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Biomarker analysis of microbial diversity in sediments of a saline groundwater seep of Salt Basin, Nebraska

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Abstract

Lipids extracted from sediments in a saline seep in the Salt Basin of Lancaster County, Nebraska included alkanes, alkenes, alkanols, phytol, $C_{27–30}$ sterols, $C_{30–32}$ hopanoids, tetrahymanol, glycolipid and phospholipid fatty acids, and lipopolysaccharide hydroxyl fatty acids. Biomarker profiles suggest that the brine seeps of Salt Basin support a microbial ecosystem adapted to a relatively highly saline and sulfidic environment. The phospholipid fatty acid (PLFA) and lipopolysaccharide hydroxyl fatty acid profiles are consistent with the presence of large numbers of sulfate-reducing bacteria (SRB) in black, sulfidic muds surrounding the seeps. In the context of field and laboratory observations, the presence of large amounts of glycolipid fatty acids is attributed to large populations of photosynthetic microorganisms (cyanobacteria, phytoplankton, and purple sulfur bacteria) that likely play important roles in the local cycling of carbon and sulfur. The sterol profile and the detection of polyunsaturated alkenes ($C_{21:6}$, $C_{21:7}$, $C_{30:4}$, and $C_{30:5}$) implicates microalgae as important contributors of organic matter at the site. Comparatively high concentrations of phytol (58.2 μg g^{-1} dry wt sediment) record the activity of photosynthetic organisms in the system. The $\delta^{13}C$ of phytol (−37.1‰) is compatible with a dominance of microalgae, cyanobacteria, or higher plants and a lesser contribution from phototrophic sulfur bacteria. The presence of various intermediate degradation products of phytol (phytenes and phytadienes) indicates that SRB likely mediate the chemical reduction of phytol in the anaerobic zone. The presence of $C_{30–32}$ hopanols can be attributed to cyanobacteria and methanotrophs in oxic regions of the water column, whereas bacterivorous ciliates and phototrophic sulfur bacteria living at the chemocline are likely sources of tetrahymanol. The carbon isotopic composition of individual fatty acids and neutral lipids helps to identify source organisms. These microorganisms and others constitute a unique and integrated ecosystem prescribed by the geochemistry of the Salt Basin.

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1. Introduction

Interest in the chemistry and biology of inland alkaline salt basins is growing for several reasons. First, they are discrete ecosystems with well-defined physical limits, contributing to the ease of studying the chemical and microbiological interactions in them. Second, they are extreme environments, offering the opportunity to study relatively unique aspects of biodiversity, microbial physiology and metabolism. Finally, the last surface aquatic habitats for life on Mars were likely to have been highly saline, making modern terrestrial salt basins good analogs for conditions under which life might have evolved on both Mars and Earth (Moore and Bullock, 1999; Joeckel and Ang Clement, 2001, 2005; Schouten et al., 2001; Zettler et al., 2002). If so, bio-molecules found in organisms adapted to high salt and high-pH environments on Earth may be reliable biomarkers for detecting life on Mars and for studying microbial evolution in general.

Lipid biomarkers, the preserved structural skeletons of biological molecules, can be used to identify potential source organisms (e.g., Summons et al., 1999), to establish possible links between modern microbial communities and their ancient counterparts (Brocks et al., 1999, 2003; Jahnke et al., 2004), and to illuminate the evolutionary histories of organisms and their environments (e.g., Brocks et al., 2003). Analysis of phospholipid fatty acids (PLFA), which are present in the form of lipid bilayers in microbial membranes, can provide a quantitative measure of the viable biomass and biological diversity of microbial communities in environmental samples (White et al., 1997). Glycolipids are unique membrane lipid components in photosynthetic organisms (Collins and Ferrier, 1995). Therefore, the analysis of glycolipids provides information on the activity of photosynthetic microorganisms in the environment (Sinninghe Damsté et al., 2001). Other lipid biomarkers, including sterols and hopanoids, are better preserved in the extended geological record and therefore offer insights into the presence of microorganisms in paleoenvironments (see Jahnke et al., 2004 and references therein).

The Salt Basin in Lancaster County, Nebraska is an inland saline basin developed around Salt Creek and its tributaries (Fig. 1). Historically at least, it incorporated multiple saline wetlands and salt flats. The Salt Basin is different from many other known salt basins in that it lies in a subhumid climate and farther northward and eastward than other well-known surficial salt accumulations in the United States (Joeckel and Ang Clement, 1999). Salt seeps and springs result from the upward discharge of groundwater through Late Pleistocene–Holocene alluvium (Joeckel and Ang Clement, 1999). Saline waters move upward into this alluvium from the Cretaceous Dakota Formation, but much of the dissolved salt content may have originated in Paleozoic strata, and probably migrated some distance (Gosselin et al., 2001). The oxidation and leaching of pyrite-bearing glacial tills underlying adjacent uplands could have contributed to the accumulation of dissolved sulfate, whereas sodium and chloride are likely to have been transported longer distances through the regional aquifer. Black sulfidic sediments are generated as a result of the saturation of surficial sediments and soils by through-flowing, sulfate-bearing groundwater around seeps and in small wetlands, where surface-water salinity varies seasonally.

Soil-surface salt efflorescences in the basin are dominated by halite (NaCl) with minor amounts of thenardite (Na_2SO_4). Sediment porewater in Salt Basin is characterized by high pH (7.8–12.1) and low redox potential (as low as −200 mV). Microbial sulfate reduction and iron sulfide precipitation are the prominent biogeochemical processes in Salt Basin. H_2S (detected by odor) and accompanying strong effervescence were produced in a reaction of sulfidic sediment with 1 N HCl (Joeckel and Ang Clement, 1999). However, iron sulfide minerals are labile and can be oxidized readily when the water table drops or surface water evaporates. Algal and cyanobacterial mats have been observed in Salt Basin (Joeckel and Ang Clement, 1999).

In the present study, we analyzed lipid biomarkers in sediments of the Salt Basin in order to provide the first-ever comprehensive assessment of the microbial community in sulfidic soils around seeps and in the overlying water column.

2. Materials and methods

2.1. Sampling and geochemical analysis

The sediment core analyzed in this study was taken from a groundwater-intercepting drainage ditch immediately west of Capitol Beach Lake, a manmade impoundment that now covers what was a large salt flat at the time of initial Euramerican settlement in the 1860s (Joeckel and Ang Clement,

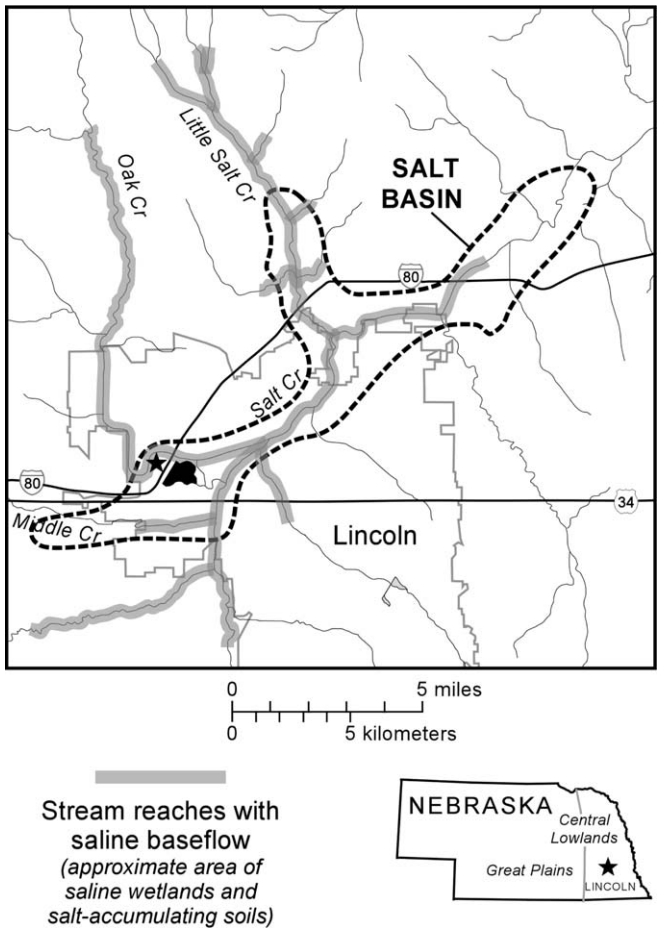

Fig. 1. Study area in Salt Basin, Lancaster County, Nebraska. Shading along streams indicates areas of saline groundwater discharge. Multiple saline wetlands and salt flats existed in the area prior to Euramerican settlement in the 1850s.

