

## Methane thermometry in deep-sea hydrothermal systems: evidence for re-ordering of doubly-substituted isotopologues during fluid cooling

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### Abstract :

Deep-sea hydrothermal fluids are often enriched in carbon dioxide, methane, and hydrogen. Methane effuses from metal-rich black smokers such as the Rainbow hydrothermal field, at temperatures higher than 200 °C. At the Lost City field, CH<sub>4</sub> emanates from alkaline fluids at < 100 °C. The abundance of the rare, mass-18 CH<sub>4</sub> isotopologues, <sup>13</sup>CH<sub>3</sub>D and <sup>12</sup>CH<sub>2</sub>D<sub>2</sub>, can mitigate degeneracies in the conventional isotopic signatures of methane. We studied the isotopologue compositions of methane from the Rainbow, Lucky Strike, Von Damm, and Lost City hydrothermal fields. At Rainbow, where the vented fluids are at ~360°C, our coupled  $\Delta^{12}\text{CH}_2\text{D}_2$  -  $\Delta^{13}\text{CH}_3\text{D}$  data establish that methane is in internal equilibrium at °C. This may track the formation temperature of abiotic methane, or it may be the result of equilibration of methane isotopologues within the carrier fluid. Lucky Strike and Von Damm have fluid temperatures < 300°C and although  $\Delta^{13}\text{CH}_3\text{D}$  values are indistinguishable from those at Rainbow, <sup>12</sup>CH<sub>2</sub>D<sub>2</sub> abundances are marginally higher. At Lost City,  $\Delta^{13}\text{CH}_3\text{D}$  data show a range of values, which at face value correspond to apparent temperatures of between °C and °C, far hotter than fluid temperatures. A unique aspect of the Lost City data is the range of large <sup>12</sup>CH<sub>2</sub>D<sub>2</sub> excesses. The  $\Delta^{12}\text{CH}_2\text{D}_2$  data correspond to temperatures of between °C and °C, showing a near-perfect match with fluid temperatures. We find that mixing scenarios involving microbial methane may not account for all of the isotope data. We suggest that  $\Delta^{12}\text{CH}_2\text{D}_2$  values, unlike  $\Delta^{13}\text{CH}_3\text{D}$  values, are prone to near-complete re-equilibration at host fluid temperatures. We suggest that <sup>13</sup>CH<sub>3</sub>D isotopologue data are consistent with abiotic methane being synthesized at ~350 °C. On the other hand, <sup>12</sup>CH<sub>2</sub>D<sub>2</sub> isotopologue ordering records post formation residence temperatures. We explore a possible mechanism decoupling the re-equilibration systematics of the doubly-substituted isotopologues.

## 36 1. Introduction

37 Deep-sea hydrothermal fluids host mantle-derived CO<sub>2</sub> and various forms of reduced carbon that include  
38 methane, C<sub>2+</sub> alkanes, formate, and a variety of unsaturated molecules (Charlou et al., 2000; Charlou et al., 2002;  
39 Charlou et al., 2010; Holm and Charlou, 2001; Konn et al., 2009; Lang et al., 2010; McDermott et al., 2015; Reeves  
40 et al., 2014; Welhan, 1988; Welhan and Craig, 1979). So-called 'black smokers' vent hydrothermal fluid with  
41 exit temperatures >300 °C and show significant amounts of molecular hydrogen (Fig. 1), which may allow for  
42 the abiotic reduction of CO and/or CO<sub>2</sub> to methane (Berndt et al., 1996; Horita and Berndt, 1999; Jones et al.,  
43 2010; McCollom, 2013; McCollom, 2016; McCollom et al., 2010; McCollom and Seewald, 2001; Neubeck et al.,  
44 2011; Okland et al., 2014; Oze et al., 2012; Seewald et al., 2006). In the laboratory, abiotic methane formation  
45 is sluggish (McCollom and Seewald, 2007) but may be enhanced by the presence of a catalyst, typically a Fe-Ni  
46 alloy, preferably in the presence of H<sub>2</sub> vapor (McCollom, 2013; McCollom, 2016; McCollom et al., 2010). In  
47 natural hydrothermal systems, the synthesis of abiotic methane may rather be catalyzed by oxides present in  
48 mafic and ultramafic rocks (Foustoukos and Seyfried, 2004) during serpentinization. However, it is unclear  
49 whether natural fluids are saturated in vapor (Welhan, 1988, Charlou et al. 2010). Whether they offer a set of  
50 conditions allowing the rapid conversion of oxidized carbon to methane remains debated. Secondary fluid  
51 inclusions may be an alternative source of abiotic methane: worldwide oceanic peridotites and gabbros host  
52 vapor H<sub>2</sub>- and CH<sub>4</sub>-rich secondary fluid inclusions (Grozeva et al., 2020; Kelley and Früh-Green, 1999; Klein et  
53 al., 2019). Within the inclusions, a complex interplay of reactions between olivine, oxidized carbon and gaseous  
54 H<sub>2</sub> can lead to the synthesis of methane. As a result, inclusions contain up to 50 mole percent CH<sub>4</sub> (Grozeva et  
55 al., 2020; Kelley, 1996; Kelley and Früh-Green, 1999; Klein et al., 2019) and could be a large reservoir where  
56 abiotic methane formation occurs at temperatures below 340 °C by reduction of trapped magmatic CO<sub>2</sub> (Klein  
57 et al. 2019, McDermott et al. 2015, Grozeva et al. 2020). Dissolution or fracturing of the olivine that hosts the  
58 inclusions could simply release abiotic methane in hot hydrothermal fluids, like at Von Damm or Rainbow sites  
59 (Klein et al., 2019; McDermott et al., 2015). Regardless of the exact process of abiotic methane synthesis, it  
60 appears associated specifically with high temperature fluid-rock interaction in deep-sea hydrothermal  
61 systems.

62 In contrast to high temperature black-smoker vents, hot-springs venting at the Lost City hydrothermal field  
63 are characterized by considerably lower temperatures. However, Lost City fluids contain relatively high CH<sub>4</sub>  
64 and H<sub>2</sub> concentrations (Fig. 1), with isotopic and chemical signatures that are consistent with alkane  
65 abiogenesis (Lang et al., 2012; Proskurowski et al., 2008), similar to observations made in hotter fluids  
66 (McDermott et al., 2015). It is unclear whether fluids at Lost City record high-temperature events  
67 (Proskurowski et al., 2006) and subsequently underwent conductive cooling, or if low-temperature chemistry  
68 allowed the synthesis of abiotic alkanes. A complication is that the coldest vents at Lost City (T < 60°C) host  
69 biofilms containing 10<sup>6</sup>-10<sup>9</sup> cells per gram of carbonate, dominated by the Lost City *Methanosarcinales*  
70 (Brazelton et al., 2006; Kelley et al., 2005; Schrenk et al., 2004). These methanogens may contribute methane  
71 to the fluids at those vents (Bradley and Summons, 2010) and the contribution

72 The concentrations of the mass-18 isotopologues of methane, <sup>13</sup>CH<sub>3</sub>D and <sup>12</sup>CH<sub>2</sub>D<sub>2</sub>, can elucidate the origins  
73 and thermal histories of methane gases in hydrothermal fields. In Δ<sup>13</sup>CH<sub>3</sub>D versus Δ<sup>12</sup>CH<sub>2</sub>D<sub>2</sub> space (Figure 2),  
74 <sup>13</sup>CH<sub>3</sub>D/<sup>12</sup>CH<sub>4</sub> and <sup>12</sup>CH<sub>2</sub>D<sub>2</sub>/<sup>12</sup>CH<sub>4</sub> ratios are reported relative to the stochastic distribution of isotopologues. In  
75 this diagram, methane in thermodynamic equilibrium with respect to isotopologue abundances falls on an  
76 equilibrium curve, where the relative abundances of rare isotopologues yield concordant temperatures (Figure  
77 2). When equilibrium signatures are observed, the assumption that methane isotopologue may constrain CH<sub>4</sub>  
78 thermometry (Stolper et al. 2014) may be valid (Young et al., 2017). In laboratory experiments, both biotic and  
79 abiotic methane exhibits disequilibrium deficits of <sup>12</sup>CH<sub>2</sub>D<sub>2</sub> at varying Δ<sup>13</sup>CH<sub>3</sub>D values (Giunta et al. 2019,  
80 Young et al. 2017). In the case of microbial methane, kinetic controls result in anomalously low values for both  
81 Δ<sup>12</sup>CH<sub>2</sub>D<sub>2</sub> and Δ<sup>13</sup>CH<sub>3</sub>D relative to formation-temperature estimates (Wang et al. 2015, Young et al. 2017). Such  
82 signatures were observed in sedimentary and lacustrine settings (Young et al. 2017, Giunta et al. 2019, Young  
83 2019). Alternatively, partial reversibility of key enzymatic reactions during microbial methanogenesis (Stolper  
84 et al., 2015; Wang et al., 2015) and/or syntrophic microbial mediation (Ash et al., 2019; Giunta et al., 2019;  
85 Young et al., 2017; Young, 2019) could lead to biogenic methane characterized by equilibrium abundances of  
86 CH<sub>4</sub> isotopologues. Abiotic methane may exhibit peculiar signatures at high temperatures (Young et al. 2017);  
87 the few experimental data suggest Δ<sup>13</sup>CH<sub>3</sub>D may preserve CH<sub>4</sub> formation temperatures of abiotic methane,  
88 while Δ<sup>12</sup>CH<sub>2</sub>D<sub>2</sub> can exhibit substantial depletions qualitatively similar to those produced by microbial

