

Extraordinary ^{13}C enrichment of diether lipids at the Lost City Hydrothermal Field indicates a carbon-limited ecosystem

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Abstract

Active and inactive carbonate chimneys from the Lost City Hydrothermal Field contain up to 0.6% organic carbon with diverse lipid assemblages. The $\delta^{13}\text{C}$ values of total organic carbon range from -21.5‰ vs. VPDB at an extinct carbonate chimney to -2.8‰ at a 70 °C, actively venting carbonate chimney. Samples collected at locations with total organic carbon with $\delta^{13}\text{C} > -15\text{‰}$ also contained high abundances of isoprenoidal and nonisoprenoidal diether lipids. Samples with TOC more depleted in ^{13}C lacked or contained lower amounts of these diethers.

Isoprenoidal diethers, including *sn*-2 hydroxyarchaeol, *sn*-3 hydroxyarchaeol, and putative dihydroxyarchaeol, are likely to derive from methanogenic archaea. These compounds have $\delta^{13}\text{C}$ values ranging from -2.9 to $+6.7\text{‰}$ vs. VPDB. Nonisoprenoidal diethers and monoethers are presumably derived from bacteria, and have structures similar to those produced by sulfate-reducing bacteria in culture and at cold seeps. In samples that also contained abundant hydroxyarchaeols, these diethers have $\delta^{13}\text{C}$ values between -11.8 and $+3.6\text{‰}$. In samples without abundant hydroxyarchaeols, the nonisoprenoidal diethers were typically more depleted in ^{13}C , with $\delta^{13}\text{C}$ as low as -28.7‰ in chimneys and -45‰ in fissures.

The diethers at Lost City are probably derived from hydrogen-consuming methanogens and bacteria. High hydrogen concentrations favor methanogenesis over methanotrophy and allow the concurrent growth of methanogens and sulfate-reducing bacteria. The unusual enrichment of ^{13}C in lipids can be attributed to nearly complete consumption of bioavailable carbon in vent fluids. Under carbon-limited conditions, the isotope effects that usually lead to ^{13}C -depletion in organic material cannot be expressed. Consequently, metabolic products such as lipids and methane have $\delta^{13}\text{C}$ values typical of abiotic carbon.

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1. INTRODUCTION

The discovery of the Lost City Hydrothermal Field (LCHF) in 2000 (Kelley et al., 2001, 2005) marked the first recognition of a warm ocean-floor hydrothermal ecosystem, hosted by peridotite, with chemistry controlled by the serpentinization of ultramafic rock. Ultramafic rocks are a significant component of the oceanic lithosphere (Fruh-Green et al., 2004), may have been widespread on early Earth

(Shock and Schulte, 1998). They can be expected on other terrestrial planets containing water (Sleep et al., 2004), such as Europa (McCullom, 1999). Because of the alkalinity and reducing power produced by serpentinization, the alteration of ultramafic rocks by water has important geochemical and biological consequences. Study of these environments may bear on prebiotic chemistry, early Earth evolution, and the potential for life elsewhere.

Reducing power, in the form of hydrogen and methane, is abundant in Lost City fluids. Hydrogen is produced directly during serpentinization (Schroeder et al., 2002) and can be coupled to reduction of CO_2 to produce methane. Formation of methane is thermodynamically favored in environments where chemistry is controlled by serpentinization (Sleep et al., 2004), and may be biologically or abiotically

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catalyzed. Both the abiotic geochemical reaction (Proskurowski et al., 2008) and production of methane by methanogenic archaea (Schrenk et al., 2004; Brazelton et al., 2006) are likely to be important processes at Lost City.

Determining the relative contributions of abiotic and biological methane to Lost City vent fluids is a challenge that holds significance for our understanding of the evolution of the biosphere. Methane is an important trace gas in the modern atmosphere and was probably abundant in the atmosphere during the first two billion years of Earth history (Catling et al., 2001; Kasting and Ono, 2006), when it may have played an important role in regulating global temperature (Pavlov et al., 2001; Lowe and Tice, 2004). The role of methanogens in moderating the hydrogen level in the atmosphere is a critical component of Precambrian climate models (Kasting, 2005) and the abundance of hydrogen in the Precambrian atmosphere is hotly debated (Tian et al., 2005, 2006; Catling, 2006). Understanding the potential for contributions to atmospheric methane by sites like Lost City, and the importance of biology in making that contribution, will influence these models. Methane has also been detected in the Martian atmosphere (Formisano et al., 2004). Its source is unknown but derivation from peridotite-hosted hydrothermal sites is not inconceivable.

Biological methanogenesis requires no direct or indirect byproducts of oxygenic photosynthesis and may be one of the more ancient metabolic strategies on Earth. There is evidence for methanogenesis as far back in Earth history as the Archean (Ueno et al., 2006), although some of this evidence has been subsequently challenged (Sherwood Lollar and McCollom, 2006), and some biologists dispute the early evolution of methanogenic archaea (Cavalier-Smith, 2002). Hydrogen as an electron donor can also be coupled to other electron acceptors such as sulfate and, when these electron acceptors are present, organisms performing these reactions generally outcompete methanogens (Kristjansson et al., 1982; Hoehler et al., 1998).

The LCHF is located on the peridotitic Atlantis massif 15 km west of the axis of the mid-Atlantic ridge at a depth of 750–900 m. Fluids are alkaline, reducing, and cool relative to those at magma-driven hydrothermal systems on the mid-ocean ridge axes. Cooling of the underlying massif is the main driver of hydrothermal circulation at Lost City (Allen and Seyfried, 2004), with additional contributions from exothermic serpentinization reactions (Kelley et al., 2001, 2005). Fluid temperatures range from <40 to 90 °C with pH between 9 and 11, Ca^{2+} concentrations up to 30 mmol/kg, CH_4 concentrations up to almost 2 mmol/kg, and hydrogen concentrations up to nearly 15 mmol/kg. Recently, Proskurowski et al. (2008) have reported the abundances of ^{13}C , ^{14}C , and ^2H in volatile hydrocarbons from the Lost City hydrothermal fluids. Their results show that the methane derives entirely from mantle carbon but cannot quantitatively resolve microbial and abiotic contributions.

The most spectacular features of the LCHF are massive calcium carbonate and brucite chimneys, which grow up to 60 m tall from the peridotite seafloor. Radiocarbon mea-

surements indicate that the carbonate has been precipitating at its present location for at least 30 ky (Fruh-Green et al., 2003). The carbonate has $\delta^{13}\text{C}$ between +1.4 and +2.9‰ and $\delta^{18}\text{O}$ between +1.5‰ and +5.2‰ relative to VPDB, while $^{87}\text{Sr}/^{86}\text{Sr}$ values are between 0.70760 and 0.70908. Calcium carbonate precipitates as vent fluids come into contact with seawater. The Ca^{2+} source is dominantly hydrothermal, while the CO_3^{2-} derives predominantly from seawater (Fruh-Green et al., 2003; Ludwig et al., 2006). Measurements of Sr isotopes and wt% Mg in carbonate chimneys have shown that mixing ratios of seawater and vent fluids vary between chimneys (Ludwig et al., 2006).

The carbonate towers host a rich microbial ecosystem. Analyses of 16S rRNA gene sequences and cell counts by fluorescence in situ hybridization (FISH) show that assemblages in active vent structures are dominated by archaea (Kelley et al., 2005; Brazelton et al., 2006), with Methanosarcinales prevalent in active, high-temperature vent structures. ANME-1 group methanotrophs are present in two samples from less active carbonate veins and at one active vent (Schrenk et al., 2004; Kelley et al., 2005; Brazelton et al., 2006). Studies of bacterial diversity indicate that Firmicutes, along with methane-oxidizing and sulfur-oxidizing bacteria, are present in fluids at higher-temperature, active vents (Kelley et al., 2005; Brazelton et al., 2006). The coexistence of obligate aerobes and obligate anaerobes in the same samples suggests that different microbial communities inhabit various environments around the vents, possibly an anaerobic community inhabiting reducing anaerobic hydrothermal fluid within carbonate structures and aerobic microbes inhabiting zones on carbonate surfaces where hydrothermal fluid mixes with oxygenated seawater.

Here we report on the chemical and isotopic composition of organic matter in the carbonate chimneys at the LCHF. These data shed light on the nature of the Lost City Methanosarcinales and on the biological production of methane.

2. ANALYTICAL METHODS

Carbonate samples were collected during Atlantis cruise AT-7-41 using the submersible *Alvin* and stored in teflon containers at $-20\text{ }^\circ\text{C}$ until processing. Marker locations refer to specific sites within the hydrothermal field and have been standardized across multiple expeditions and reports (Kelley et al., 2005). The first four digits of each sample number specify the *Alvin* dive number. The last four specify the time of collection (1640 = 2:40 PM). These numbers are also standardized across all reports.

Total organic carbon contents of carbonates were determined by weighing freeze-dried and finely crushed carbonate samples (20–40 mg) in triplicate into clean silver capsules. The silver capsules were placed in an evacuated chamber for seven days with vapor in equilibrium with concentrated HCl and several grams of P_2O_5 , which served as a desiccant. Complete removal of carbonate was verified by addition of 50–100 μl of concentrated HCl directly to the silver capsules at 60 °C. Samples were dried at 60 °C overnight, and residual CaCl_2 was allowed to remain with the samples. Silver capsules were

combusted in a Fisons Elemental Analyzer at 1030 °C coupled to a ThermoFinnigan Delta^{plus} XL isotope ratio monitoring mass spectrometer. Inspection of the resulting CO₂ traces with ThermoFinnigan IsoDat software confirmed the absence of any residual carbonate in the samples. Total organic carbon contents were calculated by integration of peak areas and comparison with an external standard with known carbon content. Stable carbon isotope ratios were determined using an external CO₂ standard calibrated to international reference materials NBS-22 oil, CH-6 sucrose, and an internal lab standard (acetanilide). They are reported relative to the Vienna Pee Dee Belemnite (VPDB) isotopic standard.