1999). At the study site (Fig. 1), patches of black sulfidic sediment under saline water, associated with seeps and small springs, were distributed along the ditch. A fifteen-centimeter push core was collected in clear polycarbonate tubing (1″ ID) and sealed with plastic caps. The core was placed on ice in a cooler and transported to the laboratory within 4 h, where it was sampled aseptically after first decanting the water covering the core. Then, the top sections of the core, 0–3.3 cm (Section 1) and 3.3–8 cm (Section 2), were removed for lipid analysis. Section 1 was black sulfidic material (BSM) (Joeckel and Ang Clement, 1999). Sediment of Section 2 was grey sand and depleted in organic matter.

Subsamples were immediately placed into test tubes with an extraction solvent mixture for lipid analysis. Pore water was extracted by placing an aliquot of the sediment into 85-ml Nalgene centrifuge tubes centrifuged at 10,000 rpm for 15 min. About 3–5 ml of the collected pore water was poisoned with sodium azide for chloride and sulfate measurements by ion chromatography. The remaining porewater was used for pH, redox potential (both by electrode probes), dissolved iron, sulfide, and dissolved oxygen analyses using CheMatrics kit (CheMatrics, Inc., Calverton, VA) (Fang et al., 2000). Concentrations of sulfate and chloride were determined using a Dionex ion chromatography system 2000. A 4-mm AS18 analytical column and a 4-mm AS18 anion guard column with an ASRS Ultra II anion suppressor operated at 96 milliamps were used for analytical separation. Potassium hydroxide (37 mM) was the mobile phase at a flow rate of 1 ml min^{-1}. The column temperatures were 30 °C and the cell temperature was 35 °C. The injection volume was 0.25 μl.

2.2. Lipid extraction and fractionation

Total sedimentary lipids were extracted at room temperature in test tubes containing a solvent mixture of methanol:dichloromethane (DCM): phosphate buffer (potassium phosphate, dibasic, 50 mM, pH 7.4) (2:1:0.8) (Fang and Findlay, 1996). Crude lipids were collected after phase partitioning by adding dichloromethane and deionized water to the test tube to the final ratio of methanol:dichloromethane:water 1:1:0.9. The aqueous phase was saved for the isolation of lipopolysaccharide (LPS) fatty acids (FA). The total lipid extract was dried under a gentle stream of nitrogen and then dissolved in hexane:dichloromethane (70:30, v/v). Total lipids were separated into different lipid classes using miniature champagne columns (Supelco Inc., Bellefonte, PA). Hydrocarbons, neutral lipids, glycolipids, and phospholipids were eluted with 5 ml of hexane, chloroform, acetone, and methanol, respectively.

The upper aqueous phase was dried in a freeze-dryer. The residue was acidified by adding 30 ml of 1 N HCl and refluxed at l00 °C for 2 h. After cooling to room temperature, the contents were transferred into a 250-ml separatory funnel with washes of 2×10 ml of methanol and 2×25 ml of DCM. The two phases were allowed to separate overnight. The organic phase containing the LPS fatty acids was collected.

2.3. Lipid analysis by gas chromatography–mass spectrometry

The neutral lipid fraction was dried under a stream of nitrogen and derivatized by adding 60 μl of *N*,*O*-bis(trimethylsilyl)-trifloroacetamide to produce TMS ethers of sterols and hopanols (heated at 75 °C for 30 min). The phospholipid fraction was subjected to a mild alkaline *trans*-methylation procedure to produce fatty acid methyl esters (Fang and Findlay, 1996). The LPS fatty acids were first methylated use BF_3/MeOH (heated at 65 °C for 25 min). The hydroxyl fatty acids were converted to their TMS ethers using the same procedures as described above.

All lipids were analyzed on an Agilent 6890 GC interfaced with an Agilent 5973N Mass Selective Detector. Analytical separation of the compounds was accomplished using a $30\ m \times 0.25$ mm i.d. DB-5 MS fused-silica capillary column (J&W Scientific, Folsom, CA). The column temperature was programmed from 50 to 120 °C at 10 °C/min, then to 310 °C at 5 °C/min, and then held for 20 min. Individual fatty acids were identified from their mass spectra. Individual sterols and triterpenoids were identified based on retention times and comparison with published mass spectra (Venkatesan, 1989; Shiojima et al., 1992). Response factors were obtained for fatty acids using duplicate injections of quantitative standards at five different concentration levels. Concentrations of individual compounds were obtained based on the GC/MS response relative to that of an internal standard (C18:0 fatty acid ethyl ester). The double-bond position and geometry of monounsaturated fatty acids were determined by using methods described by Dunkleblum et al. (1985). The concentrations of sterols and hopanols were determined based on the chromatographic response of these compounds relative to that of an internal standard, 5α-cholestane. Method blanks were extracted with each set of samples and were assumed to be free of contamination if chromatograms contained no peaks. A standard containing known concentrations of 26 fatty acids was analyzed daily on the GC/MS to check analytical accuracy (>90%). Replicate analyses (2×) of samples were done to ensure reproducibility (variation $\leqslant$10%).

Fatty acids are designated by the total number of carbon atoms:number of double bonds (i.e., a 16 carbon alkanoic acid is 16:0). The position of the double bond is indicated with a Δ number closest to the carboxyl end of the fatty acid molecule with the geometry of either *c* (cis) or *t* (trans). The terminal methyl-branching is indicated with *i* (iso) or *a* (anteiso) and the mid-branching is indicated as the position of the methyl group from the carboxyl group of the fatty acid (e.g., 10Me16:0). Cyclopropane fatty acids are indicated with cy.

2.4. Carbon isotope analysis of lipids

Carbon isotope analyses of lipids were performed at Brown University using a gas chromatograph–combustion–isotope ratio mass spectrometer (GC–C–IRMS, Thermo Finnigan) as described previously (Wang et al., 2004). An HP 6890 GC, equipped with an AS 200 autosampler, was connected to a Finnigan MAT Delta plus XL MS via the combustion interface. The temperature program and capillary column used were identical to those used for GC analysis. Helium (UHP 5.5 grade) was used as the carrier gas operating in constant

flow mode with a rate of 1.5 ml/min. Injection was performed in the splitless mode to deliver typical sample amounts (~80 ng per compound) onto the column. Compounds separated by the GC column were converted to CO_2 and H_2O through the combustion furnace (0.5 mm i.d. × 1.5 mm o.d. × 34 cm) operated at 960 °C and loaded with CuO and Pt wires as the oxidant and catalyst, respectively. Six pulses of CO_2 reference gas with known $\delta^{13}C$ values (−46.96‰) were injected via the interface to the IRMS, for the determination of $\delta^{13}C$ values of sample compounds. Typically, the standard deviation of duplicate analyses is smaller than ±0.3‰. Values are reported in the usual $\delta^{13}C$ notation relative to the Vienna Pee Dee Belemnite standard. Isotopic composition of individual fatty acids was obtained after the correction for the additional methyl carbon from methanol using a mass balance equation (Fang et al., 1993):

$$\delta^{13}C_{\mathrm{FAME}} = f_{\mathrm{FA}}\delta^{13}C_{\mathrm{FA}} + f_{\mathrm{Methanol}}\delta^{13}C_{\mathrm{Methanol}}$$

where $\delta^{13}C_{FAME}$, $\delta^{13}C_{FA}$, and $\delta^{13}C_{methanol}$ are the carbon isotopic composition of the FAME, the underivatized fatty acid, and the methanol, respectively, and f_{FA} and $f_{Methanol}$ are the fractions of carbon in the FAME due to the underivatized fatty acid and methanol, respectively.

3. Results

3.1. Sedimentary geochemistry

The groundwater of Salt Basin contains high levels of Na^+ (10.4–18.0 g/l) and Cl^- (15.3–22.6 g/l). A surface water sample taken near a saline seep contained 82 and 114 g/l of Na^+ and Cl^-, respectively. Other major ions included Ca^{2+} (0.22–0.32 g/l), SO_4^{2-} (0.66–0.84 g/l), and HCO_3^- (0.4–0.77 g/l) (Joeckel and Ang Clement, 1999). Porewater extracted from Section 1 of the sediment core contained elevated concentrations of Cl^- (20.9 g/l), SO_4^{2-} (1.7 g/l), S^{2-} (0.024 g/l), and alkalinity (0.53 g/l). The sedimentary environment was highly reducing ($E_h = -254$ mV) (see Fig. 6).