89 methanogenesis. This pronounced abiotic disequilibrium in  $\Delta^{12}\text{CH}_2\text{D}_2$  values is well illustrated by methane in  
90  $\sim 25^\circ\text{C}$  fluids from the Kidd Creek mine, where  $\text{CH}_4$  has been suggested to be abiotic in origin (Sherwood Lollar  
91 et al., 1993; Sherwood Lollar et al., 2006; Sherwood Lollar et al., 2002). There,  $\Delta^{13}\text{CH}_3\text{D}$  values are broadly  
92 consistent with environmental temperatures in the mine and inferred temperatures of methane formation  
93 (Young et al. 2017; Sherwood Lollar et al. 2002) while remarkable  $\Delta^{12}\text{CH}_2\text{D}_2$  depletions of up to  $\sim 25\%$  relative  
94 to equilibrium are observed (Young et al. 2017). Although the  $\Delta^{12}\text{CH}_2\text{D}_2$  formation temperatures are lost in this  
95 case, the disequilibrium isotopologue signature may be a diagnostic feature of abiotic methanogenesis (Young  
96 et al. 2017; Young 2019). Based on these burgeoning but still unclear signatures of methane formation  
97 pathways, methane isotopologues may provide new constraints on the origin and/or the thermal history of  $\text{CH}_4$   
98 in hydrothermal vent settings.

99 Wang et al. (2018) published the first  $\Delta^{13}\text{CH}_3\text{D}$  methane isotopologue data for deep-sea hydrothermal fields.  
100 At Rainbow, Lucky Strike, Von Damm, and Lost City, they measured a homogenous  $\Delta^{13}\text{CH}_3\text{D}$  values of  
101  $\sim 1.6 \pm 0.3\%$ . This value suggests a temperature of  $^{13}\text{CH}_3\text{D}$  ordering of  $310_{-42}^{+53}^\circ\text{C}$ , indicating abiotic methane  
102 formed at high-temperatures contributes to all studied hydrothermal fluids, including the fluids at Lost City  
103 (Wang et al., 2018). Because the homogeneous  $\Delta^{13}\text{CH}_3\text{D}$  is observed for fluids with varying exit temperatures  
104 (between  $370$  and  $96^\circ\text{C}$ ), it was suggested that  $\Delta^{13}\text{CH}_3\text{D}$  ordering is preserved upon fluid cooling (Wang et al.  
105 2018). An original aspect of the clumped  $\text{CH}_4$  systematics is that two doubly-substituted isotopologues can be  
106 measured independently (Young et al. 2016). Coupling these two species may help constrain the thermal  
107 history of methane. A current unknown is the relative behavior of  $\Delta^{12}\text{CH}_2\text{D}_2$  compared to  $\Delta^{13}\text{CH}_3\text{D}$  in  
108 hydrothermal fluids containing methane. It is unclear whether  $\Delta^{12}\text{CH}_2\text{D}_2$  may provide new constraints on the  
109 origin of  $\text{CH}_4$  in hot hydrothermal fluids. If so, we predict disequilibrium  $\Delta^{12}\text{CH}_2\text{D}_2$  signatures will help track  
110 abiotic methane in natural samples, similar to signatures observed in the  $\sim 25^\circ\text{C}$  fluids at the Kidd Creek mine  
111 (Young et al. 2017). Alternatively, any  $\Delta^{12}\text{CH}_2\text{D}_2$  abiotic signature may be erased at elevated temperatures, via  
112 re-ordering of  $^{12}\text{CH}_2\text{D}_2$  isotopologue. It is unclear whether  $\Delta^{13}\text{CH}_3\text{D}$  would also be affected by re-ordering at all.  
113 In other words, potential re-ordering processes may cloud the meaning of the  $\text{CH}_4$  systematics. In the absence  
114 of experimental data on the behaviors of  $\Delta^{12}\text{CH}_2\text{D}_2$  and  $\Delta^{13}\text{CH}_3\text{D}$  in hot fluids, we present data of  $\Delta^{13}\text{CH}_3\text{D}$  and

115  $\Delta^{12}\text{CH}_2\text{D}_2$  measured in gases from deep-sea hydrothermal fluids sampled at Lucky Strike, Rainbow, Von Damm  
116 and Lost City. These fluids experienced variable extents of conductive cooling, allowing a test of whether  
117  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  may be re-ordered in natural fluids. Ultimately, the data are used to examine how  
118  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  may constrain the origin and/or the thermal evolution of methane in hydrothermal  
119 systems. Some of our samples are the same aliquots of gas analyzed for  $\Delta^{13}\text{CH}_3\text{D}$  by laser spectroscopy by Wang  
120 et al. (2018), to which we add the first  $\Delta^{12}\text{CH}_2\text{D}_2$  measurements. For Lost City,  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$   
121 measurements are reported for four new samples. Our samples from all of the sites span a large range of  
122 measured vent temperatures, from 65 to 370°C. With these samples, we ascertain whether both mass-18  
123 methane isotopologues record concordant temperatures of bond-ordering or if they record distinctive aspects  
124 of methane thermal history.

125

## 126 **2. Samples and geological context**

127 Rainbow is a hydrothermal field hosted in ultramafic rocks at 36°14' N on the Mid-Atlantic ridge (MAR), at  
128 a water depth of 2300 meters (Charlou et al., 2002; Seyfried Jr et al., 2011). At Rainbow, hydrothermal fluids  
129 have temperatures up to 370 °C and are the hottest fluids studied here. Hydrothermal fluids show chloride  
130 concentrations significantly higher than seawater, indicative of a brine-vapor phase separation occurring at  
131 depth at a minimum temperature of 380 °C (Charlou et al. 2002). The hydrothermal endmember contains ~2  
132 mM  $\text{CH}_4$  (Fig. 1) with a  $\delta^{13}\text{C}$  of ~-15‰ (Charlou et al. 2002; Seyfried et al., 2011).

133 Lucky Strike is an unsedimented hydrothermal vent field hosted in basaltic rock, at 37°17' N on the MAR,  
134 with fluid temperatures up to 324 °C (Charlou et al., 2000; Pester et al., 2012). Lucky Strike is a relatively  
135 shallow system with a seafloor depth of 1700 meters. There, the vent fluids have low silica, metal and rare earth  
136 content and are thought to reflect a rapid upflow of fluids to the surface with short water-rock reaction times  
137 (Charlou et al., 2000). Chloride concentration of hydrothermal fluids at Lucky Strike indicate the circulating  
138 seawater experienced brine-vapor phase separation at  $T > 360^\circ\text{C}$ , but at relatively low-pressure (Charlou et al.  
139 2000). The methane concentration at Lucky Strike is ~1mM (Fig. 1) with a  $\delta^{13}\text{C}$  of ~-13‰ (Charlou et al. 2000;  
140 Pester et al., 2012).

141 The Von Damm site at 18°22' N on the Mid-Cayman rise is hosted in ultramafic, gabbroic, and basaltic rocks,  
142 at 2350 meters below seafloor (Connelly et al., 2012). There, hydrothermal fluids emanate at temperatures as  
143 high as 226 °C (McDermott et al., 2015). Evidence that fluids experienced phase separation at Von Damm is  
144 inconclusive (McDermott et al. 2015). Methane concentration in the hydrothermal endmember is ~ 3mM CH<sub>4</sub>  
145 (Fig. 1) with a δ<sup>13</sup>C of ~-15‰ (McDermott et al. 2015). For all high-temperature hydrothermal fields, δD data  
146 for CH<sub>4</sub> are scarce. To date, only Wang et al. (2018) published δD values for the studied sites, with all samples  
147 yielding values around -110±15‰. These values are consistent with a D/H equilibrium between methane and  
148 water established during methane formation, at ~300 °C (Wang et al. 2018), based on the experimental data  
149 from Horibe and Craig (1995).

