

Elevated concentrations of formate, acetate and dissolved organic carbon found at the Lost City hydrothermal field

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Received 1 September 2009; accepted in revised form 26 October 2009; available online 1 November 2009

Abstract

Fluids from the ultramafic-hosted Lost City hydrothermal field were analyzed for total dissolved organic carbon and dissolved organic acids. Formate (36–158 $\mu\text{mol/kg}$) and acetate (1–35 $\mu\text{mol/kg}$) concentrations are higher than in other fluids from unsedimented hydrothermal vents, and are a higher ratio of the total dissolved organic carbon than has been found in most marine geothermal systems. Isotopic evidence is consistent with an abiotic formation mechanism for formate, perhaps during serpentinization processes in the sub-surface. Further support comes from previous studies where the abiological formation of low molecular weight organic acids has been shown to be thermodynamically favorable during hydrothermal alteration of olivine, and laboratory studies in which the reduction of carbon dioxide to formate has been confirmed. As the second most prevalent carbon species after methane, formate may be an important substrate to microbial communities in an environment where dissolved inorganic carbon is limited. Acetate is found in locations where sulfate reduction is believed to be important and is likely to be a microbial by-product, formed either directly by autotrophic metabolic activity or indirectly during the fermentative degradation of larger organic molecules. Given the common occurrence of exposed ultramafic rocks and active serpentinization within the world's ocean basins, the abiotic formation of formate may be an important process supporting life in these high pH environments and may have critical implications to understanding the organic precursors from which life evolved.

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1. INTRODUCTION

Fluids from the Lost City hydrothermal field (LCHF) are rich in hydrogen and methane, and have high pHs (9–11), as a result of serpentinization reactions taking place in the sub-surface at moderate temperatures of approximately 120–250 °C (Kelley et al., 2001, 2005; Palandri and Reed, 2004; Allen and Seyfried, 2004; Proskurowski et al., 2006; Foustoukos et al., 2008). While Lost City is the first

of its kind to be discovered, it is unlikely to be unique. Mantle rocks are commonly exposed on the seafloor within the Mariana forearc, along spreading centers in the Arctic and Indian Oceans, and at the Mid-Atlantic Ridge; they are also a common component of on-land ophiolite deposits such as the well studied Oman and Cyprus systems (Haggerty, 1991; Charlou et al., 1998; Godard et al., 2000; Neal and Shand, 2002; Dick et al., 2003; Edmonds et al., 2003). Active serpentinization processes are ubiquitous in these areas, which potentially produce chemical environments similar to Lost City. Such alkaline systems have been suggested as possible sites for the origin and early evolution of life on Earth (Holm and Andersson, 1998; Shock and Schulte, 1998; Kelley et al., 2005; Holm et al.,

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2006; Schulte et al., 2006; Martin and Russell, 2007; Schulte, 2007; Martin et al., 2008). Additionally, serpentinization of sub-surface ultramafic minerals has been proposed as a source of CH_4 on Mars (Oze and Sharma, 2005).

Phylogenetic studies indicate microbial communities in actively venting chimneys within the Lost City field predominantly metabolize methane and sulfur species (Schrenk et al., 2004; Brazelton et al., 2006). The archaeal community is unusual in that it is dominated by a single phylotype of *Methanosarcinales* (the Lost City *Methanosarcinales* [LCMS]) (Schrenk et al., 2004; Brazelton et al., 2006). The presence of 16S rRNA genes similar to sulfur-oxidizing and sulfate-reducing bacteria has also been detected (Brazelton et al., 2006). In addition to high concentrations of methane (CH_4 ; 1–2 mM) and hydrogen (H_2 ; <1–15 mM), detectable levels of sulfate are present in the fluids (1–4 mM; Kelley et al., 2005). Fluids passing through warmer vents in the center of the field have high concentrations of hydrogen and sulfate, while fluids passing through cooler vents on the extremities of the field have lower concentrations of hydrogen and sulfate, and increased concentrations of sulfide (Proskurowski et al., 2006). Significant sulfur has also been added to the underlying mantle rocks during serpentinization, with sulfur isotopic compositions close to seawater (Delacour et al., 2004). In concert, these results indicate that sulfate reduction is an important reaction in the basement rocks beneath the field (Delacour et al., 2004; Proskurowski et al., 2006). These data indicate that the microbial communities are adapted to the novel geochemistry of Lost City although their complete metabolisms are currently unknown.

The speciation of carbon at Lost City is of particular interest for those investigating the metabolisms of local microbial communities, the chemical environment of the early Earth and other planetary bodies. Dissolved inorganic carbon (DIC) is largely removed by carbonate precipitation

and methane is the most prevalent carbon species in the fluids (Früh-Green et al., 2003; Kelley et al., 2005; Proskurowski et al., 2008). Organic carbon in the form of short-chain hydrocarbons and organic acids is predicted to form abiologically from inorganic precursors under certain hydrothermal conditions (Shock, 1992; Shock and Schulte, 1998; McCollom and Seewald, 2003, 2006; Foustoukos and Seyfried, 2004). C_1 – C_4 hydrocarbons have been detected in Lost City fluids in nano-molar concentrations, likely as a result of abiological Fischer–Tropsch type reactions (Proskurowski et al., 2008). Concentrations of long-chain (>8-carbons) aliphatic hydrocarbons, aromatic compounds and carboxylic acids are also elevated in Lost City fluids compared to deep-seawater, although their sources and quantities are as yet unknown (Konn et al., 2009). Many sulfate-reducing and methanogenic microorganisms are capable of using organic acids as their sole carbon source (Rabus et al., 2006; Hedderich and Whitman, 2006), and the abiological formation of these compounds could provide an important substrate for communities in the Lost City environment. In addition, these compounds play a central role in the hypothesis of alkaline environments hosting the emergence of life (e.g. Martin and Russell, 2007). Given the critical role that organic acids could play in supporting life in alkaline environments of the present-day and early Earth, this paper is focused on identifying the concentration of organic acids and their contribution to dissolved organic carbon (DOC) in Lost City fluids.