3.2. Hydrocarbons

Compounds in the hydrocarbon fraction of Section 1 include *n*-alkanes, pristane, pristenes, phytane, phytenes, phytadienes, sterenes, and hopanes. The *n*-alkane profile showed a bimodal distribution with C_{max} at C_{17} and C_{27} (Fig. 2a). The concentrations of most lower-molecular weight alkanes were relatively low except heptadecane ($C_{17:0}$) and heptadecenes ($C_{17:1}$), which constituted 10.2% and 10.5% of the total hydrocarbons (Table 1). The higher-molecular weight *n*-alkanes exhibited an apparent odd-over-even carbon number predominance. The concentrations of pristane and phytane were low relative to isomers of pristene, phytene and phytadiene. The three isomeric phytadienes accounted for 14.7% of the total hydrocarbons. A number of polyunsaturated alkenes were identified including $C_{21:6}$, $C_{21:7}$, $C_{30:4}$, and $C_{30:5}$. Significantly, $C_{21:7}$ is novel and its presence in sediment has never been reported. It had the highest concentration among all polyunsaturated alkenes (1.3 μg g^{-1} dry weight sediment; 13.3% of total hydrocarbons). Squalene was not detected in the sediment. However, tetrahydrosqualene, a hydrogenated derivative, was found. Although a number of sterols were detected (see below), only two sterenes were present (Table 1). These were 24-ethylcholesta-3,5-diene (5.7%) and 24-ethylcholesta-3,5,22*E*-triene (5.4%). The *m*/*z* 191 mass chromatogram shows that a number of homo-, bishomo, and trishomohopanes were present in the sediment (Fig. 2b). However, their concentrations were extremely low. The most abundant hopene was hop-22(29)-ene (diploptene) (Table 1).

3.3. Phospholipid (PLFA), glycolipid (GLFA), and lipopolysacchride (LPS) fatty acids

PLFA (Fig. 3a), GLFA (Fig. 3b), and LPS fatty acids (Fig. 4) were isolated from the sediment (Tables 2 and 3). PLFA with chain length from $C_{11–20}$ were detected in sediment from Salt Basin. The phospholipid fatty acid profiles were dominated by monounsaturated fatty acids which constituted 56% of the total fatty acids (Fig. 3a). The most abundant ones were 16:1Δ^9 and 18:1Δ^{11}. Also detected in PLFA was br-17:1Δ^9, a marker fatty acid commonly found in SRB. The saturated fatty acids accounted for 20% and 31% of the PLFA and GLFA, respectively. Terminal-branched fatty acids consisted of *iso* and *anteiso* isomers of C_{15}, C_{16}, and C_{17} fatty acids. These fatty acids account for 12% and 16% of PLFA and GLFA, respectively. The only mid-methyl branched fatty acid detected was 10Me16:0 (0.8–0.9%). Polyunsaturated fatty acids (PUFA) detected in the sediment included C16:2, three isomers of C18:2$\Delta^{9,12}$, C20:3 and 20:4, constituting

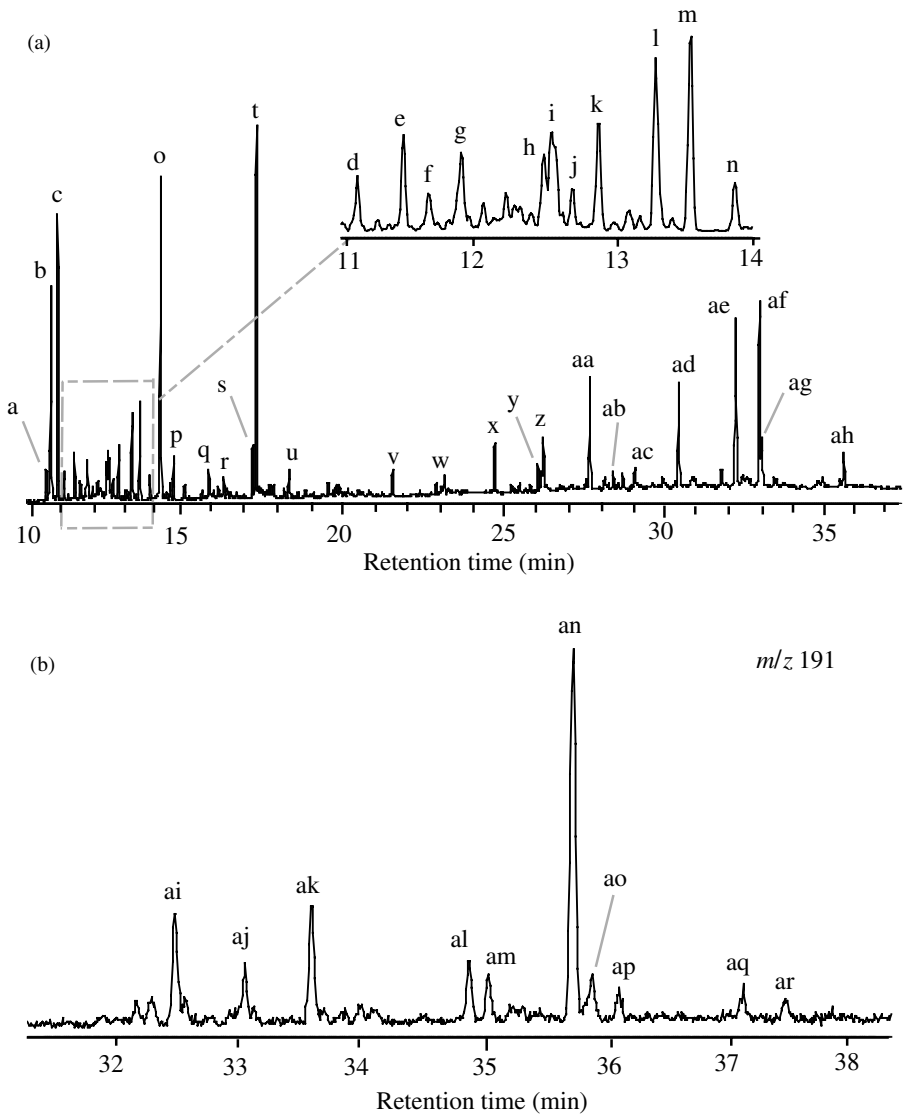

Fig. 2. Total ion chromatogram (a) and *m/z* 191 mass chromatogram (b) of the hydrocarbon fraction isolated from black sulfidic sediment, which shows macroscopic evidence for microbial sulfate reduction, around a saline seep. See Table 1 for compound identification and concentrations.

6.6% of the total fatty acids. The concentration of total PLFA was more than three times greater than that of the GLFA (209.4 vs. 62.2 μg g^{-1} dry weight sediment).

The total PLFA content of Section 2 (155 ng g^{-1}) was three orders of magnitude lower than Section 1. The C_{16} and C_{18} fatty acids constituted 74% of the total fatty acid content in Section 2 (Table 2). The PLFA abundances record the viable microbial biomass (White et al., 1997). Thus, microbial activity of Section 2 is limited. Our discussion will focus on lipids of Section 1 of the sediment core.

The lipid part (lipid A) of the lipopolysaccharide component of Gram-negative bacteria contains a variety of hydroxy and non-hydroxy (normal and branched) fatty acids (Table 3, Fig. 4). A total of 10 hydroxy fatty acids, ranging from C_{12} to C_{18}, were identified, including α- and β-hydroxy, and branched β-hydroxy compounds. The most abundant LPS FA present in Salt Basin sediment were β-hydroxy fatty acids. The concentration of individual hydroxy fatty acids ranged from 0.3% to 3.9% of the total LPS fatty acids. A polyunsaturated fatty acid, $18{:}2\Delta^{9,12}$, was also detected in the LPS lipids.

Table 1
Concentration (μg g^{-1} dry weight; percentage in parentheses) of hydrocarbons (*n*-alkanes, isoprenoid hydrocarbons, steranes and hopanes) isolated from sediment of Salt Basin

Peak labeling[a]	Compound	Concentration (μg g^{-1})
Total ion chromatogram of the hydrocarbon fraction (Fig. 2a)		
a	17:1	0.1 (1.2)
b	17:1	0.8 (9.3)
c	17:0	0.8 (10.2)
d	Pristane	0.1 (1.0)
e	Pristene	0.2 (1.6)
f	Pristene	0.1 (0.8)
g	2-Me–C_{17}	0.2 (1.8)
h	18:1	0.1 (1.4)
i	18:1	0.2 (2.8)
j	18:0	0.1 (0.7)
k	Phytane	0.2 (1.9)
l	Phytene	0.3 (3.3)
m	Phytene	0.3 (3.4)
n	Phytadiene	0.1 (0.9)
o	Phytadiene	1.2 (12.5)
p	Phytadiene	0.1 (1.3)
q	4-Me–C_{19}	0.1 (1.3)
r	20:1	0.1 (1.8)
s	21:6	0.2 (1.9)
t	21:7	1.3 (13.3)
u	21:0	0.1 (1.1)
v	23:0	0.1 (1.0)
w	24:0	0.1 (0.6)
x	25:0	0.2 (1.8)
y	30:5	0.1 (0.7)
z	30:4	0.2 (1.4)
aa	27:0	0.5 (3.5)
ab	Tetrahydrosqualene	0.2 (0.5)
ac	28:0	0.1 (0.9)
ad	29:0	0.4 (3.2)
ae	24-Ethylcholesta-3,5,22*E*-triene	0.7 (5.4)
af	24-Ethylcholesta-5,22-diene	0.8 (5.7)
ag	31:0	0.2 (1.3)
ah	Hop-22(29)-ene	0.1 (1.0)
m/z 191 mass chromatogram (Fig. 2b) (% is calculated among these compounds because of their low concentrations relative to n-alkanes)		
ai	17α(H),21β(H)-30-norhopane	0.04 (12.8)
aj	24-Ethylcholesta-3,5,22*E*-triene	0.02 (6.2)
ak	17α(H),21β(H)-hopane	0.05 (13.7)
al	(22*S*)-17α(H),21β(H)-29-homohopane	0.02 (6.6)
am	(22*R*)-17α(H),21β(H)-29-homohopane	0.02 (5.0)
an	Hop-22(29)-ene	0.14 (40.5)
ao	(22*S*)-17α(H),21β(H)-29-bishomohopane	0.02 (6.7)
ap	(22*R*)-17α(H),21β(H)-29-bishomohopane	0.01 (3.3)
aq	(22*S*)-17α(H),21β(H)-29-trishomohopane	0.01 (3.0)
ar	(22*S*)-17α(H),21β(H)-29-bishomohopane	0.01 (2.3)

[a] Peak labeling is indicated in Fig. 2.