150 Lost City is located ~15 km west of the Mid-Atlantic Ridge in 1.5 my old lithosphere dominated by ultramafic  
151 rocks with minor mafic intrusions that have been exposed through detachment faulting. It is a serpentinite-  
152 hosted hydrothermal system, venting relatively cool (20–100 °C) fluids. In contrast with other systems studied  
153 here that vent acidic fluids, Lost City is the only field venting highly alkaline fluids with pH values > 9 (Kelley et  
154 al., 2001; Kelley et al., 2005). The lower temperatures at LCHF may be due to the location of the site, a few  
155 kilometers off the mid-Atlantic ridge axis and thus not directly above a localized heat source. The chlorinity of  
156 Lost City fluid is similar to seawater, suggesting no brine separation occurred beneath the Lost City  
157 hydrothermal field (Allen and Seyfried Jr, 2004). This is generally consistent with thermodynamic modelling  
158 suggesting maximum temperatures of 200±50°C (Foustoukos et al., 2008; Seyfried Jr et al., 2015) although in  
159 the past mantle rocks in the Lost City basement experienced a higher-temperature alteration history (Früh-  
160 Green et al., 2018; Rouméjon et al., 2018). Maximum temperatures of 200±50°C for fluids currently circulating  
161 in the system appear to be lower than at the other sites, but the much cooler effusion temperatures of < 100°C  
162 nonetheless suggest that circulating fluids at Lost City underwent significant conductive cooling prior to  
163 venting.

164 Samples from Rainbow and Lucky Strike were collected by ROV Jason II during cruises to the Mid-Atlantic  
165 Ridge in 2008 (Pester et al., 2012; Reeves et al., 2014; Seyfried Jr et al., 2011). Rainbow samples are from three  
166 vents: *Guillaume*, *Auberge*, and *CMSP\_and\_P* (all venting at 365±10°C). The sample from Lucky Strike is from  
167 the vent *Isabel* (venting at 292 °C). Samples from Von Damm were collected on the Mid-Cayman Rise in 2012  
168 (McDermott et al., 2015). They are from two vents, *East Summit* (226°C) and *Ravelin* (145°C) and ~~*Old Man Tree*~~

169 (115°C). Six Lost City samples were collected during the 2008 ROV Jason II cruise (J2-361-IGT5), the 2005 Lost  
170 City expedition (H06\_GT\_15/16) and the 2018 cruise to Lost City (J2-1107-GT9, J2-1111-Titan, J2-1110-GT18,  
171 J2-1110-Titan). They are from three vents: *Beehive* (~100°C), *Marker C* (~85°C), and *Marker 2* (~65 °C). Eight  
172 of our samples were previously analyzed by laser spectroscopy at MIT (Wang et al. 2018). Those were from  
173 Rainbow (n=3), Von Damm (n=3), Lucky Strike (n=3) and Lost City (*Beehive* only, n=1). The six remaining  
174 samples were not analyzed previously for either methane bulk isotope compositions nor for the rarer mass-18  
175 methane isotopologues.

176 Due to near-zero concentrations of dissolved magnesium (Mg) in endmember hydrothermal vent fluids,  
177 measured Mg concentrations in sampled fluids can be used to estimate the extent of seawater entrainment  
178 during sampling or subsurface mixing. Concentrations of dissolved CH<sub>4</sub> concentrations plotted against  
179 dissolved Mg concentration for fluids from all sites studied are shown in Figure 1 with data from the literature  
180 (Reeves et al. 2014; McDermott et al. 2015). Despite the considerable mixing shown in Figure 1, we picked  
181 samples from Lucky Strike, Lost City, and Rainbow with low Mg concentration that can be considered to have  
182 minimal seawater contribution from sampling and thus more representative of the hydrothermal endmember.  
183 In contrast, based on Mg concentration reported in the literature, subseafloor seawater mixing before  
184 discharge occurred in some of the samples from Von Damm. One of our samples from a central vent (*Summit*)  
185 is devoid of seawater contamination and represents the local hydrothermal endmember, at 226°C, while the  
186 other (*Ravelin*) shows evidence for subseafloor mixing with seawater that is reflected by a lower exit  
187 temperature of 145°C (McDermott et al., 2015).

188

### 189 3. Measurement protocol

190 Methane was extracted from breakseals on a vacuum system at UCLA and purified by gas  
191 chromatography using procedures described previously by Young et al. (2017). Between 40 and 120  
192 micromoles of methane were available for each sample in our study (Table 1). Gases analyzed previously at  
193 MIT were subsequently trapped onto silica gel and sealed in quartz tubes for storage and transport to UCLA. At  
194 UCLA, the vacuum-line with a resting pressure of  $3 \times 10^{-5}$  mbar or lower was used to purify the samples. The

195 gases were transferred from the breakseals into a silica gel-filled stainless-steel u-trap cooled with liquid  
 196 nitrogen. Helium carrier gas was used to flush the sample to the gas chromatograph. Separation was  
 197 accomplished with a 3m long, 1/8 in. OD stainless steel column packed with 5 A molecular sieve, followed in  
 198 series by a 2m long 1/8 in. OD stainless steel column packed with HayeSep D porous polymer. Gas  
 199 chromatograph peaks were detected with an in-line, passive thermal conductivity detector (TCD). Once  
 200 methane collection was complete, methane was transferred to a glass vial filled with silica-gel at liquid nitrogen  
 201 temperature and subsequently connected to the inlet of the Panorama mass spectrometer where it was  
 202 warmed and expanded into the mass spectrometer.

203 Isotopologue measurements were made using the Panorama instrument, a Matsuda-type large radius  
 204 high-resolution mass spectrometer at UCLA with a mass resolving power that allows the simultaneous  
 205 measurement of ion currents for resolved  $^{12}\text{CH}_4^+$ ,  $^{13}\text{CH}_4^+$ ,  $^{12}\text{CH}_3\text{D}^+$ ,  $^{13}\text{CH}_3\text{D}^+$  and  $^{12}\text{CH}_2\text{D}_2^+$  ion beams. The  
 206 measured ratios of these ion currents yield values for both  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  as well as for bulk  $^{13}\text{C}/^{12}\text{C}$   
 207 and D/H. The mass spectrometer is set to a mass resolving power equal to or greater than 40,000. Both low  
 208 abundances of the mass-18 isotopologues,  $^{13}\text{CH}_3\text{D}^+$ , and  $^{12}\text{CH}_2\text{D}_2^+$ , require that measurements are made with an  
 209 electron multiplier. Isotopologues of masses 16 and 17 are measured on Faraday collectors with amplifier  
 210 resistors of  $10^{11} \Omega$ . The bulk carbon and hydrogen stable isotopic ratios are reported relative to the  
 211 international standards V- PDB and V-SMOW, respectively, and expressed in permil units using the delta  
 212 notation:

$$213 \quad \delta^{13}\text{C} = \left[ \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{VPDB}}} - 1 \right] \times 1000 \quad (1)$$

214 and

$$215 \quad \delta\text{D} = \left[ \frac{(\text{D}/\text{H})_{\text{sample}}}{(\text{D}/\text{H})_{\text{VSMOW}}} - 1 \right] \times 1000 \quad (2).$$

216  
 217 Mass-18 isotopologue compositions are reported versus a stochastic distribution expressed in permil using the  
 218 capital delta notation:

$$220 \quad \Delta^{13}\text{CH}_3\text{D} = \left[ \frac{(^{13}\text{CH}_3\text{D}/^{12}\text{CH}_4)_{\text{sample}}}{(^{13}\text{CH}_3\text{D}/^{12}\text{CH}_4)_{\text{stochastic}}} - 1 \right] \times 1000 \quad (3)$$



248 favorable counting statistics. The internal uncertainties range from 0.2‰ and 0.5‰ for  $\Delta^{13}\text{CH}_3\text{D}$ , and between  
249 0.5‰ and 2.7‰ for  $\Delta^{12}\text{CH}_2\text{D}_2$ . Note that for all samples except two gases from Von Damm that yielded the  
250 smallest  $\text{CH}_4$  quantities of the sample set, the internal uncertainties remained below 0.3‰ for  $\Delta^{13}\text{CH}_3\text{D}$ , and  
251 1.0‰ for  $\Delta^{12}\text{CH}_2\text{D}_2$ .

252

#### 253 4. Results

254 Our bulk isotope ratios are in general agreement with previously-published  $\text{CH}_4$  isotopic data for these  
255 localities (Charlou et al., 2002, Seyfried et al., 2011, 2015; Pester et al., 2012; McDermott et al., 2015; Wang et  
256 al., 2018, Proskurowski et al., 2008). In  $\delta\text{D}$  vs  $\delta^{13}\text{C}$  space the samples plot in the  $^{13}\text{C}$ - and D- enriched extremity  
257 of the abiotic methane field (Fig. 3) (Etiope and Sherwood Lollar, 2013). Comparison with the data of Wang et  
258 al. (2018) shows good agreement. Disagreement is found for one datum for sample J2-612-IGT2 from Von  
259 Damm where there is a discrepancy in  $\delta\text{D}$  between the two laboratories, with the UCLA value being lower by  
260  $\sim 5\text{‰}$ . The UCLA datum for this sample also exhibit a low  $\delta^{13}\text{C}$  value relative to the MIT value, consistent with  
261 a 1:10  $\delta^{13}\text{C}$  -  $\delta\text{D}$  fractionation that must have occurred between the moment it was measured at MIT and the  
262 moment it was measured at UCLA. We speculate that a fractionation could have occurred during incomplete  
263 sample freezing onto the silica gel in the quartz tube at MIT. Nonetheless, we determined a  $\Delta^{13}\text{CH}_3\text{D}$  value from  
264 this gas that is indistinguishable from that reported by Wang et al. (2018), within uncertainties. This suggests  
265 that if a fractionation had occurred during the trapping of the sample at MIT, or during gas processing at UCLA,  
266 it was not sufficient to affect the apparent bond ordering.

