_{16:1}$ PLFAs (Fang et al., 2000b). At Mono Lake, genetic data also showed a dominance of type I methanotrophs (*Methylobacter* as the dominant genus), whereas the variation in methane oxidation activity may correlate with changes in methanotroph community composition (Lin et al., 2005).

The large range of $\delta^{13}C$ values of PLFAs (−20 to −36‰) is consistent with a high diversity of carbon sources assimilated by the microbial communities. The lowest δ values of both bulk PLFAs and individual compounds were observed in the metalimnion (Figs. 4 and 5). Bulk isotopic values of the meta- and hypolimnion were used to estimate the relative importance of methanotrophic processes for microbial biomass production. Under the assumption of assimilating methane with $\delta^{13}C$ values of −55‰ (Fig. 5) into cell's biomass, a mean isotopic value of the hypolimnion (−27.6‰), and a 1-to-1 mixing of methane and other substrates, a contribution of methane to microbial biomass production within the metalimnion of smaller than 5% was calculated. Based on 16S rRNA investigation, the diversity and quantity of methanotrophs in the lake is estimated to range from 0.3% to 10% of total bacterial population per month and includes both, α and γ methanotrophs (Fisher, 2007).

When we examine the isotopic relationships between PLFAs and POC in the various layers of HBHA, the epilimnion stands out, i.e., $\delta^{13}C$ of PLFA is about 4‰ higher than that of POC while the relationship was reversed in deeper layers (Fig. 5). If we assume that POC consists entirely of bacterial biomass, this ^{13}C enrichment in lipids compared to bulk biomass would be indicative of an important role of autotrophic carbon fixation by green non-sulfur bacteria using the 3-hydroxylpropionate pathway for fixation of HCO_3^- or the reductive tricarboxylic acid cycle, but not

indicative for cyanobacteria (cf. Zhang et al., 2004). However, given that (i) the oxic epilimnion was an unlikely habitat for microaerophilic bacteria and (ii) the isotopic composition of PLFA was largely invariable; variations in its relationship with POC appear to be dictated by variable (isotopic) compositions of POC.

Heterotrophic carbon assimilation seems to be important for the anoxic and sub-oxic zones of HBHA. Benzene is degraded at the metalimnion and perhaps hypolimnion (Fig. 1), possibly by consortia of SRB and/or other gram-negative bacteria (see next section).

4.5. Sulfate reduction in the hypolimnion

Certain IPLs and PLFAs present exclusively in the anoxic hypolimnion strongly indicate the presence of SRB. High concentrations of PE-dialkylethers and C_{17}-PLFAs in the hypolimnion are consistent with an origin from SRB (Wilkinson, 1988; Hinrichs et al., 2000; Rütters et al., 2001; Elvert et al., 2003). The *dsrAB* sequences yielded five distinct phylotypes. The DSR Cluster III phylotype (HBHAdsr29) grouped with members of the genus *Desulfomicrobium*. These bacteria are known to be able to reduce substrates other than sulfate, including heavy metals like chromate and arsenate (Macy et al., 2000) and to incompletely oxidize organic molecules to acetate (Hippe et al., 2003). The closest cultured relative of the clones from DSR Clusters I and II (HBHAdsr27 and HBHAdsr16, respectively) was found to be *Desulfobacca acetoxidans*, a sulfate reducer known to utilize acetate as a carbon source (Oude Elferink et al., 1999). All the phylotypes had related sequences recovered from a leachate-polluted aquifer in east China (Wu and Yang, unpublished data, GenBank). This includes the DSR Cluster IV which had two clones (HBHAdsr6 and HBHAdsr21) that appear to fall outside known *dsrAB* genes and formed a distinct clade with two clones recovered from the leachate polluted aquifer. SRB are known to be able to degrade petroleum based hydrocarbons including benzene, toluene and polycyclic aromatic hydrocarbons; and common biodegrading SRB clones have been shown to be widely distributed in the environment (Perez-Jimenez et al., 2001; Perez-Jimenez and Kerkhof, 2005).