2. METHODS

Fluids from eight chimneys across the Lost City hydrothermal field (Fig. 1) were collected by *DSV Alvin* using previously described ‘major’ samplers (Von Damm et al., 1985) and the Hydrothermal Fluid and Particulate Sampler (HFPS; Butterfield et al., 2004). These samplers are well-sui-

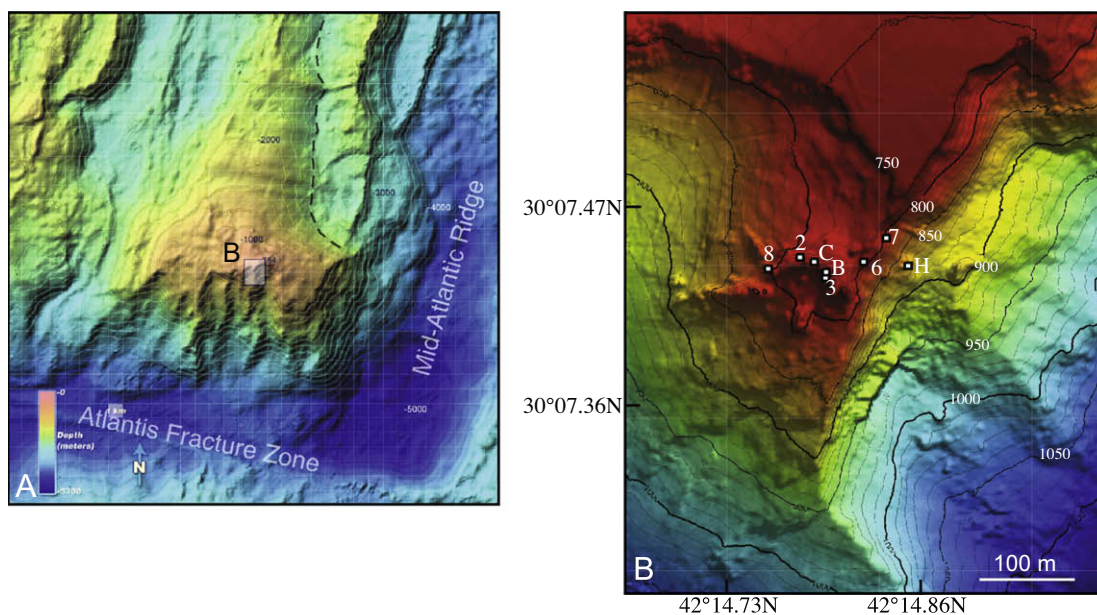


Fig. 1. Map of Lost City field with vent structures identified by their marker numbers.

ted for the analysis described here because they have low organic carbon blanks when properly cleaned (Lang et al., 2006). Both filtered (*in situ*) and unfiltered HFPS samples were collected; filters used *in situ* were either glass fiber filters (Whatman GF/F; combusted at 500 °C for 5 h) or 0.2 µm nitrocellulose filters (Millipore). Upon arrival on deck, subsamples for organic analyses were stored in 100 mL glass vials (combusted at 500 °C, 5 h) with acid-washed Teflon-lined caps (10% HCl, overnight); samples were then stored frozen (−20 °C) until analysis on shore. Samples were analyzed for Mg concentration, DOC concentration, organic acid concentration, and the $\delta^{13}\text{C}$ of DOC.

Magnesium concentrations were determined by ion chromatography on a Dionex DX500 with CS12 analytical column, 20 mmol/L NaOH eluent and CD20 conductivity detector. Precision of the Mg analysis is 2%. Dissolved organic carbon concentrations were measured with a modified MQ-1001 DOC Analyzer (Peterson et al., 2003). Samples (5 mL) were acidified to pH 2 with 12 M HCl and purged with O₂ to remove inorganic carbon. Each sample was injected 3–4 times and the reproducibility between replicate injections was typically <5%. Deep Sargasso Sea reference water (Hansell, 2001) was analyzed every 10 samples. Over the course of these samples, the average value for the reference water was $48 \pm 2 \mu\text{M}$, in good agreement with Sargasso Sea water measured by a variety of laboratories (Sharp et al., 2002) and typical for this instrument (Peterson et al., 2003; Lang et al., 2006).

Organic acids were analyzed by ion exclusion chromatography (Dionex) by the method of Xu et al., 1997. To remove salt, samples were first passed through 2.5 mL OnGuard Ba/Ag/H cartridges (Dionex) that had been pre-rinsed with Milli-Q water (60 mL). Adipic acid was used as an internal standard and recoveries were >95%. Standard deviations of duplicate samples are reported. Artificial seawater blanks analyzed as samples had formate and acetate concentrations below detection limits (1 µM).

The method for analyzing the stable carbon isotopes is described in full elsewhere (Lang et al., 2007). Briefly, the CO₂ created from a single DOC injection was trapped in a stainless steel coil with liquid nitrogen. The coil was then brought to a Finnigan Delta Plus isotope ratio mass spectrometer (IRMS) for isotopic analysis. All samples were analyzed in triplicate and the error on the measurement is approximately $\pm 0.8\%$. Reported here are the $^{13}\text{C}/^{12}\text{C}$ ratios expressed as per mil deviations from the international standard Vienna Pee Dee belemnite (VPDB):

$$\delta^{13}\text{C}_{\text{sample}} = (R_s/R_{\text{st}} - 1) \times 1000 \quad (1)$$

where R_s is the ratio of $^{13}\text{C}/^{12}\text{C}$ in the sample and R_{st} is the ratio of the VPDB standard.

Samples were taken concurrently from the same vents in gas-tight samplers for analysis of volatiles (DIC, H₂, CH₄, and corresponding isotopes), with the majority of this data reported elsewhere (Kelley et al., 2005; Proskurowski et al., 2006, 2008). Here, we report additional carbon monoxide (CO) data. Upon arrival on deck, contents of the gas-tight samplers were transferred into an evacuated gas-manifold. The gas was stripped from the fluids through addition of acid and ultrasonic agitation, dried using a water trap,

and archived for later analysis. The concentrations of archived gas samples were determined using gas chromatography techniques.

3. RESULTS

A combination of *in situ* filtered and unfiltered hydrothermal fluid samples were run for DOC concentration measurements, DOC stable isotope measurements (DO¹³C), and organic acid concentration measurements: no significant differences in results between collection types was observed. An additional unfiltered fluid sample was filtered in the lab using an acid-washed Teflon syringe and combusted, GF/F filters: again no significant concentration differences were observed between unfiltered and laboratory filtered samples.

Endmember Lost City fluids contain near-zero magnesium (Mg) concentrations, while deep seawater has a concentration of 52.5 mM (Kelley et al., 2005). Concentrations of organic species are plotted against Mg in Figs. 2 and 3, where near-zero Mg values indicated relatively pure hydrothermal fluids and concentrations near 52.5 mM indicate samples with a large fraction of local deep seawater.