267 Our methane isotopologues results (Table 1, Fig. 2) yield values of  $\Delta^{13}\text{CH}_3\text{D}$  between  $1.0\pm 0.2$  and  $3.0\pm 0.2\text{‰}$ ,  
268 expanding the range of  $1.0\pm 0.6$  to  $1.9\pm 0.3\text{‰}$  reported by Wang et al. (2018) for deep-sea hydrothermal vent  
269 systems. No direct relationship is observed between the UCLA and MIT values, because of the narrow spread  
270 of the eight samples measured in both laboratories, and because of the relative uncertainties. For the first time,  
271 we report  $\Delta^{12}\text{CH}_2\text{D}_2$  values for deep-sea hydrothermal methane, and we show the samples have values ranging  
272 from  $1.7\pm 1.0\text{‰}$  to  $11.6\pm 0.5\text{‰}$  (Table 1, Fig. 2). No  $\Delta^{12}\text{CH}_2\text{D}_2$  values below zero are observed, in contrast with

273 measurements of putative abiotic methane from the Kidd Creek mine (Young et al. 2017). Within analytical  
274 uncertainties, isotopologue abundances in samples from Rainbow are consistent with internal equilibrium with  
275 the ambient fluids (Fig. 2), with average  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values of  $1.4\pm 0.2\text{‰}$  and  $2.7\pm 0.9\text{‰}$  ( $n=3$ , both  
276 1 s.d.), respectively. Samples from Lucky Strike and Von Damm show similar signatures, with  $\Delta^{13}\text{CH}_3\text{D}$  data  
277 ranging from  $1.0\pm 0.2\text{‰}$  to  $1.7\pm 0.4\text{‰}$ , and  $\Delta^{12}\text{CH}_2\text{D}_2$  values that are slightly higher than values at Rainbow. At  
278 Von Damm,  $\Delta^{12}\text{CH}_2\text{D}_2$  values are  $+4.4\pm 1.0\text{‰}$  and  $4.5\pm 1.8\text{‰}$  for *Ravelin* and *Summit* vents, respectively. In  
279 contrast, gases from Lost City are characterized by disequilibrium isotopologue signatures (Fig. 2). The Lost  
280 City samples exhibit  $\Delta^{13}\text{CH}_3\text{D}$  values between  $1.9\pm 0.2\text{‰}$  and  $3.0\pm 0.2\text{‰}$  extending the range of values  
281 previously known for Lost City (Wang et al., 2018).  $\Delta^{12}\text{CH}_2\text{D}_2$  values are between  $10.7\pm 0.9\text{‰}$  and  $14.5\pm 1.0\text{‰}$ .  
282 The highest values are observed in *Marker 2*, the coolest vent, while *Beehive* and *Marker C* show consistent  
283 compositions. All methane from Lost City plots far above the equilibrium curve in  $\Delta^{13}\text{CH}_3\text{D}$  vs.  $\Delta^{12}\text{CH}_2\text{D}_2$  space.  
284 We find no relationships between the isotopologue signatures and host rock (whether hydrothermal fluids are  
285 hosted in basaltic versus ultramafic rocks), seafloor depth, or likely maximal pressures reached in the  
286 subsurface (Fig. 2). We argue below that the isotopologue signatures result from partial or near-complete re-  
287 equilibration of doubly-substituted isotopologues at environmental temperatures.

288

## 289 5. Discussion

### 290 5.1. Equilibrium signatures for methane isotopologues

291 The methane isotopologue data for the hydrothermal systems studied here show signatures ranging from  
292 equilibrium to disequilibrium, from the hottest to the coldest fluids, respectively (Fig. 2). Samples from  
293 Rainbow have average  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values that represent thermodynamic equilibrium among the  
294 methane isotopologues. The  $\Delta^{13}\text{CH}_3\text{D}$  values correspond to an apparent temperature of  $343^{+41}_{-35}$  °C, similar to  
295 that derived previously (Wang et al. 2018). The temperature indicated by the  $\Delta^{12}\text{CH}_2\text{D}_2$  values is  $334^{+78}_{-53}$  °C,  
296 indistinguishable from that derived from the more abundant  $^{13}\text{CH}_3\text{D}$  isotopologue, albeit less precise. This  
297 establishes that methane from Rainbow is in isotopic bond-ordering equilibrium.

298 Equilibrium could result from abiotic methane synthesis. This would be in contrast with deficits in  $\Delta^{12}\text{CH}_2\text{D}_2$   
299 where abiotic methanogenesis may have occurred at low temperatures at the Kidd Creek mine, where fluids  
300 carrying methane show measured temperatures of  $\sim 25^\circ\text{C}$  (Sherwood Lollar et al., 2002, Young et al., 2017).  
301 Equilibrium signatures in hot fluids could alternatively represent re-equilibration at high temperatures rather  
302 than being the result of the formation mechanism. Re-ordering is also suspected because disequilibrium  
303  $\Delta^{12}\text{CH}_2\text{D}_2$  signatures seem to be associated with abiotic  $\text{CH}_4$  formation in laboratory experiments with  
304 durations from 12 to 30 days (Young et al., 2017). The few data for abiotic methane synthesized experimentally  
305 suggest  $\Delta^{13}\text{CH}_3\text{D}$ -based temperatures to be roughly consistent with experimental temperatures of abiotic  $\text{CH}_4$   
306 formation, while  $\Delta^{12}\text{CH}_2\text{D}_2$  depletions of  $\sim 2$  to  $60\%$  relative to equilibrium were observed between 75 and 500  
307  $^\circ\text{C}$ . Only experiments where methane was synthesized at  $600^\circ\text{C}$  during about 12 days yielded equilibrium for  
308 both  $\Delta^{12}\text{CH}_2\text{D}_2$  and  $\Delta^{13}\text{CH}_3\text{D}$  values (Young et al. 2017). We emphasize that it is unlikely that the experiments  
309 presented in Young et al. (2017) are an accurate representation of geochemical processes in deep-sea  
310 hydrothermal fields. It is interesting, however, that the two sets of experiments explored different formation  
311 mechanisms, the Fischer-Tropsch-type Sabatier reaction and silane hydration, and yielded broadly consistent  
312 results. In the silane experiments,  $\text{Si}_5\text{C}_{12}\text{H}_{36}$  reacts with  $\text{H}_2\text{O}$  to produce  $\text{SiO}_2$ ,  $\text{CH}_4$  and  $\text{H}_2$  (Foustoukos and  
313 Mysen, 2013) In the Sabatier processes,  $\text{CO}_2$  is converted to  $\text{CH}_4$  catalyzed with ruthenium at temperature  $<$   
314  $100^\circ\text{C}$  (Etiopie and Ionescu, 2015). In both experiments, the large  $\Delta^{12}\text{CH}_2\text{D}_2$  depletions may result from a  
315 quantum tunneling effect expressed during hydrogenation of methane precursors (Young et al. 2017).  
316 Alternatively, it may result from what is termed a “combinatorial effect” caused by mixing sources of hydrogen  
317 with markedly different D/H produced by classical kinetic and equilibrium partitioning of isotopes associated  
318 with the various hydrogenation steps during reduction of oxidized carbon (Cao et al., 2019; Young et al., 2017;  
319 Young, 2019). Hydrogen with wide ranging D/H ratios is sourced from different reversible and irreversible  
320 steps during methane formation, resulting in deficits in  $\Delta^{12}\text{CH}_2\text{D}_2$  caused by mixing the various sources of  
321 hydrogen (Röckmann et al., 2016; Taenzer et al., 2020; Yeung, 2016). The two mechanistic interpretations  
322 described here both allow the preliminary experimental results from Young et al. (2017) to be extrapolated to  
323 natural settings, but further experimental work is needed.