Subsamples of carbonate were freeze-dried and crushed to a fine powder, then ultrasonically extracted three times (ca. 30 min) in a mixture of dichloromethane (DCM): methanol (3:1, v/v), and all three extracts were combined. Extracts were centrifuged at 2000 rpm for 15 min to remove residual carbonate particles and then the bulk of solvent was evaporated at 35 °C under a stream of dry nitrogen. Elemental sulfur was removed from the extracts by passing them over a column of activated copper, followed by filtration of the extract through a 40-μm combusted glass Buchner funnel. Total lipid extracts were weighed and are reported as μg of lipid per gram of dry rock extracted.

Aliquots of lipid extracts were analyzed as their trimethylsilyl ethers and esters by reacting with *N,O*-bis(trimethylsilyl)trifluoro-acetamide (BSTFA + 1% TMCS) in pyridine at 60 °C for 30 min. The remainder of the total lipid extract was separated over silica gel into five fractions using an elution scheme of solvents of increasing polarity: aliphatic hydrocarbons 1% dead column volume (DV, measured by slow addition of the first eluent with calibrated syringe) hexane, aromatic hydrocarbons 2 DV 4:1 hexane:DCM, ketones 2 DV DCM, alcohols 2 DV 4:1 DCM:ethyl acetate, fatty acids and diols 2 DV 7:3 DCM:methanol. Individual lipids were identified using a HP 6890 gas chromatograph fitted with a PTV injector operated in splitless mode and equipped with a Varian CP-Sil-5 (60-m length, 0.32-mm inner diameter, and

0.25-μm film thickness) fused silica capillary column and coupled to an Agilent 5973 mass-selective detector. Lipids were identified by comparisons of mass spectra and retention times with authentic standards or samples where these compounds have previously been characterized. Alcohols and fatty acids were analyzed as TMS derivatives. Diether lipids were identified by comparison to similar authentic standards, and we report their masses without attempting to solve their detailed structures. Lipids were quantified relative to co-injected standards.

Carbon-isotopic compositions of individual lipids were determined using a TraceGC gas chromatograph fitted with a PTV injector and equipped with a Varian DB-1 (60-m length, 0.32-mm inner diameter, and 0.25-μm film thickness) fused-silica capillary column and coupled to a ThermoFinnigan Delta^{plus} XL isotope-ratio-monitoring mass spectrometer via a combustion interface at 850 °C. Column temperatures were programmed from 60 °C at 10 °C/min to 100 °C, to 320 °C at 4 °C/min, and then held isothermal for 20 min. Carbon isotope ratios were determined relative to an external CO₂ standard that was regularly calibrated relative to a reference mixture of *n*-alkanes (Mixture B) provided by Arndt Schimmelmann (Indiana University). All lipid isotopic compositions were corrected by mass balance for the carbon present in the TMS group(s).

3. RESULTS

3.1. Total organic carbon

Thirty-seven carbonate samples had total organic carbon contents averaging about 0.2% and varying between approximately 0.05% and 0.6% TOC (Fig. 1a). The ¹³C content of the organic carbon was widely variable with δ¹³C from −27.7‰ to −2.8‰. TOC and δ¹³C were uncorrelated (Fig. 1a). Samples taken from various parts of the same carbonate tower had values of δ¹³C_{TOC} varying by up to 14.4‰, but replicate measurements for a single sample yielded variations <0.5‰. Accordingly, larger variations reflect natural heterogeneity within a tower.

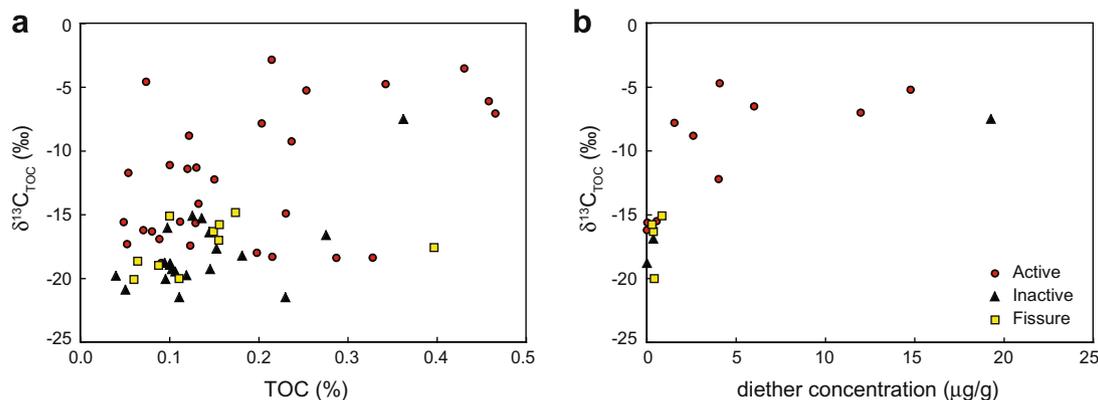


Fig. 1. (a) A cross-plot of total organic carbon versus δ¹³C_{TOC} for all carbonate chimney samples, grouped as active, inactive, and fissure samples. (b) A cross-plot of diether content versus δ¹³C_{TOC} for samples with lipid analyses, showing that samples with TOC highly enriched in ¹³C contain higher amounts of isoprenoidal and nonisoprenoidal diether lipids.

3.2. Lipid analyses

3.2.1. Isoprenoidal ether lipids and alkanes

Archaeol was detected in approximately one-half of the samples (Table 1). Concentrations of archaeol ranged up to 600 ng of archaeol per gram of dry carbonate. In most cases archaeol was enriched in ^{13}C relative to marine phytoplanktonic products, which typically have $\delta^{13}\text{C}$ near -20‰ at 30° north (Goericke and Fry, 1994). Archaeol had $\delta^{13}\text{C}$ values up to $+6.0\text{‰}$, and the average value of $\delta^{13}\text{C}_{\text{archaeol}}$ in carbonate chimneys was $+1.5\text{‰}$. Only one sample contained archaeol with $\delta^{13}\text{C} < -4.2\text{‰}$. That sample, with $\delta^{13}\text{C}_{\text{archaeol}} = -77\text{‰}$, was an inactive carbonate collected on the eastern fringe of the vent field (Marker X2, 30 m east of Marker H; sample 3880-1557).

With the single exception of sample 3880-1557, all samples containing archaeol also contained *sn*-2 hydroxyarchaeol, *sn*-3 hydroxyarchaeol, and a putative dihydroxyarchaeol. Both *sn*-2 hydroxyarchaeol and dihydroxyarchaeol were more abundant than archaeol. Concentrations of archaeol were strongly covariant with those of *sn*-2 hydroxyarchaeol and dihydroxyarchaeol. The $\delta^{13}\text{C}$ values of archaeol covaried with those of *sn*-2 hydroxyarchaeol (Fig. 2a), but not with those of dihydroxyarchaeol (Fig. 2b). The concentration of *sn*-2 hydroxyarchaeol averaged 2.6 times that of dihydroxyarchaeol, and both of these compounds were more abundant than *sn*-3 hydroxyarchaeol (Fig. 3c).

Two unidentified compounds with mass spectra similar to those of hydroxyarchaeols were common at Lost City—one eluting after *sn*-3 hydroxyarchaeol and the other just after dihydroxyarchaeol (Fig. 3a and b). The mass spectra of these compounds showed prominent ions at *m/z* 143, 237, and 341, and a smaller ion at *m/z* 515. This pattern is similar to that seen in putative dihydroxyarchaeol. A fragment at *m/z* 278 in the spectrum shown in Fig. 3a may indicate that a nonhydroxylated phytanyl moiety is bound to glycerol by an ether linkage (Hinrichs et al., 2000a). Together with the retention times, these spectra suggest that these unknown compounds are structurally related to the hydroxyarchaeols.

The hydrocarbon 2,6,10,16,19-pentamethylcosane (PMI; $+7 \leq \delta^{13}\text{C} \leq +10\text{‰}$; Table 1) was present in two samples and had $\delta^{13}\text{C}$ values that were the highest of any compound detected.

3.2.2. Nonisoprenoidal ether lipids

Nonisoprenoidal ether lipids were present in nearly all samples analyzed (Table 1). Diethers were most abundant and ranged in mass from 556 Da (C_{31} -TMS) to 664 Da ($\text{C}_{39:2}$ -TMS), with saturated and unsaturated side chains ranging in length from 13 to 18 carbons. The range of ether-lipid structures at Lost City is similar to that described in cold-seep carbonate crusts associated with Mediterranean mud volcanoes (Pancost et al., 2001a; Bouloubassi et al., 2006), including the Series I, II, and III components. Similar structures have been described from other AOM environments (Hinrichs et al., 2000a; Elvert et al., 2005), and we detect a wide range of structures, including several additional series of diethers that do not

fall into the categories described in these previous reports. Retention indices and mass spectra were consistent with the presence of unbranched alkyl chains along with *iso*-, *anteiso*-, and $\omega 7$ methylation patterns. Hydrogen deficits were commonly due to alicyclic rings (cyclopropyl, cyclohexyl) rather than to double bonds. Several samples also contained glycerol monoethers, with $\text{C}_{16:1}$ and C_{18} nonisoprenoidal side chains, with unknown double bond positions, being most abundant.