3.4. *n*-Alcohols, sterols and triterpenoids

Low amounts of even-numbered *n*-alcohols $C_{16–22}$ were detected (<1%) (Table 4, Fig. 5). Phytol was the most abundant neutral lipid in the sediment (38.1% of total) although isophytol and dihydrophytol were also detected. A number of methyl- and desmethyl-sterols as well as tetrahymanol were identified in the sediments. These sterols include $C_{27–30}$ algal and terrestrial higher plant sterols. The most abundant sterol was 24-ethylcholesta-5,22-dien-3β-ol (24.1%). Other sterols detected in sediment included

Table 2
$\delta^{13}C$ and concentration (percentage in parentheses) of phospholipid (PLFA) and glycolipid (GLFA) fatty acids isolated from sediment of Salt Basin

Peak labeling[a]	Fatty acid	PLFA			GLFA	
		Section 1		Section 2	Section 1	
		Concentration (μg g^{-1})	$\delta^{13}C$ (‰)	Concentration (ng g^{-1})	Concentration (μg g^{-1})	$\delta^{13}C$ (‰)
1	12:0	0.2 (0.1)			nd	
2	*i*14:0	3.0 (1.4)			nd	
3	*a*14:0	3.3 (1.6)	−30.0		1.3 (2.1)	−28.7
4	14:0	6.1 (2.9)	−29.4	3.2 (2.1)	3.2 (5.1)	−27.8
5	15:1$\Delta^{7}c$	0.4 (0.2)			0.0 (0.0)	
6	15:1$\Delta^{7}t$	0.5 (0.2)			0.0 (0.0)	
7	*i*15:0	9.2 (4.4)	−28.1	5.1 (3.3)	5.4 (8.7)	−27.9
8	*a*15:0	7.2 (3.4)	−29.8	9.3 (6.0)	2.1 (3.3)	−29.1
9	15:1Δ^{9}	0.4 (0.2)			0.0 (0.0)	
10	15:0	1.7 (0.8)	−27.6	1.4 (2.4)	0.7 (1.2)	−28.6
11	*i*16:0	1.5 (0.7)	−29.0	3.7 (2.4)	0.9 (1.4)	−28.4
12	16:2	0.9 (0.4)			1.4 (2.2)	
13	16:1$\Delta^{9}c$	1.3 (0.6)		11.2 (7.2)	1.1 (1.8)	
14	16:1$\Delta^{9}t$	30.8 (14.7)	−28.9		9.4 (15.0)	−27.6
15	16:1$\Delta^{11}c$	2.4 (1.1)			0.8 (1.2)	
16	16:1$\Delta^{11}t$	2.1 (1.0)			0.7 (1.1)	
17	16:0	28.0 (13.3)	−30.4	39.9 (25.7)	14.0 (22.3)	−26.5
18	br17:1Δ^{9}	0.9 (0.4)		7.7 (5.0)	0.0 (0.0)	
19	10Me16:0	1.6 (0.8)		6.4 (4.1)	0.2 (0.4)	−28.8
20	*i*17:0	0.6 (0.3)		1.4 (0.9)	0.2 (0.3)	−27.7
21	*a*17:0	1.0 (0.5)			0.0 (0.0)	
22	17:1Δ^{9}	0.9 (0.4)			0.0 (0.0)	
23	cy17:0	4.0 (1.9)	−26.8		0.7 (1.2)	−28.7
24	17:1Δ^{11}	0.3 (0.1)			0.0 (0.0)	
25	17:0	1.4 (0.7)		2.3 (1.5)	0.3 (0.5)	−30.3
26	18:2	0.6 (0.3)			0.0 (0.0)	
27	18:2	1.0 (0.5)			0.0 (0.0)	
28	18:2 $\Delta^{9,12}$	6.6 (3.1)			4.5 (7.3)	
29	18:1Δ^{9}	17.2 (8.2)		12.1 (7.8)	3.4 (5.5)	
30	18:1$\Delta^{11}c$	55.1 (26.2)	−28.8	24.6 (15.9)	8.7 (14.0)	−28.7
31	18:1$\Delta^{11}t$	5.1 (2.4)		6.8 (4.4)	0.0 (0.0)	
32	18:0	4.5 (2.1)	−30.6	20.0 (12.9)	1.0 (1.7)	−27.4
33	cy19:0	4.6 (2.2)	−30.2		0.0 (0.0)	
34	20:4	1.8 (0.9)			0.4 (0.7)	
35	20:3	2.5 (1.2)			1.0 (1.6)	
36	20:1Δ^{13}	0.4 (0.2)			0.4 (0.6)	
37	20:0	0.3 (0.1)			0.2 (0.3)	−28.7
38	22:0	nd			1.0 (0.0)	−28.7
39	24:0	nd			1.7 (0.1)	−29.0
40	26:0	nd			1.5 (0.1)	−31.0

[a] Peak labeling is indicated in Fig. 3a (PLFA) and Fig. 3b (GLFA).

cholesterol, 24-methyl and 24-ethylsterols. Small amounts of tetrahymanol, (22*R*)-17β(H),21β(H) hopan-29-ol, (22*R*)-17β(H),21β(H)homohopan-31-ol and (22*R*)-17β(H),21β(H)bishomohopan-32-ol were also detected.

3.5. Carbon isotopic composition of lipid biomarkers

The $\delta^{13}C$ values of phospholipid fatty acids varied from −26.8‰ to −30.6‰ with a mean of −28.9 ± 1.2‰. The ubiquitous fatty acids $C_{16:0}$ and $C_{18:0}$ had more negative $\delta^{13}C$ values than other fatty acids. More positive values were observed for monounsaturated, odd-numbered, terminal-branched and cyclopropane fatty acids (*i*15:0, *n*-15:0, *i*16:0, 16:1Δ^{9}, 18:1Δ^{11}, cy17:0). Glycolipid fatty acids had slightly more positive $\delta^{13}C$ values (28.3 ± 0.9‰) than PLFA. Cy19:0 in both the PLFA and GLFA fractions had more negative $\delta^{13}C$ values than other fatty acids. The neutral lipids