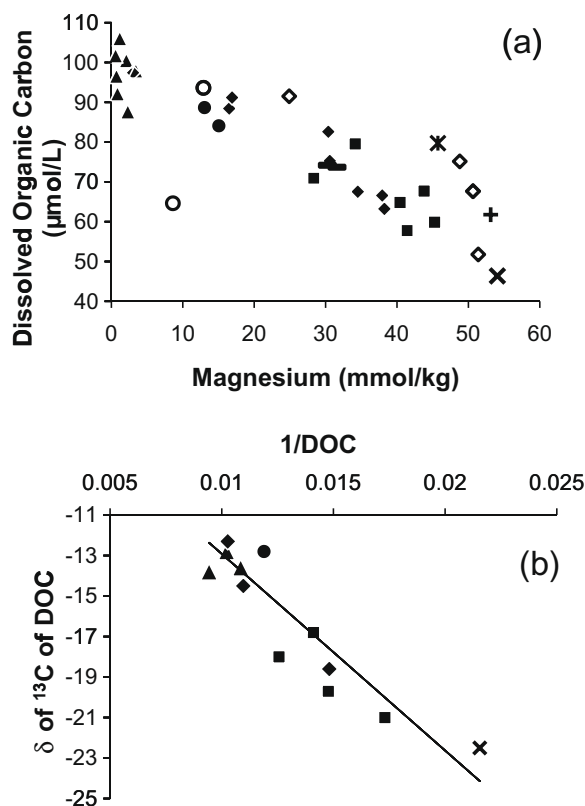


Fig. 2. (a) DOC vs. Mg for all Lost City fluid samples (linear regression $r^2 = 0.78$, $p < 0.001$). Markers from Fig. 1 are represented by the following symbols: LC-B (\blacktriangle), LC-3 (\blacklozenge), LC-IF/2 (\circ), LC-7 (\diamond), LC-8 (---), LC-6 ($*$), LC-C (\bullet), LC-H (\blacksquare), LC-CB ($+$), SW (\times). LC-CB is not identified in Fig. 1 and represents Chaff Beach, a flat area to the northeast of the field with evidence of diffuse venting; SW was a seawater sample taken adjacent to the vent field; (b) $\delta^{13}\text{C}$ of DOC vs. Mg for all Lost City fluid samples. Line represents linear regression ($r^2 = 0.78$, $p < 0.001$).

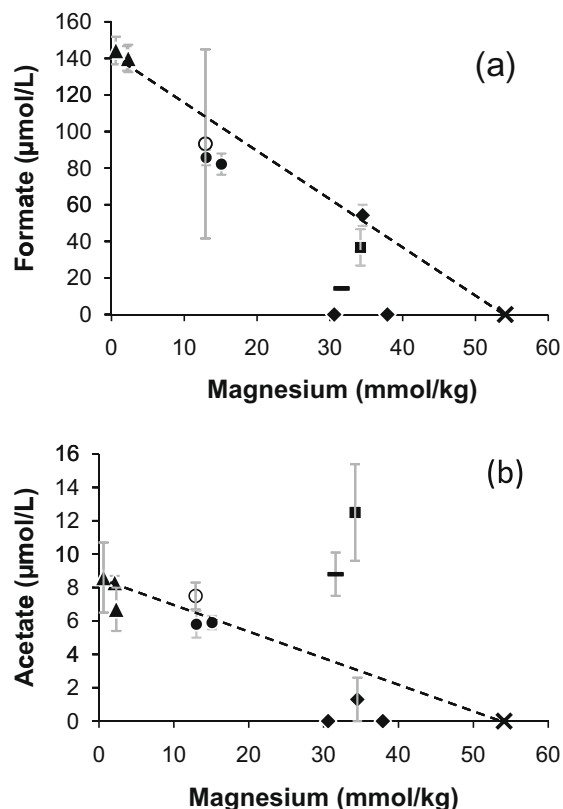


Fig. 3. (a) Formate vs. Mg and (b) acetate vs. Mg for all Lost City fluid samples. Lines representing the conservative mixing between seawater and hydrothermal endmembers have been included. Error bars are standard deviations of duplicate samples. Those symbols on the x-axis indicate samples with concentrations below detection limits (1 μM).

When concentrations of chemical species plot linearly against Mg, it indicates conservative mixing between these two end-member fluids. Concentrations of DOC in Lost City hydrothermal fluids from 10 different chimneys show a strong linear relationship with Mg (Fig. 2a). Based on a two endmember-mixing relationship, hydrothermal fluids have more than twice the DOC concentration of local background seawater (100 vs. 44 μM).

The stable carbon isotope signature ($\delta^{13}\text{C}$) of DOC can provide insight into the source of the added DOC. For example, significant $\delta^{13}\text{C}$ depletions (as low as -96%) are expected if the DOC is due to anaerobic methane oxidation (Orphan et al., 2001), and photosynthetically-derived oceanic organic matter typically has a signature of -19% to -23% (Druffel et al., 1992). The majority of samples are a mixture of hydrothermal fluids and seawater. If two endmembers with distinct isotopic compositions mix conservatively, a plot of $\delta^{13}\text{C}$ vs. $1/\text{DOC}$ will also be linear and can be used to estimate the $\delta^{13}\text{C}$ signature of DOC in end-member fluids. As is shown in Fig. 2b, the $\delta^{13}\text{C}$ of hydrothermal fluid endmembers are enriched compared to local seawater DOC endmember values (-12.6% vs. -22.5% ; $r^2 = 0.78$, $p < 0.001$) (Table 1).

Acetate and formate were the only organic acids detectable ($>1 \mu\text{M}$) and CO was below detection limits ($0.5 \mu\text{M}$)

for all samples. For the most part, formate concentrations varied linearly with Mg (Fig. 3a; $r^2 = 0.86$, $p < 0.01$) with an extrapolated concentration of $136 \mu\text{M}$ in end-member fluids. Acetate concentrations were not correlated with Mg (Fig. 3b; $r^2 = 0.22$, $p = 0.07$). The concentration of acetate in fluids with Mg concentrations $<3 \text{ mmol/kg}$ ($>95\%$ hydrothermal fluid) was $7.9 \pm 1 \mu\text{M}$ ($n = 3$). In samples with higher seawater contributions (Mg $> 25 \text{ mmol/kg}$), we have observed the loss of organic acids while samples were stored for short periods of time at $4 \text{ }^\circ\text{C}$ while awaiting analysis. Some negative deviations from the mixing line may be attributed to this analysis artifact, in particular the undetectable concentrations of formate and acetate from two of the three Marker 3 samples. Since sample contamination with high concentrations of formate is unlikely, we treat the Marker 3 sample with the highest concentration as the most representative of *in situ* conditions (Table 2).