324 In the context of the limited natural and experimental evidence, we tentatively suggest that equilibrium  
325 signatures at Rainbow (Fig. 2) might result from re-ordering rather than being inherited directly from abiotic  
326 methane synthesis. Secondary fluid-inclusions in gabbroic and ultramafic rocks likely contribute abiotic  
327 methane to hydrothermal fields (Kelley and Fruh Green, 1999, McDermott et al. 2015, McCollom, 2016, Klein  
328 et al. 2019). The inclusions may also provide conditions suitable for post-formation re-equilibration, since they  
329 contain metal oxides possibly contributing to catalysis (Klein et al. 2019, Grozeva et al. 2020) and would likely  
330 host methane over long periods of time. Alternatively, the concordant temperatures may reflect a rapid re-  
331 equilibration of all doubly-substituted isotopologues in the hot carrier fluid: at Rainbow, the  $\Delta^{13}\text{CH}_3\text{D}$ -based  
332 temperature of  $343^{+41}_{-35}$  °C and the  $\Delta^{12}\text{CH}_2\text{D}_2$  temperature of  $334^{+78}_{-33}$  °C are both statistically indistinguishable  
333 from the temperature of the carrier fluid ( $360\pm 10^\circ\text{C}$ ). Our data are therefore consistent with abiotic methane  
334 synthesis or re-equilibration in either the source fluid inclusions or in the carrier fluid at this site.

335 Methane from Lucky Strike and Von Damm show  $\Delta^{13}\text{CH}_3\text{D}$  values similar to those at Rainbow, but display  
336 marginally higher  $\Delta^{12}\text{CH}_2\text{D}_2$  values. Our one sample from Lucky Strike (from the *Isabel* vent) is virtually devoid  
337 of seawater contamination and is associated with a measured fluid temperature of 292 °C (Pester et al., 2012).  
338 The  $\Delta^{13}\text{CH}_3\text{D}$  values of  $1.0\pm 0.2\text{‰}$  suggest a temperature of  $430^{+60}_{-50}$  °C. The  $\Delta^{12}\text{CH}_2\text{D}_2$  value of  $+2.9\pm 0.7\text{‰}$   
339 corresponds to an apparent temperature of  $320^{+50}_{-39}$  °C. The latter estimate is within uncertainty of the  
340 measured fluid temperature (Fig. 4). At Von Damm, fluids are venting at a maximum temperature of 226 °C, but  
341 many of the fluids have experienced subseafloor mixing with cold seawater and are thus characterized by vent  
342 temperatures  $< 226^\circ\text{C}$  (McDermott et al., 2015). The  $\Delta^{13}\text{CH}_3\text{D}$  values are homogeneous and average  $1.5\pm 0.2\text{‰}$   
343 which yields an apparent temperature of  $325^{+40}_{-31}$  °C. The  $\Delta^{12}\text{CH}_2\text{D}_2$  values are homogeneous as well but with an  
344 average of  $\sim +4.5\pm 1.0\text{‰}$ , corresponding to a lower temperature of  $247^{+43}_{-35}$  °C, similar to the measured  
345 hydrothermal maximum temperature of 226 °C (Fig. 4). We see no correlations between methane isotopologue  
346 data and the extent of subseafloor mixing with seawater (Table 1). The methane concentration of seawater is  
347 so low that arguably, seawater contamination does not add significant amounts of methane to the bulk vent  
348 fluid. We additionally conclude that both  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values remain unaffected by the abrupt cooling  
349 associated with seawater infiltration.

350 Based on these results, we suggest that the methane isotopologue data at Rainbow, Lucky Strike and Von  
351 Damm are all consistent within  $2\sigma$  with high-temperature methane formed at  $\sim 330^\circ\text{C}$ , as recorded by  $\Delta^{13}\text{CH}_3\text{D}$ .  
352 This thermometry is consistent with methane originating from secondary fluid inclusions, where  
353 thermodynamic modeling suggests it was synthesized over geological timescales at temperatures between 300  
354 and  $400^\circ\text{C}$  (Klein et al., 2019). Our results also imply re-ordering of the original  $\Delta^{12}\text{CH}_2\text{D}_2$  values in the cooler  
355 carrier fluids. However, the  $\Delta^{12}\text{CH}_2\text{D}_2$  re-ordering is about 2‰ and thus its detection is marginal given the  
356 measurement uncertainties of  $\sim 1\%$ . We show below that data obtained on the coldest site studied here, Lost  
357 City, are more dispositive with regard to the origin of discordance between  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values.

## 358 5.2: Substantial $\Delta^{12}\text{CH}_2\text{D}_2$ re-equilibration or mixing in the cold Lost City fluids

359 Unlike at the other sites, at Lost City methane is carried to the seafloor by relatively cool fluids. The three  
360 vents examined, *Beehive* ( $\sim 100^\circ\text{C}$ ), *Marker C* ( $\sim 85^\circ\text{C}$ ), and *Marker 2* ( $\sim 65^\circ\text{C}$ ), show much lower temperatures  
361 than other vents studied here. The  $\Delta^{13}\text{CH}_3\text{D}$  values are consistent with equilibration temperatures between  
362  $265 \pm_{24}^{28}^\circ\text{C}$  and  $158 \pm_{14}^{16}^\circ\text{C}$ , far hotter than the measured fluid temperatures. The  $\Delta^{12}\text{CH}_2\text{D}_2$  values yield  
363 temperature estimates of  $101 \pm_{8}^{9}^\circ\text{C}$ ,  $93 \pm_{8}^{9}^\circ\text{C}$  and  $69 \pm_{4}^{4}^\circ\text{C}$ , showing a near-perfect match with the vent fluid  
364 temperatures (Fig. 4). The highest  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values are observed in the coldest vent, *Marker 2*.  
365 These discordant isotopologue temperatures constitute marked disequilibrium (Fig. 2).

366 Before discussing the evidence of  $\Delta^{12}\text{CH}_2\text{D}_2$  re-ordering at Lost City, we consider first whether the  
367 disequilibrium seen in the  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  data could be the result of mixing (e.g., Young et al., 2017). A  
368 relatively constant  $\text{CH}_4/{}^4\text{He}$  ratio observed in Lost City vent fluids argues in favor of a unique abiotic source  
369 (Proskurowski et al. 2008, Lang et al. 2012). However, others have suggested a possible contribution from  
370 biotic sources (Bradley et al. 2009, 2010); the coldest vents at Lost City ( $T < 60^\circ\text{C}$ ) host biofilms dominated by  
371 the Lost City *Methanosarcinales* phylotype (Brazelton et al., 2006; Kelley et al., 2005; Schrenk et al., 2004). The  
372 methanogens may contribute significant microbial methane to the fluids at those vents (Bradley and Summons,  
373 2010). In principle mixing between abiotic and microbial methane could account for the Lost City characteristic  
374 excesses in  $\Delta^{12}\text{CH}_2\text{D}_2$ . Our use of two mass-18 isotopologues affords the ability to identify mixing if it occurred

375 at Lost City: mixing trajectories in  $\Delta^{13}\text{CH}_3\text{D}$  versus  $\Delta^{12}\text{CH}_2\text{D}_2$  space may trend above the equilibrium curve into  
376 positive  $\Delta^{12}\text{CH}_2\text{D}_2$  deviations from equilibrium when endmembers are similar in  $\delta^{13}\text{C}$  values but disparate in  
377  $\delta\text{D}$  values. Multiple two-component mixing scenarios can satisfy mixing relationships in the isotopologue  
378 space. For example, in **Figure 5** we show a two-component mixing model between a gas equilibrated at 400°C  
379 and a microbial gas with characteristic  $\Delta^{12}\text{CH}_2\text{D}_2$  deficits. The high-T endmember  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values are taken  
380 to be similar to the values observed at Lucky Strike for illustration. Approximately 20 to 25% of a microbial  
381 gas mixed with high-temperature gas like that at the other vent sites can explain the methane isotopologue  
382 data at Lost City (**Figure 5, panel a**). However, for the mixing loop to reach the compositions of the Lost City  
383 samples, unusually high  $\delta^{13}\text{C}$  values of between  $-35\text{‰}$  and  $-20\text{‰}$  must be ascribed to the microbial methane  
384 component (**Fig. 5, panel b**). This is because the bulk isotopes and clumped isotopologue values, by design, do  
385 not vary independently. Such high  $\delta^{13}\text{C}$  values would be considered unreasonable for microbial methane in  
386 most systems (Whiticar, 1999), but may be conceivable at Lost City, where biomarkers were observed with  
387 extraordinarily high  $\delta^{13}\text{C}$  values (Bradley et al. 2009). This mixing scenario, however, fails to match the data  
388 trends where bulk isotope ratios are considered, including  $\delta^{13}\text{C}$  vs.  $\delta\text{D}$  and  $\Delta^{13}\text{CH}_3\text{D}$  vs.  $\delta^{13}\text{C}$  (**Figure 5, panel b**  
389 **and c respectively**). Conversely, when mixing is successful in panels comparing bulk isotopes and clumped  
390 isotopes (**Fig. 5, panel b and d**), it fails at accounting for the data in mass-18 isotopologue space (**Figure 5, panel**  
391 **a**). For this reason, we conclude that mixing with a microbial methane component is not the main source of the  
392 anomalously high  $\Delta^{12}\text{CH}_2\text{D}_2$  values at Lost City. An alternative mixing scenario involves two gases both at  
393 isotopologue thermodynamic equilibrium at two disparate temperatures (Figure 5). A ~50:50 mixing of two  
394 gases, one on the equilibrium curve at 400°C, and the other on the equilibrium curve at any temperature <  
395 150°C, could fit the Lost City isotopologue and bulk isotope data as long as the  $\delta^{13}\text{C}$  and  $\delta\text{D}$  of the endmembers  
396 are restricted to a tight range of values. An example is shown in Fig. 5, with a gas taken at 100°C for illustration  
397 (curve 2). The 100°C gas at Lost City would have to have  $\delta^{13}\text{C}$  values  $\geq -8\text{‰}$ , and corresponding  $\delta\text{D}$  values of  
398  $-170\text{‰}$  and lower. This is at the high-  $\delta^{13}\text{C}$  and low- $\delta\text{D}$  end of the empirically-defined abiotic field in Figure 2  
399 (Etiope and Sherwood Lollar, 2013; Whiticar, 1999). The high  $\delta^{13}\text{C}$  would be consistent with the near-  
400 quantitative reduction of magmatic  $\text{CO}_2$  (Horita and Berndt, 1999). The required  $\delta\text{D}$  value of  $-170\text{‰}$  would  
401 indicate a methane-water D/H equilibrium established at low temperature compared to Rainbow, Lucky Strike,