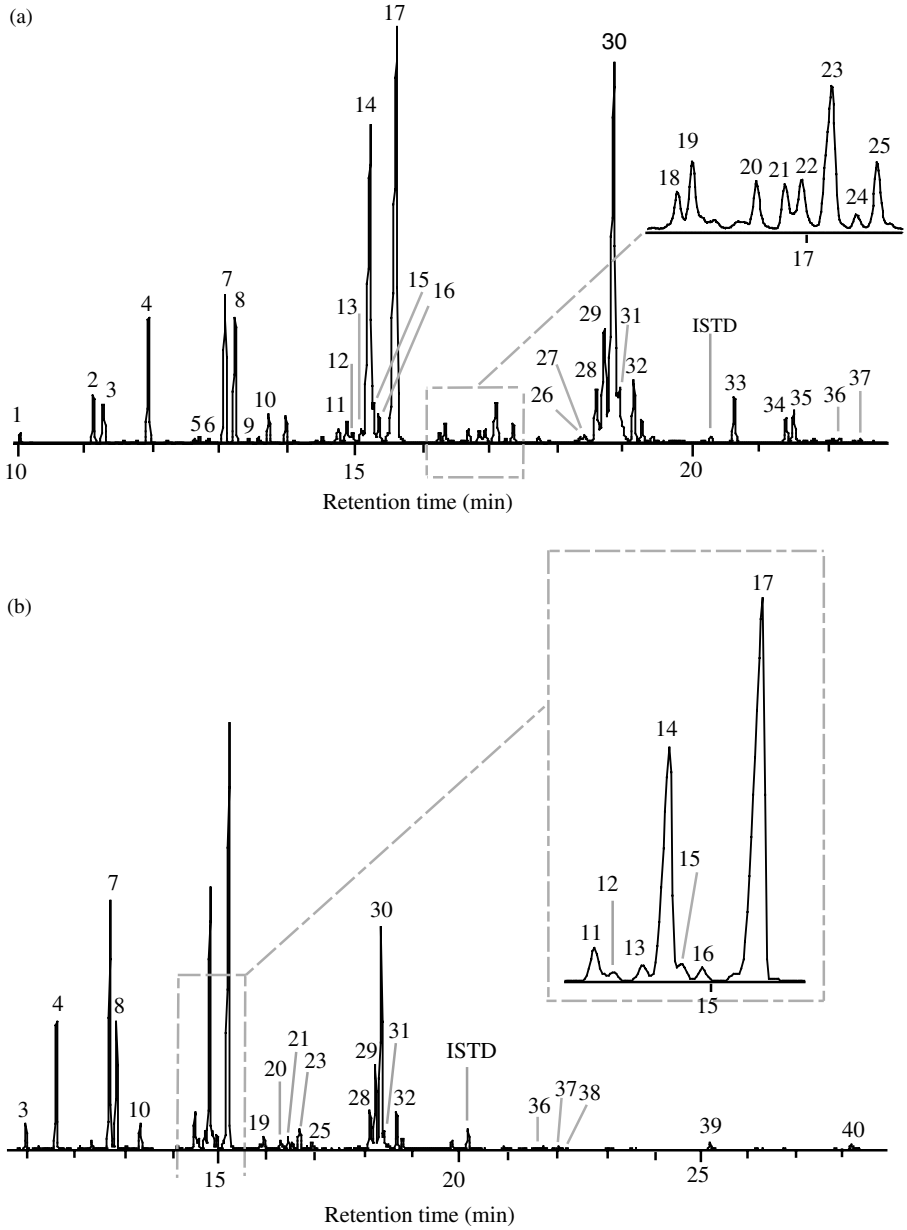

Fig. 3. Total ion chromatogram of phospholipid (a) and glycolipid (b). See Table 2 for compound identification and concentrations.

were consistently more depleted in ^{13}C than fatty acids and had a mean $\delta^{13}C$ value of $29.2 \pm 1.0‰$. Phytol and isophytol had the most negative $\delta^{13}C$, -37.1 and $-34.7‰$, respectively (Fig. 6).

4. Discussion

Because lipid profiles of many extant organisms are lacking and the abundance and distributions of biomarkers represent a mixture of contributions from all organisms present in the environment (Brocks et al., 2003; Pancost and Sinninghe Damsté, 2003), caution must be exercised in using lipid biomarkers for microbial community analysis. Nevertheless, certain molecular biomarkers are unique and diagnostic for specific microorganisms and can provide insight on overall community structure and on the physiological and biogeochemical processes that may be occurring at a location (Table 5) (White et al., 1997; Jahnke et al., 2004).

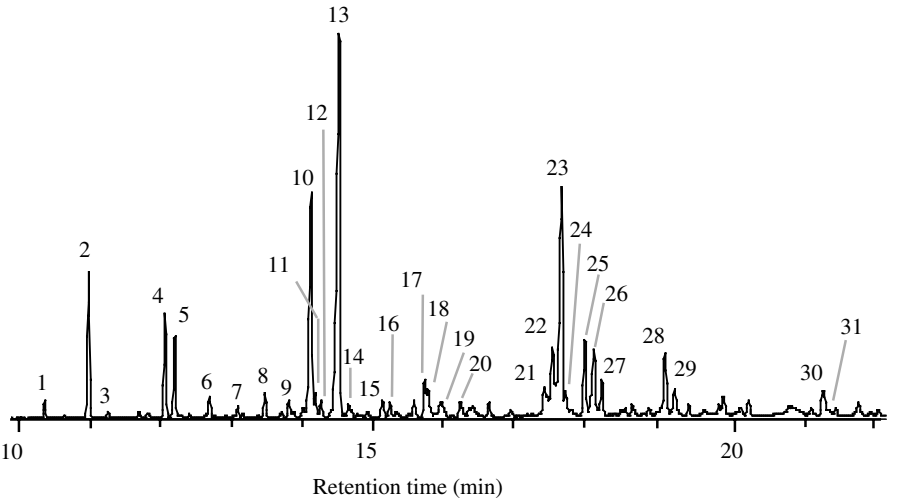

Fig. 4. Total ion chromatogram of lipopolysaccharide fatty acids. See Table 3 for compound identification and concentrations.

Table 3
Concentration (μg g^{-1} dry weight; percentage in parenthesis) of lipopolysaccharide fatty acids isolated from sediment of Salt Basin

Peak labeling[a]	Lipid	Concentration (μg g^{-1}) (%)
1	*a*14:0	1.0 (0.6)
2	14:0	10.0 (5.9)
3	β-C12	0.5 (0.3)
4	*i*15:0	7.2 (4.2)
5	*a*15:0	5.9 (3.5)
6	15:0	1.8 (1.1)
7	Phytadiene	1.1 (0.6)
8	Phytadiene	1.9 (1.1)
9	*i*16:0	1.5 (0.88)
10	16:1Δ^9*c*	19.2 (11.3)
11	16:1Δ^9*t*	1.9 (1.1)
12	16:1Δ^{11}*c*	1.4 (0.8)
13	16:0	39.2 (23.1)
14	β-C14	1.2 (0.7)
15	*i*17:0	1.7 (1.0)
16	*a*17:0	1.3 (0.8)
17	β-C15	3.2 (1.9)
18	α-C15	1.8 (1.1)
19	17:1	2.4 (1.4)
20	17:0	1.7 (1.0)
21	18:2 $\Delta^{9,12}$	4.4 (2.6)
22	18:1Δ^9	7.3 (4.3)
23	18:1Δ^{11}*c*	22.1 (13.0)
24	18:1Δ^{11}*t*	2.2 (1.3)
25	18:0	6.1 (3.6)
26	β-C16	6.6 (3.9)
27	α-C16	2.8 (1.7)
28	br-β-C17	5.6 (3.3)
29	β-C17	3.0 (1.8)
30	β-C18	3.3 (2.0)
31	α-C18	0.8 (0.5)

[a] Peak labeling is indicated in Fig. 4.

Biomarker profiles obtained in this study suggest that Salt Basin brine seeps support a unique microbial ecosystem adapted to a geochemical environment characterized by high salinity, low oxygen, and high pH (Fig. 6).

4.1. Cyanobacteria

Cyanobacteria synthesize a number of unique lipid biomarkers including *n*-alkanes, branched alkanes, fatty acids, sterols, and hopanoids (Jahnke et al., 2004 and references therein). The dominance of *n*-heptadecane ($C_{17:0}$) and the isomeric *n*-heptadecenes ($C_{17:1}$) in Salt Basin sediment suggests an input source of cyanobacteria (Table 5) (Gelpi et al., 1970; Blumer et al., 1971; Brassell et al., 1978; Shiea et al., 1990; Grimalt et al., 1992; Sakata et al., 1997; Dembitsky et al., 2000, 2001; van der Meer et al., 2003; Rontani and Volkman, 2005). This analysis is also supported by the detection of small amounts of methyl-branched alkanes (2-Me–C_{17} and 4-Me–C_{19}) commonly found in cyanobacteria (reviewed in Kenig et al., 2005).

Glycolipids constitute a significant proportion of the membrane lipids in all photosynthetic organisms including cyanobacteria (Collins and Ferrier, 1995; Kim et al., 1999; Hiroyuki et al., 2000). The detection of large amounts of glycolipid fatty acids in Salt Basin sediment suggests the presence of a large population of cyanobacteria. Indeed, filamentous cyanobacterial communities were observed in the immediate vicinity of the active saline seeps (Joeckel

Table 4
$\delta^{13}C$ and concentration (μg g^{-1} dry weight; percentage in parentheses) of neutral lipids (sterols and hopanols) isolated from sediment of Salt Basin

Peak labeling[a]	Compound	Concentration (μg g^{-1}) (%)	$\delta^{13}C$ (‰)
A	C16-ol	1.1 (0.7)	
B	Dihydrophytol	1.3 (0.8)	
C	Isophytol	3.0 (1.9)	−34.7
D	C18-ol	0.8 (0.5)	
E	Phytol	58.2 (38.1)	−37.1
F	C22-ol	1.4 (0.9)	
G	Cholest-5-en-3β-ol	5.8 (3.8)	−30.6
H	5α(H)-Cholestan-3β-ol	2.5 (1.6)	−29.7
I	24-Methylcholesta-5,22(*E*)-dien-3β-ol	2.1 (1.4)	
J	24-Methyl-5α(H)-cholest-22(*E*)-en-3β-ol	1.9 (1.2)	
K	24-Methylenecholest-5-en-3β-ol	5.6 (3.6)	−31.0
L	24-Methylcholest-5-en-3β-ol	5.9 (3.9)	−31.3
M	24-Methyl-5α(H)-cholestan-3β-ol	1.5 (1.0)	−30.7
N	24-Ethylcholesta-5,22-dien-3β-ol	5.5 (3.6)	−31.9
O	24-Ethyl-5α(H)-cholest-22(E)-en-3β-ol	1.9 (1.3)	
P	24-Ethylcholest-5-en-3β-ol	36.8 (24.1)	−32.4
Q	24-Ethyl-5α(H)-cholestan-3β-ol	4.5 (2.9)	
R	24-(*Z*)ethylidenecholest-5-en-3β-ol	6.4 (4.2)	−31.4
S	4α,24-dimethyl-5β(H)-cholest-22(*E*)-en-3β-ol	2.4 (1.6)	
T	4,4,14-trimethylcholesta-8,24-dien-3β-ol	1.1 (0.7)	
U	Tetrahymanol	0.9 (0.6)	
[b]	(22*R*)-17β(H),21β(H)-hop-29-ol	0.1 (0.1)	
[b]	(22*R*)-17β(H),21β(H)-homohop-31-ol	<0.1	
[b]	(22*R*)-17β(H),21β(H)-bishomohop-32-ol	<0.1	