Hydrogen concentrations in end-member fluids (1–15 mmol/kg) vary widely across the field, with higher concentrations found in warmer vents and lower concentrations in cooler vents; the loss of hydrogen has been attributed to biological sulfate reduction (Proskurowski et al., 2006). For this reason, the fluids with higher hydrogen concentrations are thought to be more representative of sub-surface conditions while fluids with lower hydrogen concentrations are thought to have a microbial imprint. Formate and acetate concentrations were corrected for seawater entrainment to determine if similar patterns would be observed. In fluids from vents H, 2, B, and 3, decreases in extrapolated endmember concentrations of formate (104–158 μM) and hydrogen (1.3–12.3 mmol/kg) co-vary ($r^2 = 0.98$, $p < 0.01$; Table 2). Fluids from Marker 8 and C had lower than expected concentrations of formate for the amount of hydrogen in the fluid when compared with other samples (Table 2). The sample from Marker 8 had significant seawater contribution and may have been affected by the loss of formate while stored at $4 \text{ }^\circ\text{C}$ after thawing and before analysis, although the corresponding acetate values appear unaffected. Endmember acetate concentrations also varied widely (1–35 μM), with high concentrations of acetate and low concentrations of hydrogen significantly correlated (Table 2; $r^2 = 0.70$, $p < 0.01$).

Because organic acid concentrations exceeded those of total DOC (see Section 2), experiments were performed to determine whether organic acids were removed during the purging of samples for DOC and DO^{13}C measurements. Sedimentary pore waters with high concentrations of organic acids require heating to $90 \text{ }^\circ\text{C}$ during purging to fully remove this semi-volatile component (Barcelona, 1980). Two hydrothermal fluid samples and a deep seawater sample spiked with a known concentration of formate and acetate (20 and $10 \mu\text{M}$, respectively) were treated identically to samples processed for the DOC measurements discussed above, i.e. acidified to pH 2 with HCl and purged with oxygen for a standard time. In the most hydrothermally pure sample (Mg = 0.6 mmol/kg , DOC = $101 \mu\text{M}$), $27 \pm 2\%$ of the formate and $71 \pm 17\%$ of the acetate remained in the samples after purging ($n = 2$). In a sample that was a 70:30 mixture of hydrothermal fluid:seawater (Mg = 17 mmol/kg ,

Table 1
Concentrations and isotopic compositions of measured carbon species.

Sample ID ^a	Site ^b	Measured							
		Max. Temp. (°C)	Mg (mmol/kg)	DOC ($\mu\text{mol/L}$) ^c	^{13}C (‰) ^d	Formate ($\mu\text{mol/L}$) ^d	SD ($n = 2$)	Acetate ($\mu\text{mol/L}$) ^d	SD ($n = 2$)
3865m23	Seawater		54.1	46	-22.5	bd		bd	
3870p23	LC-8	39.5	31.6	74	-	14	1	9	1
3877m23	LC-H	-	43.8	68	-19.7	-		-	
3878b8	LC-H	31.2	34.2	80	-18.0	37	10	13	3
3878b9	LC-H	24.0	41.4	58	-21.0	-		-	
3878bf11	LC-H	46.9	28.3	71	-16.8	-		-	
3870b8	LC-IF/2	56.5	12.9	94	-	93	52	7.5	0.8
3874bf14	LC-C	67.5	15.1	84	-12.8	82	6	5.9	0.4
3874p22	LC-C	68.7	13.0	89	-	86	4	5.8	0.8
3874pf23	LC-B	90.9	2.1	101	-	140	7	8.3	0.4
3874pf24	LC-B	91.0	3.4	98	-12.8	-		-	
3876m23	LC-B	90.7	2.3	88	-10.5	140	8	7	1
3876m24	LC-B	90.7	0.6	102	-	144	8	9	2
3876m14	LC-B	90.7	1.2	106	-13.8	-		-	
3876m4	LC-B	90.7	0.9	92	-13.6	-		-	
3863m24	LC-3	81.0	34.5	68	-18.6	54	6	1.3	1.3
3878p4	LC-3	37.1	37.9	67	-	bd		bd	
3878b18	LC-3	50.8	30.6	75	-	bd		bd	
3863m11	LC-3	81.0	3.0	97	-12.3	-		-	
3878pf24	LC-3	73.2	16.9	91	-14.5	-		-	

^a Samples were collected using a variety of sampling devices: m, titanium major bottles; b or p, HFPS; bf, filtered HFPS samples.

^b Marker 2 and IF are the same location; both labels have been used in the literature.

^c For brevity, not all measured DOC concentrations are reported. All are presented in Fig. 2a.

^d bd, below detection (1 μM); -, not determined.

DOC = 91 μM), similar amounts of formate and acetate remained in the samples ($38 \pm 3\%$ and $84 \pm 3\%$; $n = 2$). In deep seawater spiked with formate and acetate to achieve a final concentration of 20 and 10 μM , respectively, the majority remained after purging ($75 \pm 11\%$ and $85 \pm 12\%$; $n = 2$).

DOC is operationally defined, presenting some complexity in this case. It is the organic material that passes through a filter (0.7 μm or smaller), remains in solution after samples are acidified and purged with gas, and is successfully oxidized to CO_2 for analysis. Larger (higher molecular weight, longer chain) molecules and those with polar functional groups will be less volatile than smaller and non-polar molecules. Based on the experiments outlined above, it is estimated that 46 μM of formate ($= 158 \mu\text{M} \times 0.29$) and 6 μM of acetate ($= 8.5 \mu\text{M} \times 0.7$), or a total of 58 μM of carbon in the form of organic acids would remain in end-member fluids following sample treatment prior to analysis. Under the same conditions, all forms of DIC will be removed, as will methane. Long-chain (>8 -carbons) aliphatic hydrocarbons, aromatic compounds and carboxylic acids have been detected in Lost City fluids (Konn et al., 2009) and will contribute to DOC if they are not volatilized; since their concentrations are unknown, it is not possible to determine to what extent. Microbial communities living in the fluid pathway will add additional organic compounds such as amino acids and carbohydrates to the fluids that will also contribute to the bulk concentrations and isotopes.

Organic acids with a lighter isotopic composition will be preferentially removed during purging, and significant fractionation would skew the isotopic results. The fraction-

ation factor for this effect is small for CH_4 and CO_2 ($\alpha_{\text{CH}_4} = 0.9992$; $\alpha_{\text{CO}_2} = 0.9991$; Inoue and Sugimura, 1985; Jahne et al., 1987; Knox et al., 1992) and will be even smaller for formate and acetate because their molecular weights are larger. Using the fractionation factor for CO_2 as an upper limit, the isotopic composition of organic acids remaining in the fluids would change by $<0.5\%$ under Rayleigh fractionation conditions. To test the fractionation of formate during purging directly, six samples of Milli-Q water were spiked with formic acid to final concentrations of 10 mM. Half of these samples were treated identically to DOC samples, i.e. acidified to pH <2 with 12 N HCl and bubbled with helium for 5 min. All samples were then basified with NaOH to pH >12 (Heuer et al., 2006). Aliquots of the solution were added to tin cups, dried under a stream of N_2 , and analyzed for stable carbon isotopic composition by elemental analyzer-isotope ratio mass spectrometry. Although variable, $>65\%$ of the formic acid had been removed from the purged samples. The $\delta^{13}\text{C}$ of purged and non-purged samples ($-24.2 \pm 3.7\%$ vs. $-22.9 \pm 2.1\%$, respectively) were statistically indistinguishable.