402 or Von Damm (Horibe and Craig, 1995), consistent with the bond-ordering inferences. However, again, this  
403 type of mixing relationship fails to account for the details of the data trend in panel b of Figure 5. Where the  
404 mixing proportions needed in the isotopologue plot (Fig. 5, panel a) are roughly 50-50, they are about 80-20 in  
405 the bulk isotope space (Fig. 5 panel c), causing a mismatch in Figure 5, panel b.

406 Mixing involving a microbial endmember seems to be ruled out, and mixing between gases equilibrated at  
407 high and low temperatures yields imperfect fits to the methane isotopologue and bulk isotope data taken  
408 together. If mixing cannot satisfy the data in Fig. 5, we speculate that this is because  $\Delta^{12}\text{CH}_2\text{D}_2$  was altered by  
409 re-ordering at the temperature of the ambient fluids in the vent system at shallow depths. The near-perfect  
410 match between  $\Delta^{12}\text{CH}_2\text{D}_2$ -based temperatures and fluid temperatures (Fig. 4) argues in favor of  $\Delta^{12}\text{CH}_2\text{D}_2$  re-  
411 ordering. This conclusion is independent of the initial isotopologue composition of the abiotic methane, be it  
412 on the equilibrium curve or below the equilibrium curve, as observed with the limited experimental evidence  
413 (Young et al., 2017; see discussion above). We also suggest that the evidence for re-equilibration at Lost City  
414 (Fig. 4) allows re-equilibration to have occurred at high-temperatures sites like Rainbow (see section 5.1). The  
415 mechanism of re-equilibration remains unclear. In the laboratory, methane isotopologues reorder only in the  
416 presence of a metal catalyst or alumina (Eldridge et al., 2019; Stolper et al., 2014; Wang et al., 2019; Young et  
417 al., 2017) when methane is in a gas phase, but no experiments exist for dissolved methane in water. At Lost City  
418 (and in all other deep-sea hydrothermal sites), methane is dissolved in water. Since  $\Delta^{12}\text{CH}_2\text{D}_2$  is evidently re-  
419 ordered specifically at the exit temperature of the host fluids, the re-equilibration must have occurred in the  
420 cooling fluid upon ascent, perhaps facilitated by the metals that occur in underlying serpentinites at Lost City  
421 (Kelley et al. 2005, Fruh-Green et al., 2018) or in metal deposits in chimneys observed at other sites (Von  
422 Damm, 1990). It is also possible that the re-equilibration of  $\Delta^{12}\text{CH}_2\text{D}_2$  values is microbially mediated. The  
423 Archeal community at Lost City includes anaerobic methane-oxidizing Archaea, ANME-1 (Brazelton et al.,  
424 2010). Equilibration of  $\Delta^{12}\text{CH}_2\text{D}_2$  values has been attributed to anaerobic oxidation of methane (AOM) at  
425 several localities, notably in gases from the Baltic Sea and the Western Ontario Basin (Young et al., 2017; Giunta  
426 et al. 2019; Ash et al., 2019). However, the evidence for equilibration by AOM has been circumstantial and yet  
427 to be demonstrated conclusively in the laboratory, and would not account for the equilibrated  $\Delta^{12}\text{CH}_2\text{D}_2$  data

428 at Rainbow, Von Damm and Lucky Strike, where temperatures are above 122 °C, often considered the upper  
429 limit for life (Takai et al. 2008).

430 In all cases, the data suggest that bond re-ordering is substantially slower for  $\Delta^{13}\text{CH}_3\text{D}$ , as if re-ordering was  
431 inhibited when the isotopologue includes  $^{13}\text{C}$  rather than  $^{12}\text{C}$ . The mechanism allowing one  
432 isotopologue,  $^{12}\text{CH}_2\text{D}_2$ , to be re-ordered to fluid temperature, while  $^{13}\text{CH}_3\text{D}$  remains seemingly constant  
433 deserves experimental attention. The observation that  $\Delta^{12}\text{CH}_2\text{D}_2$  is prone to re-equilibration at lower  
434 temperatures while  $\Delta^{13}\text{CH}_3\text{D}$  is not suggests that the abundances of  $^{13}\text{CH}_3\text{D}$  may represent a geochemical  
435 indicator that preserves information about temperatures associated with methane formation, even when the  
436 methane is transported at low temperatures prior to venting. The latter point is consistent with an earlier  
437 suggestion based on  $\Delta^{13}\text{CH}_3\text{D}$  measurements alone (Wang et al. 2018), but it is verified and underscored by the  
438 contrasting behaviors of the two mass-18 isotopologues of methane at the vents studied here. The value of the  
439  $\Delta^{12}\text{CH}_2\text{D}_2$  values may lie with their sensitivity to subsequent environmental conditions.

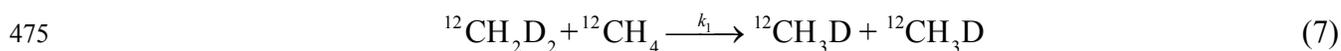
440 A caveat to the basic picture described above arises from the observation of a  $\sim 1.5\text{‰}$   $\Delta^{13}\text{CH}_3\text{D}$  variability  
441 at Lost City, when the *Beehive Marker C* and *Marker 2* vents are compared. To explain this in the context of just  
442 one high-temperature source of methane at Lost City (Lang et al., 2012), one must invoke partial re-ordering  
443 of  $\Delta^{13}\text{CH}_3\text{D}$ . The implication is that the cooling that resets  $\Delta^{12}\text{CH}_2\text{D}_2$  values also partially re-equilibrates  
444  $\Delta^{13}\text{CH}_3\text{D}$  values. High-temperature methane formed at  $\sim 330$  °C would exhibit  $\Delta^{13}\text{CH}_3\text{D}$  values of  $\sim 1.5\text{‰}$ . At 85  
445 °C, thermodynamic equilibrium drives  $\Delta^{13}\text{CH}_3\text{D}$  to  $\sim 4\text{‰}$ . *Beehive*, *Marker C*, and *Marker 2* are vented at 96°C,  
446 81 °C and 64 °C, respectively, where the expectation of a  $\Delta^{13}\text{CH}_3\text{D}$  trending towards  $\sim 4\text{‰}$  because of partial  
447 re-equilibration is valid. One may speculate that cooling allowed partial reordering of  $\Delta^{13}\text{CH}_3\text{D}$  to  $1.9\pm 0.2\text{‰}$  at  
448 *Beehive* and  $2.3\pm 0.2\text{‰}$  at *Marker C*. The ANME-1 identified at *Marker 2* (Brazelton et al., 2006) may be poorly  
449 suited to process methane in Lost City (Lang et al. 2018) but if any biological mediation of methane occurred,  
450 it might have affected  $\Delta^{13}\text{CH}_3\text{D}$  at *Marker 2*, raising the values to  $3.0\pm 0.2\text{‰}$  there. The advantage of the  
451 hypothesis for partial  $\Delta^{13}\text{CH}_3\text{D}$  re-equilibration at Lost City is that it explains why gases with higher  $\Delta^{13}\text{CH}_3\text{D}$   
452 are only observed in the coldest vents. It also removes the need for multiple methane pools, which is

453 inconsistent with the broadly constant  $\text{CH}_4/{}^4\text{He}$  ratio observed in the vents of Lost City (Proskurowski et al.  
454 2008, Lang et al. 2012).