[a] Peak labeling is indicated in Fig. 5.
[b] Compounds are not shown in Fig. 5 due to low concentrations.

and Ang Clement, 1999). Cyanobacteria form thin layers that are an integral part of the cryptogram layers developed in the salt flats of Salt Basin (Joeckel and Ang Clement, 1999).

It is possible that the polyunsaturated fatty acids were derived from cyanobacteria and/or phytoplankton. For example, 18:2$\Delta^{12,15}$, C18:2$\Delta^{10,13}$ have been found in cyanobacteria of the genus *Aphanizomenon* in freshwater lakes (Dembitsky et al., 2001). Cyanobacteria of other genera, *Dermocarpa*, *Xenococcus*, *Dermocarpella*, and *Myxosarcina*, as well as filamentous nitrogen-fixing cyanobacteria (e.g., *Anabaena variabilis*) contain similar PUFA, in addition to *iso*- and *anteiso* C_{15}, C_{16}, and C_{17} fatty acids (Caudales et al., 1998). Hopanoids have been found in aerobic bacteria including autotrophs, heterotrophs, purple nonsulfur bacteria, methanotrophic bacteria, and cyanobacteria (Rohmer et al., 1984; Zundel and Rohmer, 1985; Ourisson et al., 1987; Llopiz et al., 1996; Sakata et al., 1997; Summons et al., 1999). More recently, hopanoids were also detected in anaerobic bacteria of the bacterial phylum Planctomycetes (Sinninghe Damsté et al., 2004) and in *Geobacter metallireducens* and *G. sulfurreducens* (Härtner et al., 2005), two dissimilatory Fe(III)-reducing bacteria from the delta subgroup of the Proreobacteria. The detection of small amounts of C_{30}, C_{31}, and C_{32} hopanols in the anaerobic sediment of Salt Basin suggests that these compounds may be produced by cyanobacteria and possibly methanotrophs in oxic regions of the water column and/or by anaerobes in the black sulfidic sediment below the chemocline (Fig. 6). Diploptene can have a direct biological origin, because it is a major component in lipids of cyanobacteria (Gelpi et al., 1970; Sakata et al., 1997) and methanotrophic bacteria (Rohmer et al., 1984). Homohopanols and bishomohopanols are also commonly found in cyanobacteria (Rohmer and Ourisson, 1976). PLFA and sterol biomarkers suggest no methanotrophic bacterial signatures (e.g., C_{16} and C_{18} monounsaturated fatty acids at Δ^8, Δ^{10} and 4-methyl sterols), so an input of cyanobacteria and anaerobic bacteria is more likely.

Examining the $\delta^{13}C$ values of GLFA and PLFA reveals that all glycolipid fatty acids, except cy17:0 and cy19:0, are enriched in ^{13}C relative to the corresponding phospholipid fatty acids, suggesting

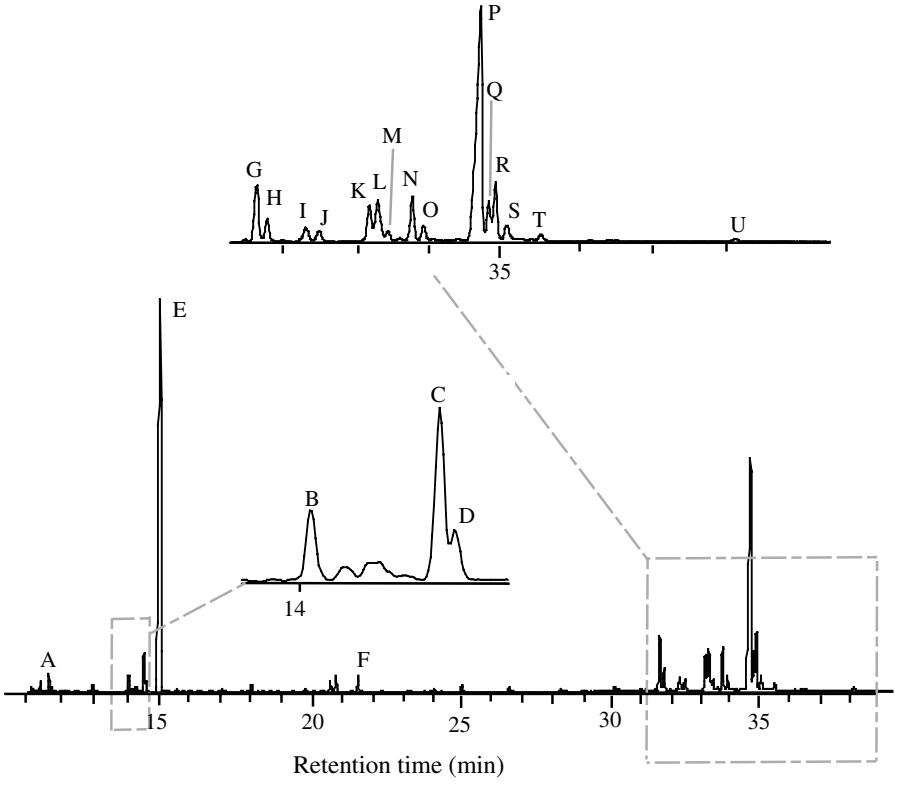

Fig. 5. Neutral lipids isolated from the black sulfidic sediment. See Table 4 for peak identification and concentrations.

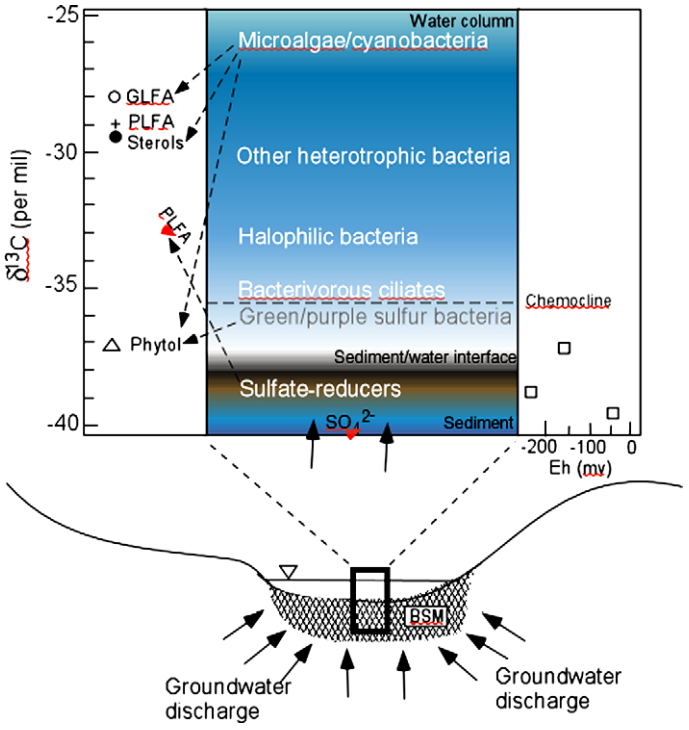

Fig. 6. Environmental conditions and microbial community structure in sediment and water column of Salt Basin.