The HBHA receives inputs of benzene and other aromatic contaminants from the adjacent Superfund site, but mass balance studies do not indicate substantial degradation rates in the HBHA hypolimnion (Wick and Gschwend, 1998; Wick et al., 2000). Since the types of sulfate reducers found in the hypolimnion are reported to be capable of utilizing aromatic compounds, this situation implies other factors operate to limit such anaerobic degradation.

5. Conclusions

1. Analyses of IPLs, compound specific carbon isotopes, BHPs and functional gene sequences provided insight into the phylogenetically diverse and metabolically complex microbial community existing in the meromictic HBHA. The chemical stratification of the lake is reflected in the community composition.
2. Bacterial IPLs constitute a significant to major fraction of total IPLs in the epilimnion, metalimnion, hypolimnion and underlying sediment. Archaeal lipids were not detected by HPLC analysis, indicating that Archaeal cell densities were low.
3. The low Archaeal biomass in the hypolimnion implies that both methanogenic and methanotrophic Archaea constitute, at the most, a minor fraction of the bulk microbial community. Given this and the profile of carbon isotopic composition of DIC, the most plausible site for methanogenesis is the underlying sediment and/or aquifer.
4. The major sink for methane appears to be aerobic methanotrophy in the metalimnion. Circumstantial evidence is provided by lowest $\delta^{13}C$ values of $C_{16:1\omega7}$ and $C_{16:1\omega9}$ PLFAs, the vertical distribution of IPLs and PLFAs, the isotope profile of DIC, and the presence of BHPs. The contribution of methanotrophy to bulk carbon assimilation in the metalimnion is lower than ~5%.
5. Reduction of sulfate in the hypolimnion was suggested by *dsrAB* genes, dialkyl IPLs and C_{17}-PLFAs. In the hypolimnion, the large pool of dissolved organic matter could serve as substrates of this sulfate reducing community.

Acknowledgments

This work was supported by the MARUM – Center for Marine Environmental Research, the Hanse Institute of Advanced Study (Delmenhorst, Germany) (financial support of sabbaticals of Colleen Cavanaugh and Philip Gschwend), by US-Grant NSF-EAR-0311937 (Ann Pearson), the US Environmental Protection Agency (EPA) (Meredith Fisher) and the Swiss National Science Foundation (Kai Udert). GenBank accession numbers for reported *dsrAB* sequences were assigned numbers: EF210715–EF210719; for *mcrA* sequences: EF210720–EF210724. We thank O. Sivan, S.R. Shah and D.P. Schrag for help with carbon isotopic analyses, and F. Schubotz for her support in IPL investigation, and the referees for reviewing this work.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.orggeochem.2008.07.009.

Associate Editor—**Brendan Keely**

References

Alain, K., Holler, T., Musat, F., Elvert, M., Treude, T., Kruger, M., 2006. Microbiological investigation of methane- and hydrocarbon-discharging mud volcanoes in the Carpathian Mountains, Romania. Environmental Microbiology 8, 574–590.

Awai, K., Marechal, E., Block, M.A., Brun, D., Masuda, T., Shimada, H., Takamiya, K., Ohta, H., Joyard, J., 2001. Two types of MGDG synthase genes, found widely in both 16:3 and 18:3 plants, differentially mediate galactolipid syntheses in photosynthetic and nonphotosynthetic tissues in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences of the United States of America 98, 10960–10965.

Aygun-Sunar, S., Mandaci, S., Koch, H.G., Murray, I.V.J., Goldfine, H., Daldal, F., 2006. Ornithine lipid is required for optimal steady-state amounts of c-type cytochromes in *Rhodobacter capsulatus*. Molecular Microbiology 61, 418–435.

Batrakov, S.G., Nikitin, D.I., Pitryuk, I.A., 1996. A novel glycolipid, 1,2-diacyl-3-(alfa)-D-glucuronopyranosyl-sn-glycerol taurineamide, from the budding seawater bacterium *Hyphomonas jannaschiana*. Biochimica et Biophysica Acta 1302, 167–176.