The nonisoprenoidal ether lipids, like the isoprenoidal diethers, were frequently enriched in ^{13}C . The most extreme enrichments in these compounds are associated with samples in which hydroxyarchaeols are also abundant. In samples containing both *sn*-2 hydroxyarchaeol and dihydroxyarchaeol, the $\delta^{13}\text{C}$ values of nonisoprenoidal diethers ranged from -11.8‰ to $+3.6\text{‰}$ (Table 1 and Fig. 4) and those of monoethers ranged from -19.2‰ to -3.9‰ . Nonisoprenoidal diethers were typically 2–10‰ more depleted in ^{13}C than hydroxyarchaeols, while nonisoprenoidal monoethers were the most ^{13}C depleted of these three compound classes.

In carbonate tower samples where hydroxyarchaeols were not abundant, nonisoprenoidal diethers had $\delta^{13}\text{C}$ ranging from -14.3 to -28.7‰ . Monoether $\delta^{13}\text{C}$ values ranged from -19.6 to -26.7‰ . There was one exception: a sample from Marker 3 (3881-1408) in which archaeal lipids were absent had nonisoprenoidal diethers with $\delta^{13}\text{C}$ averaging near -6‰ .

3.2.3. Fatty acids

Free fatty acids, potentially derived from both eukaryotes and bacteria, were detected in nearly all samples. Concentrations ranged to over 1500 ng per gram of dry rock. Abundances and $\delta^{13}\text{C}$ values of fatty acids are listed in Table 1. Fatty acids had highly variable contents of ^{13}C , spanning a range from approximately -1‰ to -27‰ . The most ^{13}C -enriched fatty acids were found in samples containing abundant hydroxyarchaeols. In many of these, the ^{13}C content of fatty acids showed a strong pattern in which saturated fatty acids were more enriched in ^{13}C than monounsaturated fatty acids (Table 1). The most ^{13}C -depleted fatty acids at Lost City were found in the carbonate veins, where all detected lipids had $\delta^{13}\text{C} < -15\text{‰}$.

3.2.4. Polycyclic triterpenoids

Sterols were detected in all samples analyzed. Cholesterol, commonly derived from animals but also from some algae such as eustigmatophytes, was the most frequently detected sterol and occurred in concentrations up to 580 ng per gram of dry carbonate. Stigmasterol and β -sitosterol, phytosterols often derived from marine algae, were also commonly detected. Ergosterol, a common fungal sterol, was detected in trace amounts in a few samples. Cycloartenol was commonly detected in concentrations approaching 500 ng per gram of dry rock. Its identity was confirmed by its mass spectrum and by coinjection with an authentic standard.

Sterols were not in any case as exceptionally ^{13}C -enriched as diethers. Cholesterol ranged in $\delta^{13}\text{C}$ from approximately -28‰ to -23.2‰ . Phytosterols had a slightly wider

Table 1

Individual lipid abundances and $\delta^{13}\text{C}$ values of selected samples. Sample number and Marker (Kelley et al., 2005) are noted and correspond to locations and samples noted in other reports (Kelley et al., 2005; Brazelton et al., 2006; Ludwig et al., 2006; Proskurowski et al., 2006). $\delta^{13}\text{C}_{\text{TOC}}$ and information about vent activity (A = active, I = inactive, or F = fissure Ludwig et al., 2006) and temperature is supplied where that information is known. All lipid concentrations are in μg lipid per gram of dry rock. Blank entries indicate that that lipid was not detected in a particular sample.

Marker	2	2	3	near 7	7	7	7	7	B	C	C	H	H	X1	X2																	
Sample number	3864-1524	3864-1537	3862-1219	3865-1322	3867-1225	3867-1228	3879-1605	3876-1436	3869-1404	3869-1446	3881-1325	3881-1228	3876-1133	3880-1557																		
Temperature	64	53.5	59	—	34	—	52	90	70	70	46	—	—	—	—																	
Vent activity	A	A	A	F	A	I	A	A	A	A	I	A	F	F	F																	
TOC (%)	0.11	0.12	0.15	0.10	0.25	0.36	0.44	0.07	0.20	0.34	0.10	0.09	0.15	0.11	0.11																	
$\delta^{13}\text{C}_{\text{TOC}}$ (‰, VS. VPDB)	-15.5	-8.8	-12.2	-15.1	-5.2	-7.5	-6.5	-16.2	-7.8	-4.7	-18.8	-16.9	-16.3	-20.0	-20.0																	
Total lipid ($\mu\text{g}/\text{g}$)	304	253	92	35	235	217	364	6	53	91	3	36	76	105																		
	Concentration	$\delta^{13}\text{C}$																														
	($\mu\text{g}/\text{g}$)	(‰)																														
archaeal lipids																																
Archaeol	0.02	-0.4	0.12	-0.8	0.01	-2.5	0.61	0.7	0.42	6.0	0.16	1.3	0.03	6.7	0.10	5.7	0.01	-4.2	0.01	-77.0												
<i>sn</i> -2 hydroxyarchaeol	0.15	2.4	0.21	0.2	0.07	1.3	5.94	1.3	6.66	4.2	1.80	0.7	0.39	5.1	0.97	4.8	0.01	-2.2	0.18	0.6												
<i>sn</i> -3 hydroxyarchaeol	0.03	1.9	—	0.01	0.2	1.25	—	-2.1	0.67	-1.5	0.39	0.5	0.03	0.8	0.12	6.0	—	0.08	4.8	—												
Dihydroxyarchaeol	0.05	3.8	0.09	1.1	0.02	1.2	3.47	-0.4	1.97	3.8	1.09	0.5	0.11	6.6	0.40	5.3	—	0.09	-2.9	—												
PMI																																
C16:1 fatty acid	0.02	-12.9	0.11	-13.5	0.26	-28.3	0.17	-13.2	0.28	-25.4	0.06	-19.5	0.10	-20.7	—	0.02	-27.3	0.08	-17.0	0.00	0.24	-14.7	0.39	-21.5								
C16 fatty acid	0.06	-7.3	1.62	-6.8	0.75	-8.8	0.15	-13.4	1.56	-7.5	0.09	-8.6	0.73	-9.1	0.01	-31.4	0.04	-25.7	0.52	-4.3	0.01	0.33	-12.0	0.12	-27.4							
C18:1 fatty acid	0.06	-13.3	0.47	-13.4	0.15	-17.6	0.08	-20.1	0.41	-22.5	0.03	-16.8	—	0.04	—	-25.4	0.14	-20.3	0.01	-20.3	0.01	0.85	-12.0	0.16	-27.5							
C18 fatty acid	0.02	-8.7	0.53	-8.9	0.10	-5.4	0.01	-20.4	0.51	-1.1	0.10	-2.2	0.21	-8.7	0.01	-30.2	0.02	-15.3	0.21	-8.0	0.01	0.11	-12.4	0.02	-25.0							
polycyclic triterpenoids																																
Cholesterol	0.04	-27.7	0.35	0.25	—	-28.5	0.10	-24.7	0.29	-26.1	0.10	-27.7	0.06	-26.3	0.49	-27.5	0.06	-23.3	0.01	-26.4	0.58	-23.0	0.01	-26.4	0.58	-27.0	0.09	-28.6				
Brassicasterol (C28:2)						0.05	—	0.03	—	0.03	—	-20.1	0.09	-25.9	0.02	-21.7	—	-21.7	—	-21.7	—	0.12	—	-28.2	0.01	-28.1						
Stigmasterol (C29:2)						0.03	—	-20.3	—	0.13	—	-24.9	—	-27.0	0.20	-24.6	0.01	-25.5	—	-25.5	—	-25.5	—	-25.5	—	-27.7						
Stigmasterol (C29)						0.10	—	-25.8	—	0.09	—	0.02	—	0.15	—	-22.3	0.01	-23.4	—	-23.4	—	0.09	—	-28.6	0.05	-26.4						
Cycloartenol	0.10	-15.4	—	0.49	—	-15.9	0.15	-17.6	—	0.04	—	-25.1	—	—	—	-22.3	0.01	-23.4	—	-23.4	—	0.27	—	-27.5	0.02	-29.8						
Diploptene						0.08	—	0.04	—	0.04	—	-19.2	—	0.03	—	-21.4	0.14	—	—	-21.4	—	0.03	—	0.03	—	0.03						
Tetrahymanol	0.03	-4.5	0.15	-3.5	—	0.04	—	-15.8	Trace	0.04	—	0.04	—	0.08	—	0.01	—	-15.7	0.14	-4.6	0.06	-16.9	0.04	-16.9	0.04	-26.5						
Diplopterol	0.01	-3.2	—	0.74	—	-18.5	Trace	0.07	—	0.03	—	0.04	—	0.23	—	0.02	—	-19.3	0.10	-20.4	0.7	-18.2	0.12	-18.2	0.12	-22.0						
C19:1 GME	0.01	-6.0	0.15	-6.0	—	0.01	—	-21.4	—	0.03	—	-9.3	0.01	-13.5	—	-6.1	0.04	-6.7	0.00	-6.7	0.00	0.04	—	-14.0	0.06	-26.4	0.02	-23.9				
C21 GME	0.02	-10.6	0.14	-8.1	—	0.02	—	-19.6	0.03	-11.5	0.07	-19.2	0.01	-12.9	—	0.004	—	-10.6	0.06	-3.9	0.00	0.13	—	-9.7	0.08	-23.3	0.03	-27.7				
C33:1 GDE						0.14	-4.3	0.42	-4.9	—	—	0.33	—	-5.0	2.37	-2.0	0.26	-5.6	—	-1.1	0.47	2.6	—	0.06	—	0.02	-30.3					
C33 GDE						0.16	-6.0	—	—	—	—	0.25	—	-6.4	0.12	-5.5	0.08	-6.1	—	0.05	—	0.8	0.14	-2.2	0.00	-18.2	0.01	-6.8	0.19	-22.1	0.20	-41.3
C35:2 GDE	0.03	-4.6	0.17	-2.9	—	0.07	—	-19.8	0.93	-6.0	3.50	-3.5	—	0.05	—	-5.3	1.03	-1.4	0.02	-25.6	—	-25.6	—	-25.6	—	-25.6	—	-25.6				
C36:1 GDE						0.34	—	-4.6	—	—	—	-4.6	—	—	—	—	—	—	—	—	—	—	—	—	—	0.01	-45.0					
C37:2 GDE	0.03	-6.4	0.28	-2.5	—	0.12	—	-18.9	—	0.24	—	-7.7	—	—	—	—	—	—	—	—	—	—	—	—	—	0.03	-36.9					

^a Representative structures are shown in the Appendix. GME and GDE refer to glycerol mono- and diethers, respectively. In CX:Y, X refers to the sum of glycerol and substituent (but not derivative) carbon atoms and Y indicates the number of rings or double bonds which must be present.