Table 5
Possible origin of lipid biomarkers isolated from sediment of Salt Basin

Biomarkers	Origin
Hydrocarbons	
$C_{17:0}$, $C_{17:1}$, 2-Me–C_{17}, 4-Me–C_{19}	Cyanobacteria
$C_{21:6}$, $C_{21:7}$, $C_{30:4}$, $C_{30:5}$	Algae
Pristane	Oxidation products of phytol
Phytane (phytenes) and phytadienes	Products of anaerobic reduction of phytol; methanogens
Tetrahydrosqualene	Methanogens; halobacteria
Hopanoids	
Diploptene	Cyanobacteria methanotrophs
Homo- bis-, and trishomohopanes	Transformation products of bacteriohopanepolyols
Fatty acids	
10Me16:0, cy17:0, cy19:0	*Desulfobacter*
cy17:0	Green sulfur bacteria
cy19:0	Purple sulfur bacteria
$17{:}1\Delta^{9}$, 15:1	*Desulfobulbus*
*i*15:0, *a*15:0	*Desulfotomaculum*
$16{:}1\Delta^{9}$, cy17:0 and LPS β-OH fatty acids	*Desulfomonile tiedjei*
10Me16:0, $17{:}1\Delta^{11}$, $16{:}1\Delta^{9}$	*Desulfobacterium*
*i*15:0, *a*15:0, $16{:}1\Delta^{9}$, $17{:}1\Delta^{9}$, $17{:}1\Delta^{11}$	*Desulfococcus*
$17{:}1\Delta^{9}$, $17{:}1\Delta^{11}$	*Desulfovibrio*
$18{:}2\Delta^{9,12}$	*Cyanobacteria*
16:2, 20:3, 20:4	Algae
Alcohols and sterols	
C_{16}, C_{18}, C_{20} alkanol	Photosynthetic sulfur bacteria
Phytol	Phototrophs (algae, plants, cyanobacteria, and phototrophic sulfur-bacteria)
Isophytol, dihydrophytol	Halobacteria; methanogens; product of anaerobic transformation of phytol
Tetrahymanol	Bacterivorous ciliates (e.g., *Tetrahymena*); purple non-sulfur bacterium *Rhodopseudomonas palustris*
$C_{27}\Delta^{5}$, $C_{27}\Delta^{5,22}$, $C_{28}\Delta^{5,22}$	Diatoms
$C_{28}\Delta^{5,22}$, $C_{29}\Delta^{5,22}$	Haptophytes
Sitosterol	Higher plants

that most of the glycolipid fatty acids are likely from cyanobacteria, with additional input from higher plants (e.g., $C_{21–26}$ fatty acids). This is in accord with the notion that cyanobacterial ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) discriminates less against ^{13}C during inorganic carbon fixation than C_3 plants (Sakata et al., 1997). Thus, the isotopic composition of the fatty acids points to an origin from autotrophic organisms in the oxic surface water and from SRB in the anoxic zone (Fig. 6).

4.2. Sulfate-reducing bacteria and halophilic bacteria

The bacterial community of Salt Basin sediment appears to be dominated by Gram-negative bacteria, indicated by the presence of high concentrations of monounsaturated fatty acids in the PLFA and the LPS FA fractions (Ratledge and Wilkinson, 1988; Findlay et al., 1990). The detection of terminal branched fatty acids suggests that Gram-positive bacteria are present as well as Gram-negative anaerobic bacteria (Parkes and Taylor, 1983; Ratledge and Wilkinson, 1988; Kaneda, 1991; White et al., 1996; Caudales et al., 1998).

The black sulfidic mud (BSM) is a distinguishing feature of Salt Basin (Joeckel and Ang Clement, 1999) compared to other freshwater sediments in the area. The BSM are waterlogged, are sandy to muddy in coarseness and are in direct association with standing water, or with continuous saturation by through-flowing groundwater from saline seeps and springs. BSM exhibited high pH (7.8–12.1), low redox potential and high sulfide content. Bacterial sulfate reduction in Salt Basin sediment has been verified in the laboratory (Joeckel and Ang Clement, 1999). Laboratory culturing of SRB from Salt Basin sediment in Medium E containing iron and sodium sulfate resulted in growth of black bacterial colonies that produced sulfide (Joeckel and Ang Clement, 1999). Thus, sulfate reduction is likely an important process in the sediment of Salt Basin (Fig. 6).

The PLFA profiles of the black sulfidic materials in Salt Basin suggest the presence of high numbers of SRB (Table 5). The high levels of *cis*-vaccenic acid ($18{:}1\Delta^{11}$), known to be formed through the anaerobic fatty acid desaturase pathway, point to the presence of anaerobic bacteria (White et al., 1997). The branched C_{15} and C_{17} fatty acids can be attributed to *Desulfococcus multivorans* (Rütters et al., 2002), whereas the MBFA, 10Me16:0 is characteristic of *Desulfobacter* (Edlund et al., 1985; Dowling et al., 1986; Konneke and Widdel, 2003) although it has also been recognized as a biomarker for *Actinomycetes* (O'Leary and Wilkinson, 1988; Vestal and White, 1989; Sundh et al., 1997). However, SRB do not contain 10Me18:0, whereas *Actinomycetes* contain significant amounts of this fatty acid (Dowling et al., 1986; Vainshtein et al., 1992). Because 10Me18:0 was not detected in Salt Basin

sediments, the 10Me16:0 fatty acid could be derived from SRB. The detection of $16{:}1\Delta^{11}$t also suggests the presence of *Desulfobacter* as well as *Desulfomonas acetoxidans* (Dowling et al., 1986). The presence of cy17:0 and cy19:0 suggest the presence of *Desulfobacter* and *Desulfobacterium* (Konneke and Widdel, 2003; Londry and Des Marais, 2004). The C_{17} monounsaturated fatty acids (e.g., $17{:}1\Delta^{9}$; $17{:}1\Delta^{10}$, $17{:}1\Delta^{11}$) are commonly associated with SRB such as *Desulfovibrio* (Taylor and Parkes, 1983; Edlund et al., 1985; Vainshtein et al., 1992; Kohring et al., 1994; Londry and Des Marais, 2004), *Desulfobulbus* (Parkes et al., 1993; Oude-Elferink et al., 1998), *Desulfococcus*, *Desulfuromonas*, *Desulfosarcina* (Kohring et al., 1994), *Desulfomicrobium* (Vainshtein et al., 1992) as well as *Desulfobacter* (Konneke and Widdel, 2003). The occurrence of $15{:}1\Delta^{9}$ and $17{:}1\Delta^{9}$ is indicative of the presence of *Desulfobulbus* in the sediment (Taylor and Parkes, 1983; Parkes et al., 1993) and *Desulfovibrio* (Londry and Des Marais, 2004), corroborating the interpretation of C_{17} monounsaturated fatty acids. SRB are also characterized by a predominance of *iso* over *anteiso* C_{15} and C_{17} fatty acids (Table 2; Dowling et al., 1986).

Hydroxy fatty acids, recovered from acid hydrolysis of the solvent extracted sediment residue, represent those linked to disaccharides by an amide linkage in the outer membranes of Gram-negative bacteria (Edlund et al., 1985; Goossens et al., 1986). Although hydroxy fatty acids have been detected in algae (Matsumoto et al., 1984) and cyanobacteria (Schmidt et al., 1980; Matsumoto et al., 1984; Wakeham et al., 2003), the detection of abundant LPS β-hydroxy fatty acids with chain-length C_{12-17} in Salt Basin provides further evidence for the presence of SRB in the sediments (Parker et al., 1982; Edlund et al., 1985). Since LPS can be rapidly degraded in the environment (Saddler and Wardlaw, 1980), the detection of LPS hydroxyl fatty acids is indicative of viable SRB in Salt Basin sediment (e.g., Parker et al., 1982).

Phytane in sediments is derived from the reduction of phytol via isomeric phytadienes as intermediates under anoxic conditions (Didyk et al., 1978; Grossi et al., 1998; Schulze et al., 2001; Rontani and Volkman, 2003). The anaerobic degradation of phytol under sulfate-reducing conditions has been studied by Grossi et al. (1998). Phytol is readily degraded by SRB to a number of isomeric phytenes and phytadienes (Grossi et al., 1998). The profile of these reduction products in Salt Basin is similar to those from in vitro sulfate reduction experiments (Grossi et al., 1998), suggesting that an active phytol reduction process mediated possibly by SRB was taking place in the sediment.

Tetrahydrosqualene was identified in Salt Basin sediment based on comparison with published mass spectrum and retention time (e.g., Kramer et al., 1972; Rontani and Volkman, 2003). The source of the hydrogenated squalene derivatives can be from methanogens (Tornabene et al., 1979; Ferrante et al., 1986) as well as halophilic bacteria such as *Halobacterium cutirubrum* and *Sarcina marina* (Tornabene et al., 1969; Hunter et al., 1981). Dihydrophytol can be derived from reduction of free phytol (Brooks et al., 1978; Schulze et al., 2001) or from methanogens (Tornabene et al., 1979) or halophilic bacteria (Kushner, 1966; Nissenbaum et al., 1972; Volkman and Maxwell, 1986).

All of the fatty acids have relatively low δ^{13}C values. This could reflect an origin from terrestrial higher plants (Rieley et al., 1991; Huang et al., 1996; Fang et al., 2002). However, given the apparent bacterial source of certain biomarkers (e.g., mid- and terminal-branched and cyclopropane fatty acids), these ^{13}C-depleted lipids could derive from recycling of organic matter by anaerobic bacteria (e.g., SRB). This has been demonstrated by Teece et al. (1999) who showed that anaerobically synthesized fatty acids were depleted by up to 11‰ relative to aerobically produced fatty acids.