Benning, C., Huang, Z.H., Gage, D.A., 1995. Accumulation of a novel glycolipid and a betaine lipid in cells of *Rhodobacter sphaeroides* grown under phosphate limitation. Archives of Biochemistry and Biophysics 317, 103–111.

Biddle, J.F., Lipp, J.S., Lever, M.A., Lloyd, K.G., Sorensen, 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 United States of America 103, 3846–3851.

Blumenberg, M., Kruger, M., Nauhaus, K., Talbot, H.M., Oppermann, B.I., Seifert, R., Pape, T., Michaelis, W., 2006. Biosynthesis of hopanoids by sulfate-reducing bacteria (genus *Desulfovibrio*). Environmental Microbiology 8, 1220–1227.

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

Bühring, S.I., Elvert, M., Witte, U., 2005. The microbial community structure of different permeable sandy sediments characterized by the investigation of bacterial fatty acids and fluorescence in situ hybridization. Environmental Microbiology 7, 281–293.

Castro, H., Reddy, K.R., Ogram, A., 2002. Composition and function of sulfate-reducing prokaryotes in eutrophic and pristine areas of the Florida Everglades. Applied and Environmental Microbiology 68, 6129–6137.

Chang, Y.J., Peacock, A.D., Long, P.E., Stephen, J.R., McKinley, J.P., MacNaughton, S., Saxton, A.M., White, D.C., 2001. Diversity and characterization of sulfate-reducing bacteria in groundwater at a uranium mill tailings site. Applied and Environmental Microbiology 67, 3149–3160.

Christie, W.W., 2003. Lipid Analysis, third ed. The Oily Press, Bridgwater.

Conrad, R., Erkel, C., Liesack, W., 2006. Rice cluster I methanogens, an important group of Archaea producing greenhouse gas in soil. Current Opinion in Biotechnology 17, 262–267.

Cork, D., Mathers, J., Maka, A., Srnak, A., 1985. Control of oxidative sulfur metabolism of *Chlorobium limicola forma thiosulfatophilum*. Applied and Environmental Microbiology 49, 269–272.

Cytryn, E., Minz, D., Oremland, R.S., Cohen, Y., 2000. Distribution and diversity of Archaea corresponding to the limnological cycle of a hypersaline stratified lake (Solar Lake, Sinai, Egypt). Applied and Environmental Microbiology 66, 3269–3276.

Dhillon, A., Teske, A., Dillon, J., Stahl, D.A., Sogin, M.L., 2003. Molecular characterization of sulfate-reducing bacteria in the Guaymas Basin. Applied and Environmental Microbiology 69, 2765–2772.

Diez, S., Noonan, G.O., MacFarlane, J.K., Gschwend, P.M., 2007. Ferrous iron oxidation rates in the pycnocline of a permanently stratified lake. Chemosphere 66, 1561–1570.

Earl, J., Hall, G., Pickup, R.W., Ritchie, D.A., Edwards, C., 2003. Analysis of methanogen diversity in a hypereutrophic lake using PCR-RFLP analysis of *mcr* sequences. Microbial Ecology 46, 270–278.

Eller, G., Kanel, L.K., Kruger, M., 2005. Cooccurrence of aerobic and anaerobic methane oxidation in the water column of lake Plusssee. Applied and Environmental Microbiology 71, 8925–8928.

Elvert, M., Boetius, A., Knittel, K., Jörgensen, B.B., 2003. Characterization of specific membrane fatty acids as chemotaxonomic markers for sulfate-reducing bacteria involved in anaerobic oxidation of methane. Geomicrobiology Journal 20, 403–419.

Elvert, M., Suess, E., Greinert, J., Whiticar, M.J., 2000. Archaea mediating anaerobic methane oxidation in deep-sea sediments at cold seeps of the eastern Aleutian subduction zone. Organic Geochemistry 31, 1175–1187.