4. DISCUSSION

4.1. Formation mechanism for formate and acetate

While these are the first DOC measurements to be made from an ultramafic-hosted vent field, some comparisons may be made to other unsedimented deep-sea hydrothermal systems. In basaltic-hosted hydrothermal systems at Axial Seamount and the Endeavour Field on the Juan de Fuca

Table 2

Averages of reported endmember concentrations from the literature and this work.

Location	Avg. E.M. pH ^a (25 °C)	Avg. E.M. H ₂ (mmol/kg) ^b	Avg. E.M. DIC (μmol/kg) ^c	Equil. Temp. Horibe modeling (°C) ^d	Avg. E.M. formate (μmol/L) ^e	Avg. E.M. acetate (μmol/L) ^e
LC-H	9.5 (Mg = 28.9)	1.3 [0.8, n = 6]	1.3 [1.0, n = 2]	55	104 [10, n = 1]	34 [3, n = 1]
LC-IF/2	10.9	3.7 [0.3, n = 4]	0.55 [0.6, n = 2]	79	124 [12, n = 1]	10 [1, n = 1]
LC-8	9.7 (Mg = 29.1)	4.2 [0.1, n = 2]	0.8 [n = 1]	75	36 [4, n = 1]	21 [2, n = 1]
LC-B	10.7	9.1 [0.1, n = 3]	0.1 [n = 1]	106	146 [7, n = 3]	8 [0.4, n = 3]
LC-C	10.0 (Mg = 12.9)	11.2 [4.6, n = 2]	0.1 [n = 1]	101	115 [8, n = 2]	8 [0.6, n = 2]
LC-3	10.8	12.3 [0.8, n = 3]	18 [11, n = 2]	105	158 [16, n = 1]	4 [0.4, n = 1]

^a Endmember pH values reported for samples with <10% seawater entrainment. For samples with >10% seawater entrainment, the pH value of the best sample is reported, with the corresponding Mg concentration (mmol/kg) in parenthesis.

^b From Proskurowski et al. (2006, 2008); averages and standard deviations are calculated from reported endmember concentrations of multiple samples.

^c From Proskurowski et al. (2008); averages and standard deviations are calculated from reported endmember concentrations of multiple samples.

^d From Proskurowski et al. (2006). Temperatures listed here the averages of H₂O–H₂ and CH₄–H₂ geothermometry calculations using the model of Horibe and Craig (1995).

^e Averages of calculated endmembers are given with the exception of LC-3, where endmember of the best sample is reported, as discussed in the Section 3. Propagated errors are reported.

Ridge, elevated concentrations of DOC (max. = 71 μM) are found in areas of low-temperature diffuse flow vents that have high microbial cell counts and a presumed increased sub-surface autotrophy (Lang et al., 2006). At these two sites, DOC concentrations do not correlate linearly with Mg and this variation is believed to reflect differing degrees of microbial DOC production. In contrast, Lost City DOC concentrations behave conservatively with Mg, indicating that the majority of the additional carbon is added in the sub-surface and alterations in the chimneys are minor compared to the primary signal.

If all of the DOC in recharge seawater that enters the Lost City system survives (45 μM, −22.5‰), an “additional” 55 μM (C) with a δ¹³C of −4.5‰ is present in end-member fluids. In this case, the excess DOC can be entirely attributed to the organic acids that remain in the sample following purging of the sample, and the combined δ¹³C of the acetate and formate would be roughly equal to that of the excess DOC (Fig. 4). This calculation becomes more complicated if a portion of the DOC in recharge seawater is removed from fluids in the sub-surface due to microbial uptake, sorption or thermal cracking. Approximately 70% of the DOC entering high temperature and ridge-flank hydrothermal systems is removed before fluids exit the seafloor (Lang et al., 2006). Basement rocks drilled from the central dome of the Atlantis Massif contain hydrocarbons with an isotopic composition consistent with a marine origin, suggesting at least a portion of DOC is removed due to sorption during recharge (Delacour et al., 2008). As an extreme case, if only 30% of the DOC in recharge fluids survives passage through the basement rocks below Lost City (13 μM), and if this removal occurs without significant fractionation, an “additional” 87 μM (C) with a δ¹³C of −11‰ is present in end-member fluids (Fig. 4). When measured for isotopic composition, the majority (60%) of this “additional” DOC is still accounted for by incompletely purged organic acids. Depending on the amount of seawater DOC that survives passage through the seafloor, the excess organic matter will have an isotopic composition of −4.5‰

to −11‰ (Fig. 4). This composition is similar to the δ¹³C of methane in the Lost City fluids (−9‰ to −14‰; Kelley et al., 2005), archaeal lipids in the carbonate chimneys (−2.9 to +6.7‰; Bradley et al., 2009), and total organic carbon in the chimneys (−3.1 to −18.1‰; Kelley et al., 2005; Fig. 4).

Formate and acetate occur in high concentrations in sub-surface environments including oil fields, sedimentary pore fluids, groundwater, hot springs, peat bogs, and fluid inclusions of metamorphic and felsic igneous rocks (McMahon and Chapelle, 1991; Pittman and Lewan, 1994; Amend et al., 1998; Zeng and Liu, 2000; Burdige, 2002 and references therein). In most cases, their presence in these environments is attributed to the decomposition of organic matter and acetate concentrations are elevated over those of formate. Interaction with degrading organic matter should add other dissolved carbon molecules to fluids as well. For example, in anoxic sediment pore waters with high concentrations of formate, the concentration of this species is still <6% of the total DOC (Barcelona, 1980). The majority of oceanic organic matter, both dissolved and particulate, is derived from photosynthesis in the upper ocean and therefore has an isotopic composition between δ¹³C −19‰ to −23‰ (Druffel et al., 1992). Organic molecules de-sorbing from sediments and entering the hydrothermal fluid pathway would be expected to have a similar signature. Thus, while decomposition of larger organic molecules may explain the addition of acetate in Lost City fluids, it is an unlikely mechanism for the formation of formate which is present at much higher concentrations.