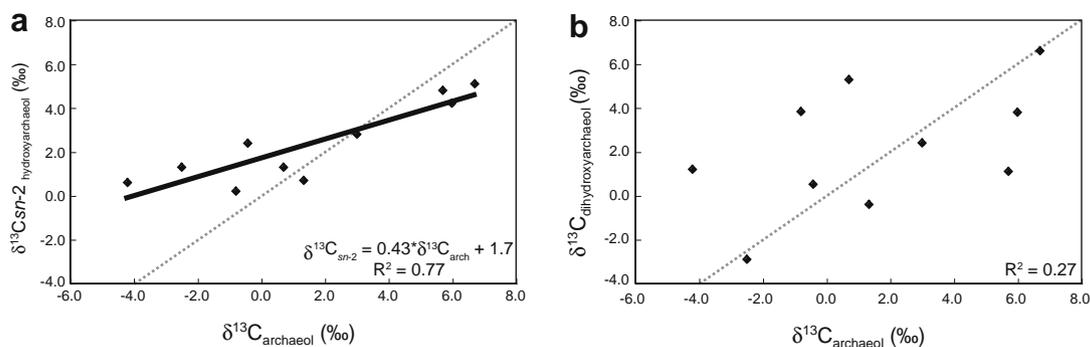


Fig. 2. (a) Correlations of $\delta^{13}\text{C}$ values of archaeol vs. $sn-2$ hydroxyarchaeol, (b) correlations of $\delta^{13}\text{C}$ values of archaeol vs. dihydroxyarchaeol. Black lines are 1:1 lines.

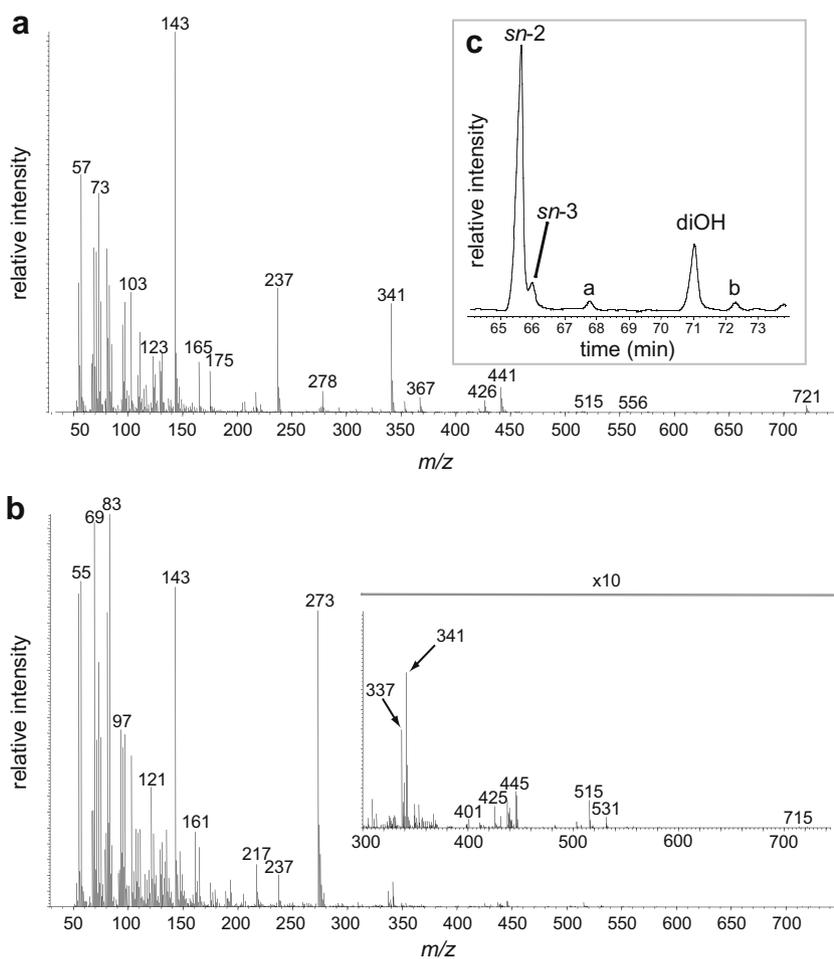


Fig. 3. Mass spectra of unknown compounds detected eluting after $sn-3$ hydroxyarchaeol (a) and after dihydroxyarchaeol (b). (c) Chromatogram showing relative elution times and peak heights of compounds a, b, $sn-2$ hydroxyarchaeol ($sn-2$), $sn-3$ hydroxyarchaeol ($sn-3$), and dihydroxyarchaeol (diOH).

range of $\delta^{13}\text{C}$, from approximately -20‰ to -29‰ . The ^{13}C contents of cycloartenol were more variable, with the lowest $\delta^{13}\text{C}$ value near -30‰ in the carbonate vein X2, while cycloartenol in carbonate chimneys containing abundant archaeol lipids had $\delta^{13}\text{C}$ as high as -15.4‰ .

The C_{30} hopanoids diploptene and diplopterol, which are derived from bacteria, were also detected in a number of the vent chimneys. Concentrations of these hopanoid products ranged up to 740 ng per gram of dry carbonate. Their $\delta^{13}\text{C}$ values ranged from -26‰ to -2‰ . The polycyclic triterpenoid tetrahymanol, often derived from marine

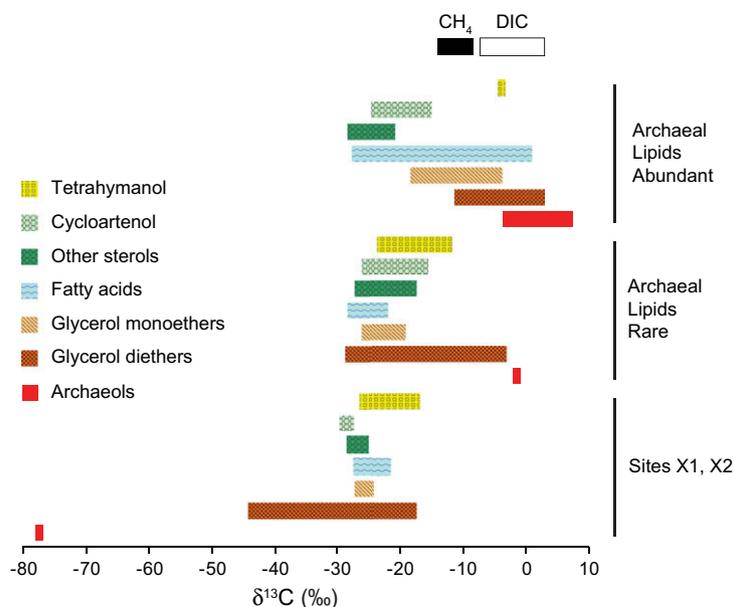


Fig. 4. Ranges of carbon isotopic compositions for different compound classes at the LCHF. Ranges for methane and DIC are measured from fluid samples over the entire field (Kelley et al., 2005). Ranges for lipids are reported by grouping carbonate samples into those with abundant archaeal lipids (3862-1219, 3864,1524, 3864-1537, 3867-1225, 3867-1228, 3869-1404, 3869-1443, 3869-1446, 3879-1605), rare archaeal lipids (3865-1322, 3876-1133, 3876-1219, 3876-1436, 3881-1132, 3881-1325, 3881-1408), and the ANME-dominated samples (Brazelton et al., 2006) at markers X1 and X2 (3880-1557).

ciliates, was detected in concentrations up to 150 ng per gram of dry carbonate. The ¹³C content of tetrahymanol ranged from -3.5 to -26.5‰, similar to the range in bacterial lipids.

4. DISCUSSION

Organic material in Lost City carbonates must derive from a combination of sources: from the microbial communities inhabiting the carbonate chimney interiors, from organic detritus in the marine water column which is trapped in carbonate chimneys as they precipitate, from microbial and animal communities on the chimney exteriors, and potentially from the subsurface from which fluids are derived. Examination of the structures and isotopic compositions of biomarker lipids allows us to begin to disentangle these sources and to improve our understanding of the microbial communities and carbon cycling in Lost City vents.

4.1. Total organic carbon

Exceptional enrichment in the ¹³C content of total organic carbon is most prevalent in actively venting structures (Fig. 1a). Of samples that are characterized as active or inactive, with one exception (3867-1228) only active structures contained total organic carbon with δ¹³C > -10‰, and inactive structures (except 3867-1228) all contained TOC with δ¹³C < -15‰. This strongly implies that the microbial community within active vents is the source of ¹³C-enriched organic carbon.