Fang, J.S., Barcelona, M.J., Nogi, Y., Kato, C., 2000a. Biochemical implications and geochemical significance of novel phospholipids of the extremely barophilic bacteria from the Marianas Trench at 11,000 m. Deep-Sea Research Part I-Oceanographic Research Papers 47, 1173–1182.

Fang, J.S., Barcelona, M.J., Semrau, J.D., 2000b. Characterization of methanotrophic bacteria on the basis of intact phospholipid profiles. FEMS Microbiology Letters 189, 67–72.

Fischer, W.W., Summons, R.E., Pearson, A., 2005. Targeted genomic detection of biosynthetic pathways: anaerobic production of hopanoid biomarkers by a common sedimentary microbe. Geobiology 3, 33–40.

Fisher, M.C., 2007. The microbial ecology of a polluted, stratified lake: methanotrophy, biodegradation, sulfate reduction, and methanogenesis. PhD Thesis, Harvard University, Cambridge, MA, USA.

Francisco, D.E., Mah, R.A., Rabin, A.C., 1973. Acridine orange-epifluorescence technique for counting bacteria in natural waters. Transactions of the American Microscopical Society 92, 416–421.

Friedrich, U., Schallenberg, M., Holliger, C., 1999. Pelagic bacteria-particle interactions and community-specific growth rates in four lakes along a trophic gradient. Microbial Ecology 37, 49–61.

Froelich, P.N., Klinkhammer, G.P., Bender, M.L., Luedtke, N.A., Heath, G.R., Cullen, D., Dauphin, P., Hammond, D., Hartman, B., Maynard, V., 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. Geochimica et Cosmochimica Acta 43, 1075–1090.

Goldfine, H., 1984. Bacterial membranes and lipid packing theory. Journal of Lipid Research 25, 1501–1507.

Gorchein, A., 1968. Studies on the structure of an ornithine-containing lipid from non-sulphur purple bacteria. Biochimica et Biophysica Acta 152, 358–367.

Härtner, T., Straub, K.L., Kannenberg, E., 2005. Occurrence of hopanoid lipids in anaerobic *Geobacter* species. FEMS Microbiology Letters 243, 59–64.

Hales, B.A., Edwards, C., Ritchie, D.A., Hall, G., Pickup, R.W., Saunders, J.R., 1996. Isolation and identification of methanogen-specific DNA from blanket bog feat by PCR amplification and sequence analysis. Applied and Environmental Microbiology 62, 668–675.

Hallam, S.J., Girguis, P.R., Preston, C.M., Richardson, P.M., DeLong, E.F., 2003. Identification of methyl coenzyme M reductase A (mcrA) genes associated with methane-oxidizing Archaea. Applied and Environmental Microbiology 69, 5483–5491.

Hanson, R.S., Hanson, T.E., 1996. Methanotrophic bacteria. Microbiological Reviews 60, 439–471.

Hinrichs, K.-U., Boetius, A., 2002. The anaerobic oxidation of methane: new insights in microbial ecology and biogeochemistry. In: Wefer, G., Billet, D., Hebbeln, D., Jorgensen, B.B., Schlueter, M., Weering, T.V. (Eds.), Ocean Margin Systems. Springer-Verlag, Berlin, Heidelberg, pp. 457–477.

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 analyses of anaerobic methane-oxidizing communities in marine sediments. Organic Geochemistry 31, 1685–1701.

Hippe, H., Vainshtein, M., Gogotova, G.I., Stackebrandt, E., 2003. Reclassification of *Desulfobacterium macestii* as *Desulfomicrobium macestii comb. nov.*. International Journal of Systematic and Evolutionary Microbiology 53, 1127–1130.

Hoehler, T.M., Alperin, M.J., Albert, D.B., Martens, C.S., 2001. Apparent minimum free energy requirements for methanogenic Archaea and sulfate-reducing bacteria in an anoxic marine sediment. FEMS Microbiology Ecology 38, 33–41.

Hölzl, G., Dörmann, P., 2007. Structure and function of glycoglycerolipids in plants and bacteria. Progress in Lipid Research 46, 225–243.