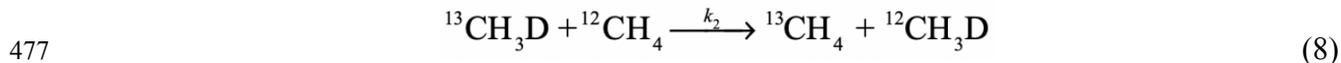
### 455 5.3 The relative vulnerability of ${}^{12}\text{CH}_2\text{D}_2$ to re-ordering

456 A process allowing  $\Delta^{12}\text{CH}_2\text{D}_2$  to be equilibrated at fluid temperature during conductive cooling, while  
457  $\Delta^{13}\text{CH}_3\text{D}$  remains broadly constant, appears to account for natural data. This process calls for differences in re-  
458 equilibration rates. Here we explore this possibility with a simple kinetic model for exchange. Sophisticated  
459 models, where re-equilibration is driven by D/H exchange between methane and water, could be envisioned.  
460 In this context, water would be a reactive intermediate for re-ordering of methane isotopologues. At Lost City,  
461 this approach would be consistent with the overall lower D/H ratio for vented methane compared to other  
462 sites, suggesting some (but rather limited) D/H exchange occurred between methane and water during fluid  
463 cooling (Proskurowski et al., 2006, Reeves et al., 2012). As a first approximation, we explore the specific case  
464 of exchange of H and D between methane molecules. Our result based on molecular symmetry turns out to be  
465 the same if we use water as the exchange partner. In either case, the stretched bond permitting the fleeing of  
466 a protium or deuterium from a methane molecule is the reaction coordinate. We can describe the transition  
467 state formed by this stretching symbolically, in the case of  $\text{CH}_4$ , as  $\text{CH}_3\text{-H}$ . The relative magnitudes of rate  
468 constants for exchange reactions among isotopologues are dominated by differences in symmetry numbers, as  
469 can be deduced from the analysis by Haghnegahdar et al. (2017). Indeed, both kinetic rate constants and  
470 equilibrium constants for the mass-18 isotopologues are controlled to first order by differences in symmetry  
471 numbers of the molecules. For example, the high-temperature limits for the equilibrium constants shown in  
472 Equations (5) and (6) are 1 and 3/8, respectively, both corresponding to the relevant ratios of reactant and  
473 product symmetry numbers.

474 Consider the reactions



476 and



478 One may apply transition state theory and write the reaction in terms of the transition state for one molecule  
 479 that will control the rate of the reaction, treating the deuterated reactant as rate limiting in a matrix of  
 480 exchangeable CH<sub>4</sub> gas (or, as it turns out, H<sub>2</sub>O). While the full equation that includes vibrational and rotational  
 481 partition functions is necessary for capturing isotope ratios in detail at the per mil level of precision, the rate  
 482 constant ratio can be well approximated for our purposes using the ratio of symmetry numbers for the  
 483 transition states and the reactants where  $\sigma_i$  are the symmetry numbers. For this purpose, we use the known  
 484 symmetry numbers for the reactants:  $\sigma_{12\text{CH}_4} = 12$ ,  $\sigma_{13\text{CH}_3\text{D}} = 3$ , and  $\sigma_{12\text{CH}_2\text{D}_2} = 2$  and recall that the rotational  
 485 partition functions are proportional to the inverse of the symmetry numbers. We estimate the likely symmetry  
 486 numbers for the transition states CH<sub>2</sub>D-D\* and <sup>13</sup>CH<sub>3</sub>-D\* based on previous work (Haghnegahdar et al., 2017).  
 487 Stretching of the <sup>12</sup>CH<sub>2</sub>D<sub>2</sub> molecule to donate a D to <sup>12</sup>CH<sub>4</sub> should result in a highly asymmetric transition state  
 488 with  $\sigma_{\text{CH}_2\text{D-D}^*} = 1$ . The transition state for <sup>13</sup>CH<sub>3</sub>D involves stretching the one <sup>13</sup>C-D bond, forming <sup>13</sup>CH<sub>3</sub>-D\*, and  
 489 preserving the symmetry of <sup>13</sup>CH<sub>3</sub>D, yielding  $\sigma_{\text{CH}_3\text{-D}^*} = 3$ . We therefore have for the rate constant ratio

$$490 \quad \frac{k_{\text{CH}_2\text{D}_2}}{k_{^{13}\text{CH}_3\text{D}}} \sim \frac{\sigma_{\text{CH}_2\text{D}_2} \sigma_{\text{CH}_4}}{\sigma_{\text{CH}_2\text{D-D}^*}} \frac{\sigma_{^{13}\text{CH}_3\text{-D}^*}}{\sigma_{^{13}\text{CH}_3\text{D}} \sigma_{\text{CH}_4}} = \frac{2 \times 12}{1} \frac{3}{3 \times 12} = 2 \quad (9)$$

491 This result shows that the reaction involving the re-equilibration of <sup>12</sup>CH<sub>2</sub>D<sub>2</sub> can be expected to have a rate  
 492 constant that is twice that for <sup>13</sup>CH<sub>3</sub>D. In practice, this means that during conductive cooling of a fluid, simple  
 493 symmetry effects will lead  $\Delta^{12}\text{CH}_2\text{D}_2$  to equilibrate twice as fast to environmental temperature than  $\Delta^{13}\text{CH}_3\text{D}$ .  
 494 Because the symmetry number of the exchange partner (<sup>12</sup>CH<sub>4</sub> as written) cancels in Equation (9), substituting  
 495 H<sub>2</sub>O for <sup>12</sup>CH<sub>4</sub>, where  $\sigma_{\text{H}_2\text{O}} = 2$ , gives the same ratio of rate constants. We note, however, that the geometries  
 496 associated with CH<sub>4</sub>-H<sub>2</sub>O exchange are highly uncertain (e.g., Mourik and Duijneveldt 1995; Cappelletti et al.  
 497 2015; Richardson et al. 2016).

498 In order to apply these rate constants to the exchange process, we make use of rate equations for  
 499 kinetic isotope exchange expressed in terms of fractional approaches to equilibrium (Criss et al., 1987) for the  
 500 reactions in Equations (7) and (8), respectively:

$$501 \quad \frac{[\text{}^{12}\text{CH}_2\text{D}_2]_t - [\text{}^{12}\text{CH}_2\text{D}_2]_{\text{EQ}}}{[\text{}^{12}\text{CH}_2\text{D}_2]_0 - [\text{}^{12}\text{CH}_2\text{D}_2]_{\text{EQ}}} = e^{-k_{\text{CH}_2\text{D}_2} t} \quad (10)$$

502 and

$$\frac{[^{13}\text{CH}_3\text{D}]_t - [^{13}\text{CH}_3\text{D}]_{\text{EQ}}}{[^{13}\text{CH}_3\text{D}]_0 - [^{13}\text{CH}_3\text{D}]_{\text{EQ}}} = e^{-k_{^{13}\text{CH}_3\text{D}} t} \quad (11)$$

503  
504 where subscript  $t$  refers to time, subscript  $o$  refers to initial concentrations, and subscript EQ refers to the  
505 equilibrium concentrations for the isotopologues.

506 The re-equilibration mechanism with various rate constant ratios is plotted on figure 2, alongside the  
507 data. We explore whether the trajectory of a gas equilibrated at high temperatures undergoing cooling matches  
508 the Lost City observation. We take the low-temperature endpoint to be constrained by the measured  
509 temperature in the relevant Lost City vents, at 65 °C. Although the rate constant ratio of 2 resulting from  
510 Equation (9) yields a trajectory in the right direction, it does not fully account for the data. We find that a rate  
511 constant ratio  $k_{\text{CH}_2\text{D}_2}/k_{^{13}\text{CH}_3\text{D}}$  of  $\sim 5$  is required to explain the data (Fig. 2). Again, our data highlight the need for  
512 experimental work to explore whether these re-equilibration patterns are to be expected on a physicochemical  
513 basis. The simple theoretical framework explored here may offer a first order guide to how methane  
514 isotopologues behave during fluid cooling in natural settings.

## 515 Conclusion

516 We report  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  data in methane samples from deep-sea hydrothermal systems. We show  
517 that in the hottest fluids at the Rainbow site, concordant temperatures are recorded by the two mass-18  
518 isotopologues. Thus, methane is in internal thermodynamic equilibrium. In the other sites, we observe variable  
519  $\Delta^{12}\text{CH}_2\text{D}_2$  at nearly constant  $\Delta^{13}\text{CH}_3\text{D}$ . The data exclude mixing with microbial gas; mixing produces imperfect  
520 fits to the bulk isotope ratios and isotopologue abundances. The variable  $\Delta^{12}\text{CH}_2\text{D}_2$  values correlate  
521 surprisingly well with fluid temperatures and thus we suggest they record rapid  $^{12}\text{CH}_2\text{D}_2$  re-equilibration in  
522 cooling hydrothermal fluids upon ascent. None of the isotopologue signatures resemble those obtained from  
523 experimental investigations of abiotic  $\text{CH}_4$  synthesis in the laboratory, nor do they resemble the isotopologue  
524 signatures of abiotic methane formation in natural settings to date. The mechanism of re-equilibration in the  
525 case of these deep-sea hydrothermal fluids but not in the case of Kidd Creek cold fluids deserves experimental  
526 investigation. The discovery of resetting of  $\Delta^{12}\text{CH}_2\text{D}_2$  values during transport affords the opportunity to use  
527 these values in concert with  $\Delta^{13}\text{CH}_3\text{D}$  values to obtain a more complete picture of the thermal evolution of

528 methane gases. This is because  $\Delta^{12}\text{CH}_2\text{D}_2$  appears to be completely reset to fluid temperatures in a wide  
529 temperature range, between 360 and 65°C. In contrast,  $\Delta^{13}\text{CH}_3\text{D}$  shows limited evidence for re-ordering. We  
530 suggest that to first order, equilibration of  $\Delta^{12}\text{CH}_2\text{D}_2$  should be faster than that for  $\Delta^{13}\text{CH}_3\text{D}$  by a factor of  $\sim 2$ ,  
531 but further investigation on relative rates of reaction is warranted. Because the apparent  $\Delta^{13}\text{CH}_3\text{D}$ -based  
532 temperatures greatly exceed those amenable to microbial life in all sites, and because the isotopologue data  
533 rule out mixing with microbial gases, our data indicate that methane formation at all of the sites, including Lost  
534 City, is probably dominantly abiotic.