Examination of the biomarker content of the samples containing ¹³C-enriched organic carbon revealed another pattern: ¹³C-enrichment was detected only in those samples in which diether lipids are abundant. In Fig. 1b, the δ¹³C of total organic carbon is plotted versus the total content of diether lipids (isoprenoidal and nonisoprenoidal) in a range of samples. It is evident that total organic carbon has δ¹³C > -10‰ only in those samples in which concentrations of diethers exceed ca. 1 microgram per gram of rock. This suggests that biomass derived from diether-producing organisms is the source of ¹³C-enriched organic material, as discussed below.

4.2. Archaeal Methanogenesis at Lost City

In environmental samples, hydroxyarchaeols, particularly *sn*-2 hydroxyarchaeol, are usually related to the presence of the archaeal orders Methanosarcinales or Methanococcales, including the ANME-2 group of archaeal methanotrophs (Hinrichs et al., 2000b; Pancost et al., 2001b; Blumenberg et al., 2004). There are a few exceptions to this rule, however. Within the Methanobacteriales, *Methanosphaera* species have been reported to synthesize *sn*-2 hydroxyarchaeol (Koga et al., 1998; Spratt et al., 1999), as has *Methanobrevibacter* (Spratt et al., 1999). One halophilic archaeon, *Natronobacterium*, is also reported to synthesize the compound (Upasani et al., 1994). The compound *sn*-3 hydroxyarchaeol is presumed to derive predominantly from methanogens and has been reported from lipid extracts of *Methanothermococcus thermolithotrophicus* (Summons et al., 2006b).

In many Lost City carbonates, *sn*-2 hydroxyarchaeol was the single most abundant lipid detected. Its concentration was strongly covariant with those of archaeol and dihydroxyarchaeol, suggesting that perhaps all of these compounds are synthesized by a single archaeal strain, or at least by organisms that commonly co-occur. Carbon isotopic patterns confirmed the close association of archaeol and *sn*-2 hydroxyarchaeol, although they failed to demonstrate a similar relationship between archaeol and dihydroxyarchaeol (Fig. 2), suggesting an additional source for one of these compounds.

Unknown compounds with mass spectra indicating a structural relationship to hydroxyarchaeols were also detected at Lost City (Fig. 3). Compounds with similar retention times and spectra have been detected in sediments at the Haakon Mosby Mud Volcano (Elvert and Niemann, 2008), which contains ANME-3 methanotrophs. The ANME-3 group is very closely related to the methanogens found at Lost City (Losekann et al., 2007). These compounds may be specific to this clade.

Microbiological evidence from Lost City carbonates is consistent with the derivation of hydroxyarchaeols from Methanosarcinales. Archaeal diversity is low in Lost City carbonates (Schrenk et al., 2004; Brazelton et al., 2006), which are dominated by a phylotype denoted as LCMS (Lost City Methanosarcinales). It is the sole archaeal phylotype in most carbonate chimneys. Exceptions are samples from Marker C, which contain 16S rRNA gene sequences corresponding to both LCMS and ANME-1, and two samples from carbonate veins hosted in serpentinite, which contain ANME-1 only (X2) or ANME-1 together with a very small amount of LCMS (X1). At high temperatures, Methanosarcinales are generally outcompeted by methanogens that conserve energy without the involvement of cyto-

chromes (Thauer et al., 2008). However, Methanosarcinales are the group of methanogens that can achieve the highest growth yields on hydrogen and CO_2 (Thauer et al., 2008), and the very high concentrations of hydrogen may help to explain their success at Lost City.

In marine sediments and cold seeps, hydroxyarchaeols from methanotrophic archaea commonly are extremely depleted in ^{13}C , with $\delta^{13}\text{C}$ values ranging from about -80 to -130‰ (Hinrichs et al., 1999, 2000b; Boetius et al., 2000; Pancost et al., 2000). In sharp contrast, the hydroxyarchaeols at Lost City are highly enriched in ^{13}C , with an average $\delta^{13}\text{C}$ value near $+2\text{‰}$ (Table 1). PMI, also likely derived from Methanosarcinales, has $\delta^{13}\text{C} = +10\text{‰}$, the most ^{13}C -enriched lipid yet reported to date from a natural environment.

The striking enrichment must hold clues to the nature of the Methanosarcinales at Lost City. The LCMS phylotype has, in some cases, been included in the newly defined ANME-3 group, apparently on the basis of the close correspondence of its 16S rRNA gene sequence to sequences from environments where methane is being oxidized anaerobically, such as the Haakon Mosby Mud Volcano (Knittel et al., 2005). In such cases, products of the ANME-3 group, notably archaeol and *sn*-2 hydroxyarchaeol, are highly depleted in ^{13}C (Niemann et al., 2006). Later reports have defined the ANME-3 clade more narrowly, and exclude LCMS (Losekann et al., 2007). The isotopic enrichment observed here supports this view, particularly indicating that the LCMS phylotype is not methanotrophic. Three convergent lines of evidence support this deduction.

First, values of $\delta^{13}\text{C}$ for methane at Lost City range from -13.6 to -9.4‰ (Kelley et al., 2005). The archaeal lipids are significantly enriched in ^{13}C relative to methane (Fig. 5), reversing the relationship observed in archaeal

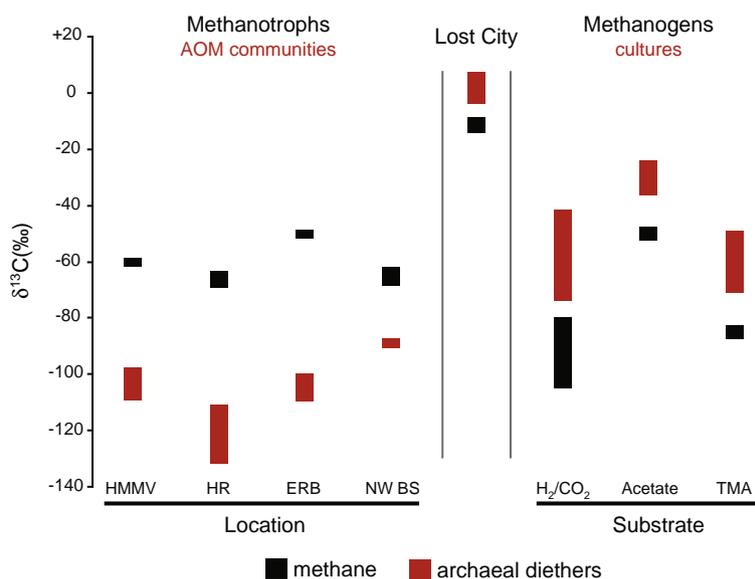


Fig. 5. Ranges of the $\delta^{13}\text{C}$ values of archaeal diethers and of methane in AOM communities (HMMV: Haakon Mosby Mud Volcano (Niemann et al., 2006; Losekann et al., 2007); HR: Hydrate Ridge (Elvert et al., 1999; Boetius et al., 2000; Heeschen et al., 2005; Teichert et al., 2005); ERB: Eel River Basin (Hinrichs et al., 1999); NW BS: Dniepr Canyon, Northwest Black Sea (Michaelis et al., 2002), Lost City (this study) and methanogen cultures grown on several substrates (Summons et al., 1998; Londry et al., 2008).

methanotrophs (Elvert et al., 1999; Hinrichs et al., 1999). Second, DIC is lower in abundance than methane but more enriched in ^{13}C . This pattern would not occur if large amounts of methane were being oxidized to DIC, although small amounts of methane oxidation could occur without disrupting this pattern. Third, concentrations of hydrogen within the vent fluids approach 15 mM, providing a strong thermodynamic drive for methanogenesis (Amend and Shock, 2001).

Accordingly, the LCMS phylotype is likely methanogenic. Methanosarcinales are physiologically diverse and capable of producing methane from a variety of substrates including CO_2 , acetate, methanol, and trimethylamine (Thauer, 1998). The extraordinary abundance of hydrogen in Lost City vent fluids suggests that it may be the most viable electron donor for methanogenesis. It follows that methanogens are likely autotrophic, although heterotrophic methanogenesis using substrates such as acetate, formate, or methanol cannot be absolutely excluded. Formate or methanol might form by reduction of CO formed from CO_2 under some hydrothermal conditions, but the very high pH at Lost City limits CO_2 availability and these substrates are unlikely to be available in abundance (Seewald et al., 2006).

Naturally occurring lipids with ^{13}C -enrichments on the order of those detected at Lost City are unusual. Lipids with $\delta^{13}\text{C} = +4\text{‰}$ have been detected in a geothermal sinter in New Zealand (Pancost et al., 2006). At that site, ^{13}C -enrichment occurs in fatty acids and ether lipids likely derived from Aquificales, as well as in archaeol derived from archaea (Pancost et al., 2006). Although similar lipids are detected at Lost City, Aquificales are not detected in surveys of microbial diversity (Brazelton et al., 2006).

Unusual enrichment of ^{13}C in natural samples may derive from the presence of unusually enriched substrates. For example, the ^{13}C -enrichment of archaeols in New Zealand sinters (Pancost et al., 2006) is postulated to potentially derive from ^{13}C -enriched DIC. Another possibility is that ^{13}C -enriched lipids are derived from organisms with carbon assimilation pathways that do not strongly discriminate against the heavier isotope of carbon. Most photosynthetic organisms fix CO_2 via the Calvin cycle, which discriminates against the heavier isotope of carbon by 22–30‰ (Hayes, 2001). However, within Bacteria both the reductive tricarboxylic acid (rTCA) cycle and the 3-hydroxypropionate pathways are carbon-fixation pathways that do not strongly discriminate against the ^{13}C compared to the enzymes of the Calvin cycle (Hayes, 2001).