Formate and acetate can also be formed as microbial metabolites. Acetogenic bacteria have been shown to grow using molecular hydrogen and carbon dioxide as sole substrates, producing both acetate and formate as by-products (e.g. Chapelle and Bradley, 1996). The isotopic fractionation associated with the conversion of carbonate to acetate is −58.6 ± 0.7‰ (Gelwicks et al., 1989; Zerkle et al., 2005). Within the Lost City system, there are numerous potential sources of CO₂ with similar δ¹³C signatures: seawater

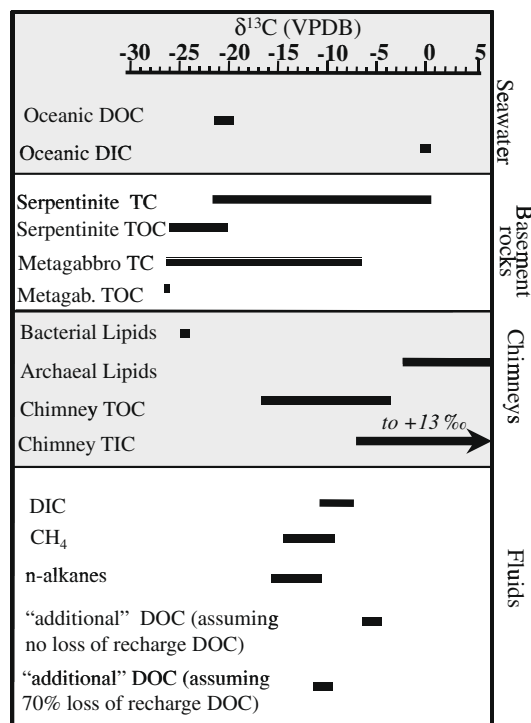


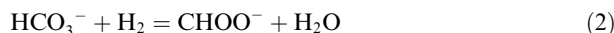
Fig. 4. Isotopic distribution of inorganic and organic carbon reservoirs at Lost City, organized according to reaction pathway with recharge seawater at the top, followed by basement rocks, chimneys, and fluids. Seawater data from Druffel et al. (1992); basement rock data from Kelley et al. (2005), Delacour et al. (2008); Chimney data from Kelley et al. (2005), Bradley et al. (2009); Fluid data from Kelley et al. (2005); Proskurowski et al. (2008); this work. Abbreviations: DOC, dissolved organic carbon; DIC, dissolved inorganic carbon; TOC, total organic carbon; TIC, total inorganic carbon; TC, total carbon.

bicarbonate ($\sim 0\text{‰}$), mantle CO_2 (-9‰ ; Proskurowski et al., 2008), and chimney carbonate (-7 to $+13\text{‰}$, with $>70\%$ of samples in the range of -2 to $+2\text{‰}$; Kelley et al., 2005; Fig. 4). If acetogenic CO_2 reduction by bacteria were responsible for the elevated concentrations of formate and acetate, the $\delta^{13}\text{C}$ isotopic composition of these organic acids would be expected to approach $\sim -55\text{‰}$. While the isotopic composition of formate and acetate have not been directly determined, this value is significantly depleted compared with their potential estimated isotopic range of ~ -4.5 to -11‰ .

Methanogenic archaea growing on CO have been shown to also produce formate, acetate and methylated thiols (Henstra et al., 2007; Oelgeschlager and Rother, 2008). Analysis of fluids from across the field contained no detectable CO, although it could be consumed before the point of sampling. However, the formation of CO is not thermodynamically favored at Lost City sub-surface conditions, as is discussed below.

Alternatively, the compounds may be formed abiotically. When highly reducing fluids such as those found at Lost City are mixed with seawater, the abiological formation of organic acids from inorganic precursors can be thermodynamically favored (Shock, 1992; Shock and Schulte,

1998). In fluids with high concentrations of hydrogen (H_2) and an alkaline pH, formate is predicted to be more thermodynamically stable than DIC (McCollom and Seewald, 2003). The reduction of carbon dioxide to formate has been demonstrated when seawater is reacted with the magnesium- and iron-bearing mineral olivine at 300 °C and 350 bars (McCollom and Seewald, 2003):



The equilibration of these chemical species proceeds rapidly under laboratory conditions at temperatures $>175\text{ °C}$ (McCollom and Seewald, 2001, 2003, 2006; Seewald et al., 2006). Despite attempts to do so, the abiological production of acetate under similar conditions has not been observed. High concentrations of organic acids found in pore fluids from a seamount-forming serpentinite diapir were originally attributed to decomposition of organic matter (Haggerty, 1991; Haggerty and Fisher, 1992), but it has since been suggested that abiotic production is the more likely mechanism of formation in that environment (Holms, 1996; McCollom and Seewald, 2003). As with Lost City fluids, concentrations of formate were higher than those of acetate (Haggerty, 1991; Haggerty and Fisher, 1992).

An abiogenic source for formate within Lost City is suggested by several lines of evidence: (1) formate is a small organic acid that is predicted to be more thermodynamically stable than DIC under the high pH and high-hydrogen conditions of Lost City (Shock, 1992; Shock and Schulte, 1998; explored further below); (2) formate has been shown to rapidly equilibrate with DIC in laboratory experiments (McCollom and Seewald, 2003); (3) formate is present in significantly higher concentrations than other organic species that would also be expected if formed by the degradation of organic matter; (4) large concentrations of organic acids produced by acetogenic CO_2 reduction would have an isotopic signature inconsistent with that of the excess DOC (Gelwicks et al., 1989; Zerkle et al., 2005).

Several of these lines of evidence cannot be extended to support the abiological formation of acetate. While acetate is thermodynamically favored over DIC in high pH, high-hydrogen conditions (Shock, 1992; Shock and Schulte, 1998), no laboratory experiments have yet borne out predictions. The contribution of acetate to DOC concentrations, not accounting for loss from purging, is on average only $\sim 6\%$ (maximum 17%) while that of formate is 76% (maximum 160%). This lower percentage cannot be used to argue against a contribution from organic matter degradation. Finally, while formate concentrations largely vary linearly with Mg, suggesting a sub-surface source, those of acetate do not. Instead, endmember acetate concentrations increase with decreasing hydrogen concentrations. Lost City fluids with low concentrations of hydrogen are believed to have been significantly microbially altered as a result of sulfate reduction (Proskurowski et al., 2006). It is within these low-hydrogen fluids that elevated acetate concentrations are also observed (Table 2).

One possibility is that an autotrophic microbial community not based on the acetogenic reduction of CO_2 could be directly producing acetate as a by-product of their metabolism. Some autotrophic sulfate-reducing microorganisms

excrete traces of acetate (Brysch et al., 1987) and acetate has been proposed as the metabolite passed between anaerobic methane oxidizers and their metabolic, sulfate-reducing bacterial partners (Valentine and Reeburgh, 2000). The phylogenetic markers of sulfate-reducing bacteria have not been found in direct association with methane-cycling microorganisms at Lost City (Brazelton et al., 2006), although strong gradients in hydrogen and sulfate concentrations are observed (Proskurowski et al., 2006).