Imhoff, J.F., Bias-Imhoff, U., 1995. Lipids, quinones and fatty acids of anoxygenic phototrophic bacteria. In: Blankenship, R.E., Madigan, M.T., Bauer, C.E. (Eds.), Anoxygenic Photosynthetic Bacteria. Kluwer Academic Publishers, Dordrecht, pp. 179–205.

Imhoff, J.F., Kushner, D.J., Kushwaha, S.C., Kates, M., 1982. Polar lipids in phototrophic bacteria of the *Rhodospirillaceae* and *Chromatiaceae* families. Journal of Bacteriology 150, 1192–1201.

Innes, H.E., Bishop, A.N., Head, I.M., Farrimond, P., 1997. Preservation and diagenesis of hopanoids in recent lacustrine sediments of Priest Pot, England. Organic Geochemistry 26, 565–576.

Iversen, N., Oremland, R.S., Klug, M.J., 1987. Big Soda Lake (Nevada). 3. Pelagic methanogenesis and anaerobic methane oxidation. Limnology and Oceanography 32, 804–814.

Joyard, J., Maréchal, E., Block, M.A., Douce, R., 1996. In: Smallwood, M., Knox, P., Bowles, D.J. (Eds.), Membranes: Specialized Functions in Plants. BIOS Scientific, Oxford, pp. 179–194.

Joye, S.B., Connell, T.L., Miller, G., Oremland, R.S., Jellison, R.S., 1999. Oxidation of ammonia and methane in an alkaline, saline lake. Limnology and Oceanography 44, 178–188.