Neither assimilation of ^{13}C -enriched substrates, nor a unique carbon-fixation pathway is likely to be responsible for the enrichment in ^{13}C in lipids of the organisms at Lost City. Delta values of inorganic carbon in Lost City vent fluids range between -8‰ and $+3\text{‰}$ (Kelley et al., 2005) and thus coincide with those of the archaeal lipids. To date there has been no reported evidence at Lost City for carbon substrates more enriched in ^{13}C than DIC, so extreme enrichment in substrate is not the most attractive explanation for the enrichment in lipid. Small discrimina-

tions by the carbon fixation pathway can also be excluded, at least for the archaeal lipids, which are the most ^{13}C -enriched lipids detected. These lipids are likely derived from methanogenic Methanosarcinales, in which the carbon assimilation pathway is well known. The biochemical pathway of methanogenesis is well studied and in all cases the final production of methane is catalyzed by methyl-coenzyme M reductase (Thauer, 1998). DNA sequences corresponding to this gene have been amplified from Lost City carbonates (Brazelton et al., 2006), confirming that Methanosarcinales are cycling methane. Autotrophic methanogenesis is understood to proceed via a pathway similar to the acetyl-CoA pathway, and carbon isotope fractionations by methanogens have been well studied (Games et al., 1978; Botz et al., 1996; Hayes, 2001; House et al., 2003; Valentine et al., 2004; Conrad, 2005; Penning et al., 2005, 2006; Londry et al., 2008). Taken together, these lines of evidence strongly suggest that no alternate carbon-fixation pathway is responsible for the ^{13}C enrichment seen in archaeal lipids. Diminished isotopic fractionation between CO_2 and biological methane has been reported as a result of very high temperatures and pressures (Takai et al., 2008), but occurs well beyond the ranges relevant at Lost City.

The absence of isotopic fractionation can be explained by carbon limitation. If available inorganic carbon is consumed nearly completely, isotopic discrimination will be minimized. Indeed, at the marker-7 and marker-C vents (the locations at which archaeal lipids were particularly abundant), concentrations of dissolved inorganic carbon in the vent fluids were 4.0 and 0.1 micromolar, respectively (Proskurowski et al., 2008). Moreover, at pH 11 and 70 °C more than 99% of DIC is carbonate and conversion of carbonate to CO_2 is not enzymatically catalyzed.

Although the carbonate in the towers derives from seawater (Fruh-Green et al., 2003), methane carbon contains no ^{14}C and must primarily derive from the mantle (Proskurowski et al., 2008). Two situations are thus possible: (1) a significant portion of the methane is biogenic and the Lost City Methanosarcinales are scavenging residual mantle carbon from the fluids ascending in the vent channels. (2) The methanogens producing the biomass sampled here are utilizing seawater DIC and the resulting CH_4 is diffusing from the exterior walls of the carbonate towers rather than mixing into the methane-rich fluids in the axial channels.

The interpretations offered by Proskurowski et al. (2008) do not exclude microbial contributions to the methane in the Lost City hydrothermal fluids. The absence of radiocarbon in the CH_4 pertains to the source of the carbon, not the source of the methane. It excludes production of methane from seawater DIC but does not require an exclusively abiotic synthesis. Abundances of abiotic hydrocarbons produced by Fischer–Tropsch reactions are predicted to conform to the Anderson–Schulz–Flory (ASF) distribution: a log-linear relationship showing decreasing hydrocarbon concentration with increasing chain length (McCollom and Seewald, 2007). When compared to concentrations of other volatile hydrocarbons,

the concentration of methane at Lost City is elevated by at least two orders of magnitude relative to that predicted by an ASF distribution (Proskurowski et al., 2008). The $\delta^{13}\text{C}$ values of volatile hydrocarbons ethane, propane, and butane are lower than that of methane: between -13‰ and -16‰ . This is consistent with abiotic formation, perhaps through Fischer–Tropsch type reactions (Proskurowski et al., 2008). Lost City methane has $\delta^2\text{H}$ between -99‰ and -147‰ vs. SMOW (Proskurowski et al., 2006, 2008). Ethane and propane have similar ^2H contents (Proskurowski et al., 2008). Microbial methane is typically depleted by 150‰ – 275‰ vs. SMOW (Whiticar, 1999; Valentine et al., 2004), so the methane at Lost City is more enriched in ^2H than would be expected from a purely biological source. However, the activity of methanogens suggests that biological methane must be present. Lost City methane is likely a mixture, consisting of biological and abiotic inputs. The $\delta^2\text{H}$ content of measured methane would be a function of the mixing proportions and δ values of each endmember. Constraining the δ values of these endmembers is difficult. Unusual conditions produce unusual carbon isotope ratios in biologically produced methane (Takai et al., 2008). The assumption that hydrogen isotope ratios in biologically produced methane from Lost City fall within the usual ranges might not be valid.

Organic compounds produced abiotically by hydrothermal synthesis can be depleted in ^{13}C relative to their source material (McCollom and Seewald, 2006). Organic carbon or methane in hydrothermal deposits cannot be distinguished as biotic or abiotic based solely on a $\delta^{13}\text{C}$ value of less than -25‰ (McCollom and Seewald, 2006; Sherwood Lollar and McCollom, 2006). The isotope results at Lost City extend this concept to the full range of ^{13}C contents found in nature. If ultramafic ecosystems rich in hydrogen were important on the early Earth, preserved organic compounds of biological origin detected in those systems may not have the characteristic ^{13}C depletion that is commonly associated with life.

4.3. Bacterial lipid distribution and ^{13}C content

The co-occurrence of isoprenoidal and nonisoprenoidal ether lipids, with the former slightly more depleted in ^{13}C , is common at sites where methane is being consumed anaerobically. At such locations, a combination of geochemical and microbiological techniques has confirmed that the nonisoprenoidal ether lipids are produced by sulfate-reducing bacteria that are the syntrophic partners of archaea that are consuming methane anaerobically (Hinrichs et al., 2000b; Orphan et al., 2001; Orphan et al., 2002; Blumenberg et al., 2004). The archaeal members of these consortia that produce isoprenoidal diethers typically belong to the ANME-1 phylogenetic cluster. Bacteria associated with ANME-2 archaea tend to produce nonisoprenoidal glycerol ether lipids. The bacterial members in either case belong to the sulfate-reducing *Desulfosarcina/Desulfococcus* group.

At Lost City the archaea are methanogenic and microbiological studies fail to detect δ -proteobacterial sulfate-

reducers (Brazelton et al., 2006). Instead, the dominant sulfate-reducing bacteria in these environments are Firmicutes related to the hydrogen-utilizing sulfate reducer *Desulfotomaculum* (Brazelton et al., 2006). Accordingly, Clostridia are candidates for producers of the nonisoprenoidal diethers. Clostridia commonly produce ether–lipid plasmalogens and incorporate them in their cellular membranes (Goldfine, 1997). However, studies of *Desulfotomaculum* have not detected diethers (Pikuta et al., 2000; Londry et al., 2004). At least one Clostridium, *Ammonifex*, does produce diethers (Huber et al., 1996), and the Firmicute lipid biosynthesis pathway shares many characteristics with archaea (Skophammer et al., 2007). At many hydrothermal settings, diether lipids are produced by Aquificales (Jahnke et al., 2001), but rRNA gene sequences related to this group were undetected at Lost City (Brazelton et al., 2006).

The coexistence of sulfate-reducing bacteria and methanogens is unusual. In marine sediments, sulfate-reducing bacteria outcompete methanogens for hydrogen due to the higher energetic yield of sulfate reduction compared to CO_2 reduction (Hoehler et al., 1998) and the greater substrate affinity of the enzymes of sulfate-reducing bacteria for hydrogen (Kristjansson et al., 1982). However, under very high hydrogen concentrations, methanogens and sulfate-reducing bacteria can coexist. Enzymatic affinities for hydrogen are in the micromolar range for both sulfate-reducers and methanogens (Kristjansson et al., 1982); the millimolar hydrogen concentrations at Lost City exceed this threshold and allow coexistence.

Bacterial diethers are slightly depleted in ^{13}C relative to archaeal diethers in most Lost City carbonates (Fig. 4). The fact that $\delta^{13}\text{C}$ values are still highly positive relative to typical marine organic material suggests that carbon-limitation may be playing a key role in bacterial ecology at Lost City, just as it does with the archaea.

Another possibility is that bacteria are acquiring carbon via an alternative pathway that discriminates against ^{13}C to a different degree than archaea. Bacteria are unlike methanogens in that they have a wide range of plausible carbon-acquisition pathways. It is conceivable that major bacterial community members are fixing carbon autotrophically via a pathway that does not discriminate strongly against ^{13}C . For example, the Aquificales species *Thermococcus* fixes CO_2 via the rTCA acid cycle, and when grown autotrophically its lipids are depleted in ^{13}C by only 2‰ relative to CO_2 (Jahnke et al., 2001). The small discrimination conferred by this pathway has been invoked (along with ^{13}C -enriched DIC) to explain the ^{13}C -enriched lipids with $\delta^{13}\text{C}$ up to $+4\text{‰}$ likely to be derived from Aquificales in New Zealand hot springs (Pancost et al., 2006) and a variety of ^{13}C -enriched lipids with $\delta^{13}\text{C}$ up to $+2\text{‰}$ from the Turtle Pits hydrothermal field at the mid-Atlantic Ridge (Blumenberg et al., 2007). Aquificales species produce diether lipids that are similar to the nonisoprenoidal diethers detected at Lost City, but microbial diversity studies fail to detect these taxa at Lost City (Brazelton et al., 2006).

A third possibility is that bacteria and archaea are obtaining carbon from different substrates. The main

sulfate-reducing bacteria at Lost City, *Desulfotomaculum* (Brazelton et al., 2006), is capable of growth on a variety of carbon substrates, including acetate, formate, ethanol, lactate, and pyruvate. Bacteria may be able to outcompete methanogens for competitive substrates like acetate (Kristjansson and Schonheit, 1983). Information regarding the concentrations of these species in Lost City vent fluids is lacking, however.