Alternatively, acetate may be produced during biomass degradation. High concentrations of total organic carbon are found in the chimneys, likely as a result of microbial mats (Kelley et al., 2005). Acetate is a primary degradation product of fermentative anaerobes during the break-down of complex organic matter (Zinder, 1984). For example, the oxidation of organic compounds by sulfate reducers is frequently incomplete, leading to acetate as an end-product (Rabus et al., 2006). Its presence in anoxic pore waters is often attributed to the degradation of organic matter (e.g. Blair et al., 1987; Burdige, 2002). This degradation could also occur thermally; in laboratory experiments where organic rich sediment was heated under hydrothermal conditions, acetate concentrations increased dramatically and the compound was proposed as an intermediary during the transformation of organic matter to CH₄ and CO₂ (Seewald et al., 1990). If acetate results from biomass degradation, its isotopic signature should be similar to that of the starting material. In structures with high acetate concentrations the $\delta^{13}\text{C}$ of TOC ranges from -3‰ to -12‰ (Kelley et al., 2005; Fig. 4). In anaerobic sediments with high organic loads, acetate in pore waters was 5‰ lighter than the corresponding TOC (Blair et al., 1987). If this relationship held at Lost City, acetate would have an isotopic signature of -8‰ to -17‰ .

4.2. Thermodynamic calculations

We used our results as well as previously published measurements to further explore the predicted speciation of single-carbon compounds under the specific temperature, pressure, and chemical conditions of Lost City. The equilibration of formate and DIC by Eq. (1) above is highly dependent on hydrogen concentrations, which vary widely across the field, with lower concentrations attributed to microbial utilization (Proskurowski et al., 2006). For this reason, fluids with the highest hydrogen concentrations are believed to reflect most accurately sub-surface geochemical conditions.

Fig. 5 shows the expected metastable equilibrium speciation of single-carbon compounds in fluids from Marker 3, the vent with the highest hydrogen concentrations, after the conventions of McCollom and Seewald (2003) and Seewald et al. (2006). We assume a pressure of 100 bar (10 MPa) and the endmember chemical compositions found in Table 2, with a total dissolved carbon concentration of 180 μmolal . Predicted temperatures based on models of serpentinization are similar ($200 \pm 50\text{ °C}$, Allen and Seyfried, 2004; $200\text{--}300\text{ °C}$, Foustoukos et al., 2008) while oxygen isotope data from carbonate veins and the lack of anhydrite precipitation suggest temperatures $<150\text{ °C}$ (Kelley et al., 2005).

These temperatures are somewhat higher than those calculated for the temperature at which H₂O–H₂ or CH₄–H₂ last equilibrated ($55\text{--}106\text{ °C}$ for fluids considered here; Proskurowski et al., 2006). This discrepancy may be due to conductive cooling of the fluids after they leave the reaction zone (Proskurowski et al., 2006; Foustoukos et al., 2008). Because of the dependence of the formate–DIC equilibrium on H₂ concentrations, the temperatures calculated by the H₂O–H₂ equilibrations are likely to be the most representative of the final equilibration temperature of formate–DIC (105 °C for Marker 3; Table 2). Methane formation has been excluded from the equilibrium assemblage; while its formation is thermodynamically favored over formate or DIC, the detectable presence of these latter compounds in fluids and sluggish reaction rates in laboratory experiments indicates methane does not equilibrate with other single-carbon compounds at low temperatures at timescales relevant to Lost City discharge zones (Shock, 1992; Foustoukos and Seyfried, 2004; McCollom and Seewald, 2006).

At the sub-surface conditions of Lost City, formate formation is indeed thermodynamically favored (Fig. 5). While the pH of end-member fluids is 10.7 at 25 °C, the pH at *in situ* temperatures and pressures will be somewhat lower (Bach et al., 2004; Foustoukos et al., 2008). Under these conditions, the predicted relative concentrations of formate and DIC are reasonably well reflected by the measured concentrations. It should be noted, however, that DIC concentrations from Marker 3 fluids are 10–100 times higher than fluids from other locations (Table 2). As an example, Boomin' Beehive (LC-B) has similar hydrogen and formate concentrations as Marker 3, but $<1\%$ of the DIC content. One explanation is that there is a loss of DIC at some point along the fluid pathway, either due to precipitation of carbonate (Kelley et al., 2001, 2005; Früh-Green et al., 2003; Ludwig et al., 2006) or uptake by microbial communities (Bradley et al., 2009).

If formate, DIC and hydrogen concentrations reflected equilibrium at sub-surface conditions, it would be possible to calculate the temperature at which they came into equilibration. For reaction (1) above, we use the relations:

$$\Delta G_r = \Delta G_r^\circ + 2.303RT \log Q \quad (3)$$

where ΔG_r is the overall Gibbs free energy of reaction, ΔG_r° is the standard Gibbs free energy of reaction, R is the gas constant, T is temperature and Q is the activity product, given for equilibration between carbon dioxide and formate by

$$Q = a(\text{HCOO}^-)a(\text{H}_2\text{O})/a(\text{HCO}_3^-)a(\text{H}_2) \quad (4)$$

At the temperature of equilibrium, the value calculated for Q from the fluid data will be equal to the equilibrium constant (K). Values of K are calculated by using the SUPCRT92 software (Johnson et al., 1992), with additional data from Shock (1995).

Assuming a pressure of 100 bars (10 MPa), and the concentrations reported in Table 2, calculated equilibration temperatures for reaction (1) are $<25\text{ °C}$ for all vents, much lower than temperatures measured at the time of fluid sampling or sub-surface temperatures calculated by other means (Allen and Seyfried, 2004; Kelley et al., 2005;

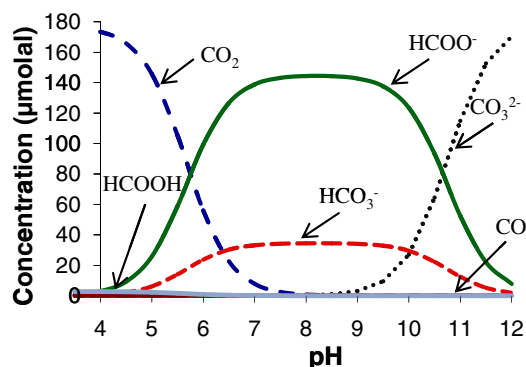


Fig. 5. Calculated equilibrium distribution of single-carbon compounds excluding methane ($\text{CO}_{2(\text{aq})}$, HCO_3^- , CO_3^{2-} , CO , $\text{HCOO-H}_{(\text{aq})}$, and HCOO^-) at 105 °C and 100 bars at Marker 3. Total dissolved carbon concentration is the sum of measured formate and DIC (Table 2), or 180 μmolal . Hydrogen concentration held constant at 12.3 mmolal (Proskurowski et al., 2006).