Kannenberg, E.L., Poralla, K., 1999. Hopanoid biosynthesis and function in bacteria. Naturwissenschaften 86, 168–176.
Kato, M., Sakai, M., Adachi, K., Ikemoto, H., Sano, H., 1996. Distribution of betaine lipids in marine algae. Phytochemistry 42, 1341–1345.
Khotimchenko, S.V., 2002. Distribution of glyceroglycolipids in marine algae and grasses. Chemistry of Natural Compounds 38, 223–229.
Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B., Stahl, D.A., 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437, 543–546.
Kuhner, C.H., Frank, C., Griesshammer, A., Schmittroth, M., Acker, G., Gossner, A., Drake, H.L., 1997. *Sporomusa silvacetica* sp *nov*., an acetogenic bacterium isolated from aggregated forest soil. International Journal of Systematic Bacteriology 47, 352–358.
Lin, J.L., Joye, S.B., Scholten, J.C.M., Schafer, H., McDonald, I.R., Murrell, J.C., 2005. Analysis of methane monooxygenase genes in mono lake suggests that increased methane oxidation activity may correlate with a change in methanotroph community structure. Applied and Environmental Microbiology 71, 6458–6462.
Lin, X.J., Wakeham, S.G., Putnam, I.F., Astor, Y.M., Scranton, M.I., Chistoserdov, A.Y., Taylor, G.T., 2006. Comparison of vertical distributions of prokaryotic assemblages in the anoxic Cariaco Basin and Black Sea by use of fluorescence in situ hybridization. Applied and Environmental Microbiology 72, 2679–2690.
Londry, K.L., Des Marais, D.J., 2003. Stable carbon isotope fractionation by sulfate-reducing bacteria. Applied Environmental Microbiology 69, 2942–2949.
Lopez-Lara, I.M., Sohlenkamp, C., Geiger, O., 2003. Membrane lipids in plant-associated bacteria: their biosyntheses and possible functions. Molecular Plant–Microbe Interactions 16, 567–579.
Luton, P.E., Wayne, J.M., Sharp, R.J., Riley, P.W., 2002. The *mcrA* gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. Microbiology 148, 3521–3530.
Macy, J.M., Santini, J.M., Pauling, B.V., O'Neill, A.H., Sly, L.I., 2000. Two new arsenate/sulfate-reducing bacteria: mechanisms of arsenate reduction. Archives of Microbiology 173, 49–57.
Makula, R.A., 1978. Phospholipid composition of methane-utilizing bacteria. Journal of Bacteriology 134, 771–777.
Makula, R.A., Finnerty, W.R., 1975. Isolation and characterization of an ornithine-containing lipid from *Desulfovibrio gigas*. Journal of Bacteriology 123, 523–529.
Martens, C.S., Berner, R.A., 1977. Interstitial water chemistry of anoxic Long Island Sound sediments. 1. Dissolved gases. Limnology and Oceanography 22, 10–25.
Masai, E., Yamada, A., Healy, J.M., Hatta, T., Kimbara, K., Fukuda, M., Yano, K., 1995. Characterization of biphenyl catabolic genes of gram-positive polychlorinated biphenyl degrader *Rhodococcus* sp. strain RHA1. Applied and Environmental Microbiology 61, 2079–2085.
Orphan, V.J., 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 United States of America 99, 7663–7668.
Oude Elferink, S., Akkermans-van Vliet, W.M., Bogte, J.J., Stams, A.J.M., 1999. *Desulfobacca acetoxidans gen. nov*., sp., *nov*., a novel acetate-degrading sulfate reducer isolated from sulfidogenic granular sludge. International Journal of Systematic Bacteriology 49, 345–350.
Pearson, A., Flood Page, S.R., Jorgenson, T.L., Fischer, W.W., Higgins, M.B., 2007. Novel hopanoid cyclases from the environment. Environmental Microbiology 9, 2175–2188.
Perez-Jimenez, J.R., Kerkhof, L.J., 2005. Phylogeography of sulfate-reducing bacteria among disturbed sediments, disclosed by analysis of the dissimilatory sulfite reductase genes (*dsrAB*). Applied and Environmental Microbiology 71, 1004–1011.
Perez-Jimenez, J.R., Young, L.Y., Kerkhof, L.J., 2001. Molecular characterization of sulfate-reducing bacteria in anaerobic hydrocarbon-degrading consortia and pure cultures using the dissimilatory sulfite reductase (*dsrAB*) genes. FEMS Microbiology Ecology 35, 145–150.
Prescott, L.M., Harley, J.P., Klein, D.A., 2005. Microbiology, sixth edition. McGraw-Hill, Boston.
Rohmer, M., Bouvier-Nave, P., Ourisson, G., 1984. Distribution of hopanoid triterpenes in prokaryotes. Journal of General Microbiology 130, 1137–1150.
Rossel, P.E., Lipp, J.S., Fredricks, H.F., Arnds, J., Boetius, A., Elvert, M., Hinrichs, K.-U., 2008. Intact polar lipids of anaerobic methanotrophic Archaea and associated bacteria. Organic Geochemistry. doi:10.1016/j.orggeochem.2008.02.02.
Rütters, H., Sass, H., Cypionka, H., Rullkoetter, 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.