One relationship that may cast light on these isotopic values is shown in Fig. 4, which shows that bacterial diethers are slightly depleted relative to archaeal diethers in samples in which archaeal diethers are abundant, but more depleted in ^{13}C relative to archaeal diethers in samples in which archaea-derived material is rare. In samples with abundant archaeal lipids, bacterial diethers have a range of $\delta^{13}\text{C}$ values between about +4‰ and -12‰, while in chimneys with rare archaeal lipids the range of $\delta^{13}\text{C}$ extends down to about -29‰. Excluding the two samples 3881-1408 and 3876-1436, each of which contains only low concentrations of nonisoprenoidal diethers and no detected archaeal lipids, the maximum $\delta^{13}\text{C}$ for nonisoprenoidal diethers in samples with rare archaeal biomarkers is near -14‰—lower than the minimum $\delta^{13}\text{C}$ for samples that contain abundant archaeal biomarkers. Although archaeal lipids were undetected in sample 3881-1408, this sample contained 16S rRNA sequences corresponding to Lost City Methanosarcinales (Brazelton et al., 2006). The implication is that the presence of this methanogenic archaeon, particularly at high abundance, is responsible for ^{13}C enrichment for all organisms in the vent fluids—perhaps through draw-down to limitation of a limiting substrate, most likely inorganic carbon.

Other bacterial biomarkers in Lost City carbonate, such as fatty acids and hopanoids, are less taxonomically specific than diether lipids and have a greater range of $\delta^{13}\text{C}$ values. These compounds are probably derived from a greater range of sources. The hopanoids diploptene and diplopterol have $\delta^{13}\text{C}$ values that range from -15‰ to -22‰. The $\delta^{13}\text{C}$ values of hopanoids do not correlate with the abundances of archaeal lipids, as those of the nonisoprenoidal diethers do. A likely interpretation is that hopanoids are dominantly derived from aerobes living at the interface of reduced vent fluids and oxidized seawater. Most hopanoids are derived from aerobic bacteria, although a number of recent studies have demonstrated that some anaerobes are also capable of producing them (Sinninghe Damste et al., 2004; Fischer et al., 2005; Hartner et al., 2005; Blumenberg et al., 2006). Organisms in contact with aerobic seawater could be expected to have ^{13}C contents typical of marine organic material, while those in the anaerobic vent fluids would have ^{13}C contents reflecting the conditions, in many cases carbon-limited, in the vent fluids.

The isotopic compositions of fatty acids may also be consistent with this model. While samples with rare archaeal lipids contain fatty acids with $\delta^{13}\text{C}$ values between -20‰ and -30‰, the $\delta^{13}\text{C}$ values of fatty acids in samples with abundant archaeal lipids extend over a much wider range, with $\delta^{13}\text{C}$ values up to -1‰. However, there is a pattern to these results, with saturated fatty acids tending

to be more ^{13}C -enriched than unsaturated fatty acids. This is interpreted to mean that saturated fatty acids in these samples are predominantly derived, along with the ether lipids, from the carbon-limited anaerobic environment inside the carbonate chimneys. Mono-unsaturated fatty acids probably originate both from this environment and from organisms inhabiting the chimney exterior and overlying water column. This pattern may have to do with the regulation of membrane fluidity, which is increased by either increasing degree of unsaturation or increasing temperature. Organisms inhabiting lower temperature environments can be expected to have a higher degree of unsaturation.

4.4. Anaerobic methanotrophy at Lost City

Microbial community analysis suggests that methane-oxidizing ANME-1 archaea dominate the archaeal community at two sites, X1 and X2, and were present at Marker C (Brazelton et al., 2006). Sample 3880-1557 from site X2 contained an archaeol with a $\delta^{13}\text{C}$ value unlike those detected from the rest of the Lost City field (Fig. 4). The low value of $\delta^{13}\text{C}_{\text{archaeol}}$ implies that archaea at this site consume methane. No hydroxyarchaeols were detected in this sample. Archaeal lipids from environments containing abundant ANME-1 methanotrophic consortia are characterized by a low ratio of *sn*-2 hydroxyarchaeol to archaeol (Blumenberg et al., 2004), so the lack of detected *sn*-2 hydroxyarchaeol is not inconsistent with ANME-1 dominating this sample.

The origin of methane at this site is unknown. Archaeol at site X2 has $\delta^{13}\text{C} = -77\text{‰}$. In several studies from AOM environments that report the ^{13}C content of both methane and lipids, archaeol is depleted in ^{13}C relative to methane by 7‰ to 55‰ (Hinrichs et al., 1999; Michaelis et al., 2002; Teske et al., 2002; Reitner et al., 2005). The $\delta^{13}\text{C}$ of Lost City methane at the main area of venting is -13.6‰ to -9.4‰ (Kelley et al., 2005), but was not measured at X2. It is possible that methane at site X2 derives from a different pool, with a $\delta^{13}\text{C}$ value distinct from methane at the main area of venting. Based on the $\delta^{13}\text{C}$ value of archaeol, we hypothesize that methane at site X2 should fall in approximately the range -27‰ to -70‰.

The $\delta^{13}\text{C}$ values of bacterial diethers found in samples at X1 and X2 span a range of nearly 30‰. This could indicate that these compounds derive from a mixture of bacteria with different carbon acquisition physiologies or different carbon sources, potentially including carbon derived from methane. At site X2, diethers with the most positive values of $\delta^{13}\text{C}$ are enriched relative to archaeol by slightly less than 50‰. While not diagnostic, this difference is within the range found between archaeal and bacterial diethers at AOM sites (Hinrichs et al., 2000b). However, no bacterial syntrophic partners or sulfate-reducing Firmicutes have been detected in the carbonate vein sites (Brazelton et al., 2006), so the source of the nonisoprenoidal ether lipids remains uncertain. Conceivably, the ANME-1 organisms at these sites are operating without a syntrophic partner (Orphan et al., 2002).

4.5. Eukaryotic lipid distribution and ^{13}C content

Cholesterol is the most frequently detected sterol at Lost City and is probably derived from animals inhabiting the surfaces of the carbonate chimneys (Kelley et al., 2005; DeChaine et al., 2006) and from detritus trapped from the water column. C_{28} and C_{29} sterols and stanols, also frequently detected in carbonates, are likely derived from phytoplankton or other eukaryotic inputs. Ergosterol was detected in a few samples and is likely to be a product of one of the two fungal lineages detected at Lost City (Lopez-Garcia et al., 2007).

In several samples the most abundant sterol was cycloartenol. Cycloartenol, the immediate product of the cyclization of oxidosqualene in plants and algae as well as in several groups of protists, is a 'protosterol' (Summons et al., 2006a). In most organisms cycloartenol is a biosynthetic intermediate that is modified to yield a demethylated sterol. To date it has been reported as the accumulating final product in only one organism, the myxobacterium *Stigmatella aurantiaca* Sg a15 (Bode et al., 2003). In other organisms concentrations of cycloartenol detected in cellular extracts are typically minor, reflecting its role as a biosynthetic intermediate. Its accumulation can be enhanced by metabolic inhibitors (Hata et al., 1987; Haughan et al., 1988) or by genetic mutation of the downstream sterol modification pathway.

The high abundance of cycloartenol in Lost City carbonates implies that an organism inhabiting this environment is accumulating cycloartenol as a final product. Given that the animal protosterol is always lanosterol and that C_{28} and C_{29} phytosterols are dominant in marine algae, animals and algae can probably be excluded as the source. Myxobacteria are not detected in 16S rRNA gene surveys at Lost City (Brazelton et al., 2006), and can probably also be excluded. The most probable source of abundant cycloartenol is one or more of the protists inhabiting the carbonate chimneys.

A diverse population of protists has been detected in Lost City carbonates. Ciliates are the most dominant, and other alveolates, fungi, heterokonts, radiolaria and other cercozoa, and heterolobosea have also been detected (Lopez-Garcia et al., 2007). Detailed information about sterol biosynthetic pathways within these groups of protists is sparse. Among protists in which the sterol synthetic pathway is known, most make cycloartenol as their protosterol (Summons et al., 2006a). The alternate pathway—the lanosterol pathway—is ubiquitous among opisthokonts and also occurs among kinetoplastids and dinoflagellates (Summons et al., 2006a). These groups can be ruled out as possible sources for cycloartenol, but large protistan diversity remains (Lopez-Garcia et al., 2007). Given the abundance of cycloartenol in some carbonates, its source is likely to be an important part of the microbial ecology.

Synthesis of cycloartenol requires only one molecule of O_2 (Summons et al., 2006a), but subsequent modification to a sterol that is fully demethylated on its α -face, such as stigmaterol or cholesterol, requires an additional ten molecules of O_2 . For this reason we speculate that cycloartenol

might be a favored sterol from an amphiaerobic eukaryote inhabiting vent fluids with only sporadic access to oxygenated seawater.

The triterpenoid tetrahymanol (Ten Haven et al., 1989; Harvey and McManus, 1991), commonly attributed to ciliates, was also detected in several Lost City vent carbonates. Tetrahymanol has a structure similar to sterols, but one respect in which it differs is that oxygen is not required for tetrahymanol biosynthesis. Gene sequences representative of ciliates have been found in Lost City carbonates (Lopez-Garcia et al., 2007), and ciliates are the most plausible source for tetrahymanol. Consumption of bacteria by ciliates in AOM environments at the Kazan mud volcano (Werne et al., 2002), and consumption of methanogens by thermophilic anaerobic ciliates (Baumgartner et al., 2002) have also been reported. The co-occurrence of cycloartenol and tetrahymanol is likely not fortuitous.