Proskurowski et al., 2006; Foustoukos et al., 2008). Marker 3 is the only exception, with a calculated temperature of ~ 75 °C. Calculated temperatures using formate equilibrium are likely too low due to loss of DIC after equilibration.

4.3. Implications

Several lines of evidence support the abiotic formation of formate at Lost City including its isotopic composition, its rapid equilibration with CO_2 in laboratory experiments, and the ratio of formate to total DOC. This small organic acid is the second most prevalent carbon species in Lost City fluids and may provide a necessary carbon source to local microbial communities in the face of low DIC concentrations ($<1\text{--}26$ μM ; Proskurowski et al., 2008). A single archaeal phylotype dominates microbial communities in the chimneys, the Lost City *Methanosarcinales* [LCMS] (Schrenk et al., 2004; Brazelton et al., 2006). Lipids believed to be associated with LCMS are unusually isotopically enriched (-2.9‰ to $+6.7\text{‰}$; Bradley et al., 2009) compared to almost any of the potential inorganic carbon sources (DIC: more depleted than -8‰ ; Proskurowski et al., 2008; CH_4 : -9‰ to -14‰ ; Kelley et al., 2005). Bradley et al. (2009) argue that these lipids were produced by archaea reducing CO_2 to methane, and attribute the extreme isotopic enrichment to carbon limitation, which does not allow the consumer to express the expected isotopic fractionation. If methanogenesis is a dominant community metabolism, formate may also play an important role. About half of all known methanogens can utilize formate as an electron donor (Hedderich and Whitman, 2006), and for numerous isolates it can be the sole source for carbon and energy (Jones and Stadtman, 1977; Morii et al., 1983; Belay et al., 1986; Sparling and Daniels, 1986). As yet, however, no species of *Methanosarcinales* has been shown capable utilizing formate. Methanogenesis from formate involves oxidation of the substrate to produce CO_2 , catalyzed by the formate dehydrogenase gene (Sparling and Daniels, 1986). A recently sequenced species of *Methanosarcina* does contain the necessary formate dehydrogenase gene, but attempts

to grow this species on formate were unsuccessful (Maeder et al., 2006). Phylogenetic studies indicate sulfur-cycling metabolisms may also be important and chemical gradients in fluids across the field are consistent with the occurrence of sulfate-reduction (Brazelton et al., 2006; Proskurowski et al., 2006). Several sulfate-reducing microorganisms are capable of growth on formate as the sole carbon source (Brysch et al., 1987). The direct determination of the isotopic signature of formate would greatly assist in testing the hypothesis that it is a carbon source to Lost City microbial communities.

If formate is being used as a carbon source at Lost City, microbial uptake would be expected to result in concentration decreases across the field. Of the four vents across which formate and hydrogen co-vary (H, 2, B, and 3) there is a ~ 60 μM decrease in the organic acid for a ~ 11 mmol/kg decrease in hydrogen. For many anaerobic bacteria, chemical species used for cell synthesis are only a fraction of chemical species used for energy metabolism (Thauer et al., 1977). Therefore, large variations in hydrogen (mmol/kg) used for thermodynamic energy may result in only relatively minor variations (μM) in chemical species used for carbon biomass. Alternatively, this decrease of formate may be a simple thermodynamic equilibration back to DIC as a result of lower hydrogen concentrations.

Acetate has a strong negative correlation with hydrogen across the Lost City field and is likely produced as a result of microbial activity. While it is possible it is produced as a by-product of an autotrophic community, it is more likely produced by the heterotrophic or thermal break-down of larger organic molecules. Some contribution of abiologically formed acetate cannot be discounted, as thermodynamic studies indicate its formation is energetically favorable at high pHs and high hydrogen (Shock and Schulte, 1998). More work must be done to distinguish these sources.

Because acetate is an excellent carbon and energy source for a wide range of microbial species, including sulfate reducers and methanogens (Rabus et al., 2006; Hedderich and Whitman, 2006), it is surprising this compound is not fully utilized. Some uptake of acetate may be occurring before the point of sampling. However, if vent fluids with high hydrogen are the least microbially altered and the most representative of deep sub-surface conditions, then the majority of acetate production also appears to be a result of microbial influence within the chimneys. The anoxic microbial populations best able to thrive at Lost City are presumably those able to take advantage of the resources available in pure end-member fluids, namely high concentrations of hydrogen, methane, sulfate, and formate but low concentrations of acetate. Indeed, phylogenetic studies indicate microbial communities in actively venting chimneys predominantly metabolize methane and sulfur species (Schrenk et al., 2004; Brazelton et al., 2006). Acetate produced by these communities may be removed by fluid flow before it can be utilized by other populations.

Alkaline hydrothermal systems have been proposed as potential sites to the origin of life and organic acids play an important role in this hypothesis. Hydrothermal systems within ultramafic environments are thought to have been operative for much of Earth's history (Suror and Arfa,

1997; Kelley et al., 2005). The hydrogen-rich, anaerobic, alkaline environment within the sub-surface of Lost City is favorable for some aspects of prebiotic chemistry such as the formation of nucleotide constituents and RNA-bearing vesicles (Russell, 2003; Holm et al., 2006). The initial reduction of CO₂ has been described as one of the most difficult chemical steps in the development of the acetyl-CoA cycle, and formate is a critical intermediate towards the kinds of reduced carbon species found in metabolic cycles (Martin and Russell, 2007; Martin et al., 2008). Given that similar high pH, carbonate-bearing environments are common in systems where active serpentinization is occurring, or has occurred in the past, investigation into the origin and cycling of dissolved organic carbon is critical to understanding biological processes in these systems, and the formation of the organic precursors from which life evolved.

ACKNOWLEDGMENTS

We thank the captains and crews of the R/V Atlantis, DSV Alvin and ABE for their invaluable assistance, Rick Keil and Lihini Aluwihare for informative conversations and Sunny Remington for assistance with method development. This manuscript benefited from helpful comments from Gretchen Früh-Green and three anonymous reviewers. We thank Tom McCollom for his editorial handling. This work was supported by NSF Grant OCE0137206 and partially funded by the PMEL Vents Program and the Joint Institute for the Study of the Atmosphere and Ocean (JISAO) under NOAA Cooperative Agreement No. NA17RJ1232, JISAO contribution #1740, PMEL contribution #3223.

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Associate editor: Tom McCollom