4.3. *Tetrahymena* and anaerobic phototrophic sulfur bacteria

Tetrahymanol has been found in bacteriovorous ciliates such as *Tetrahymena* (Mallory et al., 1963; Ourisson et al., 1987; Harvey and McManus, 1991) and the anaerobic phototrophic purple bacterium *Rhodopseudomonas palustris* (Kleemann et al., 1994) as well as in an anaerobic fungus (Kemp et al., 1984) and a fern (Zander et al., 1969). *Rhodopseudomonas palustris* is a purple, non-sulfur, phototrophic bacterium (Kleemann et al., 1990;Kleemann et al., 1994). This species is a metabolically versatile Gram-negative bacterium belonging to the alpha subgroup of the Proteobacteria commonly found in soil and sediment (Larimer et al., 2003).

Tetrahymena pyriformitis is widely distributed in freshwater ponds, lakes, and stream and evaporative environments (Hill, 1972; Elliot, 1973; Venkatesan, 1989). It is believed that tetrahymanol plays the same role as sterols as membrane inserts in lower

eukaryotes and some bacteria (Ourisson et al., 1987). Ciliates biosynthesize tetrahymanol only when their dietary supplies do not contain sterols which would otherwise be used as cell membrane constituents (Harvey and McManus, 1991). Thus, tetrahymanol is a biomarker for stratified environments where the bacterivorous ciliates thrive at the chemocline (oxic–anoxic interface) feeding on the anaerobic phototrophs (green and purple sulfur bacteria) (Zubkov et al., 1992; Schoell et al., 1994; Sinninghe Damsté et al., 1995; Thiel et al., 1997). In Salt Basin, chemical stratification of the water column can occur due to freshwater runoff over the dense, saline groundwater where dense populations of microaerophilic and perhaps anaerobic bacteria are expected to develop at the oxic–anoxic interface. In fact, in certain sites where chemical stratification seems to have occurred, large populations of magnetotactic bacteria, known to be both microaerophilic and anaerobic (Bazylinski and Frankel, 2004), were present (D.A. Bazylinski and J. Fang, unpublished). These organisms thrive at the oxic–anoxic interface and their presence is indicative of chemical stratification with oxygen concentration and redox gradients (Bazylinski and Frankel, 2004). It is therefore possible that ciliates and anaerobic phototrophic bacteria are the major sources of tetrahymanol in Salt Basin.

The presence of C_{16}, C_{18} and C_{20} *n*-alkanols and the absence of high-molecular weight counterparts in the sediment is consistent with the presence of photosynthetic, green non-sulfur and sulfur bacteria such as *Chloroflexus aurantiacus* and *Chlorobium* sp. (green non-sulfur and sulfur, respectively) (Shiea et al., 1991; van der Meer et al., 1998; Rontani and Volkman, 2005). Furthermore, the LPS β-14:0 fatty acid and phytol may also derive from phototrophic sulfur bacteria such as *Chromatium*, *Thiocapsa*, and *Thiocystis* (Meissner et al., 1988; Imhoff and Bias-Imhoff, 1995).

Cyclopropane fatty acids (cy17:0 and cy19:0) have commonly been used as biomarkers for SRB such as *Desulfobacterium autotrophicum* and *Desulfobacter hydrogenophilus* (Parkes and Taylor, 1983; Parkes et al., 1993; Londry and Des Marais, 2004). Cy17:0 is a major component in lipids of green sulfur bacteria (GSB) of the Chlorobiaceae (e.g., Kenyon and Gray, 1974). Cy19:0 was detected in purple sulfur phototrophic bacteria (PSB) *Ectothiorhodospira* (Grimalt et al., 1992). The green sulfur bacteria utilize a reverse or reductive tricarboxylic acid (TCA) cycle (for autotrophy) and the purple sulfur bacteria use a normal or forward TCA cycle (for processing of carbonaceous compounds) (Holo and Sirevåg, 1986; Madigan et al., 1989; van der Meer et al., 1998). Examining the $\delta^{13}C$ values of cy17:0 and cy19:0 suggest that cy17:0 in both PLFA and GLFA fractions is enriched in ^{13}C relative to cy19:0 by 3.4–4.2‰ (Table 2). It is therefore possible that part of the cy17:0 was from GSB and part of the cy19:0 was from PSB.

The $\delta^{13}C$ of phytol can provide further insight into the distribution of phototrophs in Salt Basin. Phytol can be contributed from plants, algae, cyanobacteria, and phototrophic sulfur bacteria. Putschew et al. (1996) measured the $\delta^{13}C$ value of phytol from green algae (−33.9‰), grass (−33.9‰) and purple sulfur bacteria (−47.9‰) in Lake Cadagno in the Swiss Alps. This lake is similar to Salt Basin in many ways, e.g., receiving constant inflow of groundwater enriched in sulfate and having a permanent layer of anoxic bottom water. Phytol isolated in Salt Basin sediment has a $\delta^{13}C$ value of −37.1‰ (Table 5). Apparently, microalgae and vegetation cannot be the sole source of phytol detected in Salt Basin sediments, as phytol would have similar $\delta^{13}C$ values as or be enriched in ^{13}C relative to the sterols (Bidigare et al., 1997) and fatty acids (Bach, 1995; Schouten et al., 1998). Thus, an isotopically depleted source, i.e., phototrophic purple sulfur bacteria living at the chemocline, may have contributed phytol to the sediment (Fig. 6).

4.4. Microalgae and higher plants

The distribution patterns of fatty acids and sterols in the sediment suggest that diatoms and haptophytes may be two of the main contributing sources of organic matter to Salt Basin sediment. Polyunsaturated fatty acids 16:2, 20:3 and 20:4 are typically found in diatoms (Schouten et al., 1998). Diatoms are characterized by $C_{27}\Delta^{5}$, $C_{27}\Delta^{5,22}$, $C_{28}\Delta^{5,22}$ (diatomsterol), whereas haptophytes by $C_{28}\Delta^{5,22}$ and $C_{29}\Delta^{5,22}$ (Volkman, 1986; Conte et al., 1994). $C_{29}\Delta^{5,22}$ has also been found in Chrysophyceae (Volkman, 1986). The C_{29} sterols (24-ethylcholesterol, 24-ethylcholesta-5,22*E*-dien-3b-ol) are also known as major sterols in diatoms (Volkman, 1986; Volkman et al., 1998), although these and other C_{29} sterols (Table 4) are commonly associated with higher plants (Volkman, 1986).

Marine planktonic algae (particularly diatoms) are known to contain various polyunsaturated alkenes in the $C_{21–37}$ range with up to seven double bonds (Lee and Loeblich, 1971; Volkman et al., 1998; Sinninghe Damsté et al., 2000). The most commonly found polyunsaturated alkene is *n*-$C_{21:6}$ and to a lesser extent *n*-$C_{21:5}$. However, these compounds are rarely found in sediments because they can be degraded rapidly. Thus, detecting these polyunsaturated alkenes in Salt Basin sediments further suggests the presence of algal input, perhaps from intact algal cells (Volkman et al., 1998). To the best of our knowledge, this is the first report of $C_{21:7}$ in sediment.

The contributions of organic matter from the surrounding vegetation can be inferred from sterol and high-molecular weight *n*-alkane compositions. The strong odd-number carbon preference of *n*-alkanes in the $C_{21–31}$ range indicates an origin from higher plants (Eglinton and Hamilton, 1967; Lockheart et al., 1997). The dominance of sitosterol (24-ethylcholest-5-en-3β-ol) could also suggest input from higher plants (Huang and Meinschein, 1976), and the most likely source is the vegetation growing around the basin.

5. Conclusions

Biomarkers of cyanobacteria, phototrophic sulfur bacteria, SRB, sulfide-oxidizing bacteria, and bacterivorous ciliates were identified in sediments near saline seeps in the Salt Basin of Lancaster County, Nebraska. The identification of these compounds verifies and greatly amplifies the results achieved by more conventional means (Joeckel and Ang Clement, 1999). Our results also demonstrate that distinct and comparatively unique microbial assemblages exist in shallow geological environments and in the even shallower overlying water column within a microenvironment characterized by relatively extreme chemical conditions (Fig. 6). The biomarker distributions also provide strong evidence for the type of biogeochemical processes taking place in the sediment. Microbial sulfate reduction occurs primarily in the top few centimeters of the sediment (Section 1 of the core) and is coupled with seeping saline groundwater from below and driven by production of organic matter in the water column. In contrast, the lower layer (Section 2) has equally abundant sulfate, but the concentration of PLFA in this layer is three orders of magnitude lower than that of the top layer, suggesting a drastically reduced microbial biomass and the detachment of the carbon and sulfur cycles in the lower layer. It is clear that carbon and sulfur cycles, mediated by microbes with diverse physiologies, are actively at work within mere centimeters of sediment, as long as fluxes of saline groundwater are maintained.

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