Variability in triterpenoid $\delta^{13}\text{C}$ is greater than would be expected if all sterol inputs were marine. Cycloartenol has $\delta^{13}\text{C}$ values as high as -15‰ (Fig. 4), consistent with derivation from protists feeding in part on ^{13}C -enriched archaea and bacteria in the carbonate chimneys. In samples in which its $\delta^{13}\text{C}$ values is as high as -3.5‰ , tetrahymanol shows very strong evidence of being derived from an organism obtaining its carbon from ^{13}C -enriched vent material (Fig. 4). However there is no strong correlation between $\delta^{13}\text{C}$ of tetrahymanol and total organic carbon. To a lesser extent, C_{28} and C_{29} sterols are also enriched in some samples with $\delta^{13}\text{C}$ up to a maximum of near -20‰ at Marker C, suggesting that their sources may not entirely derive from the water column.

In some samples, tetrahymanol with high $\delta^{13}\text{C}$ values occurs together with ^{13}C -enriched archaeal lipids, suggesting a close relationship between methanogens and ciliates. Some anaerobic ciliates possess hydrogenosomes—cellular organelles related to mitochondria that generate hydrogen. Hydrogenosomes often host symbiotic methanogens, which serve to keep intracellular hydrogen pressures low (Hackstein and Vogels, 1997; van Hoek et al., 2000). However, the millimolar concentrations of hydrogen in Lost City vent fluids may be too high for methanogens to consume at a rate high enough for hydrogenosomes to function. Strong covariation between the $\delta^{13}\text{C}$ of tetrahymanol and *sn*-2 hydroxyarchaeol is lacking, suggesting that the relationship between these organisms is not ubiquitous. The relationship between the organisms may be predator and prey, similar to that of *Trimyema minutum* and *M. thermolithotrophicus* (Baumgartner et al., 2002), but the ^{13}C depletion of tetrahymanol relative to archaeal lipids in the samples implies that methanogens are not the sole food source for ciliates.

4.6. Implications for Earth history and astrobiology

The hydrogen-rich, anaerobic environment within the carbonate towers at Lost City is likely to be similar to some early Earth environments. Autotrophic methanogenesis using CO_2 or bicarbonate as an electron acceptor proceeds exergonically without need for any products of

oxygenic photosynthesis. Thus, the dominant primary producer in this ecosystem may be independent of solar energy. This is in contrast to the organisms in many other anaerobic ecosystems that usually require indirect byproducts of photosynthesis such as sulfate or nitrate. Ecosystems independent of the sun, and certainly independent of the byproducts of oxygenic photosynthesis, would have been important on the early Earth. Bacterial components of the ecosystem at Lost City partly rely on sulfate derived from seawater, but sulfate may also have been present on early Earth, and was present in ecosystems before the origin of oxygenic photosynthesis (Shen and Buick, 2004). The microbial community at Lost City therefore may offer an analogue for early Earth ecosystems.

Genomic studies offer further evidence that the microbial community at Lost City might be relevant to understanding ecosystems on the early Earth. Recent attempts to root the universal tree of life based on analyses of insertions and deletions within sets of paralogous genes have supported the view that the widely accepted root between archaea and bacteria may be an artifact of long branch attraction (Skophammer et al., 2007). Instead, several alternative sites for the root have been proposed within the Gram Positive Bacteria, including a root on the branch leading to the Firmicutes plus Archaea (Skophammer et al., 2007). While this placement of the root is untraditional, it argues for a mesophilic cenancestor that may have inhabited a site like Lost City (Skophammer et al., 2007). Furthermore, Firmicutes and Archaea play major roles in the Lost City ecosystem (Brazelton et al., 2006). Further work on microbial and genomic evolution will have to be undertaken to determine if this correspondence is coincidental.

The chemistry of the Lost City vents suggests that similar ancient systems may have been important not only for their ecological role, but also as a reactor in which some critical aspects of life may have emerged. The alkaline conditions at Lost City are favorable for some aspects of prebiotic chemistry, such as formation of RNA-bearing vesicles (Russell, 2003). The warm, reducing, and alkaline conditions are appropriate for the emergence of biochemical pathways such as methanogenesis and acetogenesis. It has been suggested that these biochemistries originated in such an environment (Martin and Russell, 2007). The thermodynamic drive for methanogenesis at Lost City is underscored by the fact that the reaction occurs both with and without biological catalysis.

Quantifying the relative flux of biologically derived methane versus abiotic methane at Lost City is a major challenge for future study. Solving this problem will be important for constraining the composition of the early atmosphere. Methane is often invoked to solve the faint young sun problem (Kasting, 2005), but models applying this to the Hadean and Archean Earth require an accurate understanding of the potential roles for archaeal methanogens, including the environments in which they live, the timing of their evolution, and the amount of methane that can

be produced in their absence. The possible production of abundant biological methane that is ^{13}C -enriched complicates our understanding of the timing of the evolution of biological methanogenesis, which is based, in part, on the appearance of ^{13}C depleted organic carbon in the geologic record (Hayes, 1994).

5. CONCLUSIONS

The hydrogen-rich chemistry of Lost City vent fluids and the isotopic compositions of the archaeal and bacterial diether lipids lead us to conclude that the Lost City Methanosarcinales phylotype (LCMS) is a methanogen. Similar ^{13}C enrichments in nonisoprenoidal diethers demonstrate that the conditions causing unusual $\delta^{13}\text{C}$ values are not restricted to archaea, but are most prevalent where archaea are dominant. The most plausible explanation for these data is that the archaea-rich vent communities are carbon-limited and the full extent of fractionation between DIC and biomass is not being expressed.

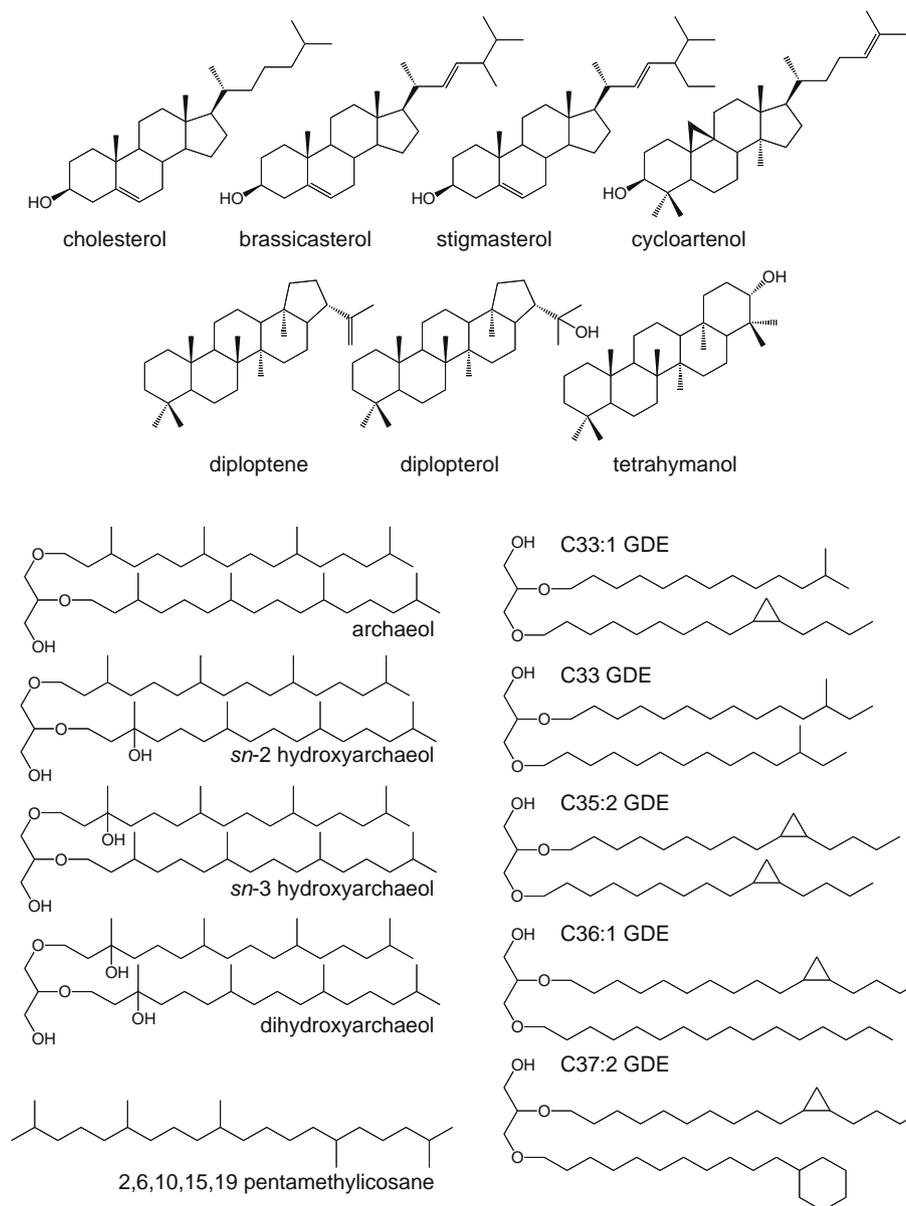
Conditions of CO_2 limitation are rare on Earth. As a result, depletion in ^{13}C is usually necessary (but not sufficient) evidence that preserved organic carbon is biological in origin. The results from Lost City suggest that even this bias may not be appropriate for all environments. Ultramafic ecosystems may have been important on early Earth, and may be present elsewhere in the solar system. The results presented here suggest that biological organic carbon in such systems can have a wide range of ^{13}C contents. Many investigations have emphasized that ^{13}C -depleted organic carbon is not necessarily a signal of life. Similarly, our results show that enrichment of ^{13}C is not a reliable indicator of abiotic origin.

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APPENDIX A

Structures of compounds mentioned in the text.



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