Sanina, N.M., Goncharova, S.N., Kostetsky, E.Y., 2004. Fatty acid composition of individual polar lipid classes from marine macrophytes. Phytochemistry 65, 721–730.
Scholten, J.C.M., Joye, S.B., Hollibaugh, J.T., Murrell, J.C., 2005. Molecular analysis of the sulfate reducing and archaeal community in a meromictic soda lake (Mono Lake, California) by targeting 16S rRNA, *mcrA*, *apsA*, and *dsrAB* genes. Microbial Ecology 50, 29–39.
Schubert, C.J., Coolen, M.J.L., Neretin, L.N., Schippers, A., Abbas, B., Durisch-Kaiser, E., Wehrli, B., Hopmans, E.C., Damste, J.S.S., Wakeham, S., Kuypers, M.M.M., 2006. Aerobic and anaerobic methanotrophs in the Black Sea water column. Environmental Microbiology 8, 1844–1856.
Sohlenkamp, C., Lopez-Lara, I.M., Geiger, O., 2003. Biosynthesis of phosphatidylcholine in bacteria. Progress in Lipid Research 42, 115–162.
Southworth, B.A. 1999. Hydrogen peroxide in the Halls Brook Holding Area, a small meromictic lake. MSc. Thesis, Massachusetts Institute of Technology, Cambridge, USA.
Sprague, S.G., Staehelin, L.A., Dibartolomeis, M.J., Fuller, R.C., 1981. Isolation and development of chromosomes in the green bacterium *Chloroflexus–Aurantiacus*. Journal of Bacteriology 147, 1021–1031.
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.
Talbot, H.M., Farrimond, P., 2007. Bacterial populations recorded in diverse sedimentary biohopanoid distributions. Organic Geochemistry 38, 1212–1225.
Talbot, H.M., Squier, A.H., Keely, B.J., Farrimond, P., 2003. Atmospheric pressure chemical ionization reversed-phase liquid chromatography/ion trap mass spectrometry of intact bacteriohopanepolyols. Rapid Communications in Mass Spectrometry 17, 728–737.
Talbot, H.M., Watson, D.F., Murrell, J.C., Carter, J.F., Farrimond, P., 2001. Analysis of intact bacteriohopanepolyols from methanotrophic bacteria by reversed-phase high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. Journal of Chromatography A 921, 175–185.
Taylor, G.T., Iabichella, M., Ho, T.-Y., Scranton, M.I., Thunell, R.C., Muller-Karger, F., Varela, R., 2001. Chemoautotrophy in the redox transition zone of the Cariaco Basin: a significant midwater source of organic carbon production. Limnology and Oceanography 46, 148–163.
Thiele, O.W., Oulevey, J., Hunneman, D.H., 1984. Ornithine-containing lipids in *Thiobacillus-A2* and *Achromobacter* sp.. European Journal of Biochemistry 139, 131–135.
Wakeham, S.G., Lewis, C.M., Hopmans, E.C., Schouten, S., Sinninghe Damsté, J.S., 2003. Archaea mediate anaerobic oxidation of methane in deep euxinic waters of the Black Sea. Geochimica et Cosmochimica Acta 67, 1359–1374.
White, D.C., Davis, W.M., Nickels, J.S., King, J.D., Bobbie, R.J., 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologica 40, 51–62.
Wick, L.Y., Gschwend, P.M., 1998. Source and chemodynamic behaviour of diphenyl sulfone and ortho- and para-hydroxybiphenyl in a small lake receiving discharges from an adjacent superfund site. Environmental Science and Technology 32, 1319–1328.
Wick, L.Y., McNeill, K., Rojo, M., Medilanski, E., Gschwend, P.M., 2000. Fate of benzene in a stratified lake receiving contaminated groundwater discharges from a Superfund Site. Environmental Science and Technology 34, 4354–4362.
Wilkinson, S.G., 1988. Gram-negative bacteria. In: Ratledge, C., Wilkinson, S.G. (Eds.), Microbial Lipids. Academic Press, London, pp. 299–488.
Wille, A., Sonntag, B., Sattler, B., Psenner, R., 1999. Abundance, biomass and size-structure of the microbial assemblage in the high mountain lake Gossenkoellesee (Tyrol, Austria) during the ice-free period. Journal of Limnology 58, 117–126.
Zhang, C.L., Li, Y., Ye, Q., Fong, J., Peacock, A.D., Blunt, E., Fang, J., Lovley, D.R., White, D.C., 2003. Carbon isotope signatures of fatty acids in *Geobacter metallireducens* and *Shewanella* algae. Chemical Geology 195, 17–28.

Zhang, C.L., Fouke, B.W., Bonheyo, G.T., Peacock, A.D., White, D.C., Huang, Y.S., Romanek, C.S., 2004. Lipid biomarkers and carbon-isotopes of modern travertine deposits (Yellowstone National Park, USA): implications for biogeochemical dynamics in hot-spring systems. Geochimica et Cosmochimica Acta 68, 3157–3169.

Zink, K.-G., Mangelsdorf, K., 2004. Efficient and rapid method for extraction of intact phospholipids from sediments combined with molecular structure elucidation using LC–ESI-MS–MS analysis. Analytical and Bioanalytical Chemistry 380, 798–812.