535

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543

### 544 Captions

545

546 Table 1: Methane isotope data for hydrothermal fluids. bulk  $^{13}\text{C}/^{12}\text{C}$  and D/H are reported alongside  $\Delta^{13}\text{CH}_3\text{D}$   
547 and  $\Delta^{12}\text{CH}_2\text{D}_2$ .

548 Analytical uncertainties are below 0.1‰ (95% confidence intervals) for  $\delta^{13}\text{C}$  and  $\delta\text{D}$ . Propagated uncertainties  
549 are listed for  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  in  $1\sigma$ . They are variable from a sample to another, depending mostly on  
550 counting statistics. Hence the magnitude of the uncertainties for both  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  are a simple  
551 function of the processed  $\text{CH}_4$  quantity (in  $\mu\text{mol}$ ). Temperature data are extracted from the literature

552 (McDermott et al. 2015, Reeves et al. 2014, but see also Pester et al. 2012, Charlou et al. 2010, Seyfried et al.,  
553 2011).

554

555

556

557 Figure 1: Dissolved methane and hydrogen concentrations in hydrothermal fluids from the five locations  
558 studied here plotted against Mg concentrations. Data are from Reeves et al. (2014) and McDermott et al. (2015).  
559 With the exception of a sample from Vom Damm, *Ravelin*, all the samples studied here have Mg concentrations  
560 5 mmol/kg (Data in Reeves et al., 2014, McDermott et al., 2015, and this study). *Ravelin*, however, shows a  
561 concentration of ~16 mmol/kg, together with a lower temperature than the local fluid endmember (Reeves et  
562 al., 2014). This one gas can be accounted for by seawater infiltration, abruptly lowering temperature venting.

563

564

565 Figure 2: Plot of  $\Delta^{12}\text{CH}_2\text{D}_2$  vs.  $\Delta^{13}\text{CH}_3\text{D}$  for methane samples from the hydrothermal vent systems that are part  
566 of this study. The curve representing thermodynamic equilibrium is shown for reference, along with  
567 corresponding temperatures. While Rainbow shows equilibrium signatures, Von Damm and Lucky Strike  
568 display marginally high  $\Delta^{12}\text{CH}_2\text{D}_2$ . The Lost City samples display considerable  $\Delta^{12}\text{CH}_2\text{D}_2$  enrichments at a given  
569  $\Delta^{13}\text{CH}_3\text{D}$  value. Data and uncertainties are from Table 1. Re-equilibration trajectories are plotted using the  
570 equations in section 5.3. Three scenarios are shown with three different ratios for the exchange rate constants  
571 for  $\Delta^{12}\text{CH}_2\text{D}_2$  and  $\Delta^{13}\text{CH}_3\text{D}$  (see text). Note that the endmember low temperature is picked at 65 °C for  
572 illustration. This corresponds to the approximate temperature of the fluid from *marker 2*, the coldest vent  
573 studied here. For the Beehive vent, a temperature of 96 °C would be required for the low-temperature  
574 endmember. Although not shown here for the sake of clarity, such an endmember temperature fits the Beehive  
575 data if  $k_1 \sim 5 \times k_2$ .

576

577 Figure 3:  $\delta\text{D}$  and  $\delta^{13}\text{C}$  values of methane samples in the “Schoell” plot (Schoell, 1988). Fields are suggested by  
578 those from Etiope and Sherwood Lollar (2013). As suggested in previous work (Wang et al., 2018,

579 Proskurowski et al. 2008, Charlou et al., 2010, McDermott et al., 2015), methane in deep-sea hydrothermal field  
580 plots defines the extremity of the abiotic field. Data and uncertainties are from Table 1.

581  
582 Figure 4: Plot showing the relationship between the two mass-18 isotopologues of methane samples from the  
583 hydrothermal systems and the endmember temperatures of the fluid containing the methane. Note the  
584 correlation with fluid temperatures exhibited by  $\Delta^{12}\text{CH}_2\text{D}_2$  but not  $\Delta^{13}\text{CH}_3\text{D}$  values. Data and uncertainties are  
585 from Table 1.

586  
587 Figure 5. Mixing scenarios for methane from Lost City, examined in 4 different  $\text{CH}_4$  isotopic spaces. Dots  
588 represent 25% increases in mixing increments. The bold curve is mixing between high-temperature gas and a  
589 microbial gas (see text) and shown by the bold plain curve. The dashed curve represents a two-component  
590 mixing between two equilibrated gases, at distinct temperatures, with endmember composition fit to account  
591 for the data in panel A and C. The plain thin curve is a two-component mixing between two equilibrated gases,  
592 at distinct temperatures, with endmember composition fit to account for the data in panel B, C and D. The panel  
593 A shows the two endmember possibilities that could fit the clumped isotopologue data, where  $\Delta^{12}\text{CH}_2\text{D}_2$  is  
594 plotted vs.  $\Delta^{13}\text{CH}_3\text{D}$ . Panel B, C and D shows the various bulk-isotope trajectories of the model that is seemingly  
595 successful in panel A. We find no mixing scenario that accounts for all of the trends defined by the data.

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787

788 **Declaration of interests**

789

790  The authors declare that they have no known competing financial interests or personal  
791 relationships that could have appeared to influence the work reported in this paper.

792

793  The authors declare the following financial interests/personal relationships which may be considered  
794 as potential competing interests:

795

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Table 1

Methane isotope data for hydrothermal fluids. Bulk  $^{13}\text{C}/^{12}\text{C}$  and D/H are reported alongside  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$ .

Site	Vent	Sample ID	CH <sub>4</sub> quantity (μmol)	Endmember temperature (C)	$\delta^{13}\text{C}$ PDB	$\delta\text{D}$ SMOW	$\Delta^{13}\text{CH}_3\text{D}$	1 se	$\Delta^{12}\text{CH}_2\text{D}_2$	1 se
Rainbow	guillaume	J2-352-IGT4	38.8	361	-16.71	-98.06	1.6	0.3	2.7	0.8
	CMSP_and_P	J2-354-IGT3	51.3	365	-17.03	-98.49	1.2	0.2	3.5	0.8
	Auberge	J2-352-IGT3	46.8	370	-16.96	-98.07	1.3	0.2	1.7	1.0
Von Damm	Ravelin	J2-617-IGT6	45.0	226	-15.72	-107.26	1.4	0.3	4.4	1.0
	Summit	J2-612-IGT2	40.0	228	-16.50	-113.08	1.7	0.4	4.5	1.8
Lost City	Beehive	J2-361-IGT5	62.5	96	-10.80	-127.11	1.9	0.2	10.7	0.9
	Beehive	H06_GT_15/16	85.0	96	-10.84	-127.84	1.9	0.2	11.6	0.5
	Marker 2	j1107 GT	75.0	64	-9.62	-143.31	3.0	0.2	14.5	1.0
	Marker 2	J1111 titan	120.0	64	-7.58	-141.48	2.3	0.2	13.8	0.6
	Marker c	j1110 titan	90.0	81	-10.61	-129.70	2.3	0.2	11.3	0.8
	Marker c	j1110 GT	50.0	81	-10.97	-130.19	2.0	0.2	12.3	1.4
Lucky Strike	Isabel	J2-357-IGT5	47.5	292	-12.03	-100.72	1.0	0.2	2.9	0.7

Analytical uncertainties are below 0.1‰ (95% confidence intervals) for  $\delta^{13}\text{C}$  and  $\delta\text{D}$ . Propagated uncertainties are listed for  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  in 1 $\sigma$ . They are variable from a sample to another, depending mostly on counting statistics. Hence the magnitude of the uncertainties for both  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  are a simple function of the processed CH<sub>4</sub> quantity (in μmol). Temperature data are extracted from the literature (McDermott et al., 2015; Reeves et al., 2014, but see also Pester et al., 2012; Charlou et al., 2010; Seyfried Jr. et al., 2011).

Figure 1

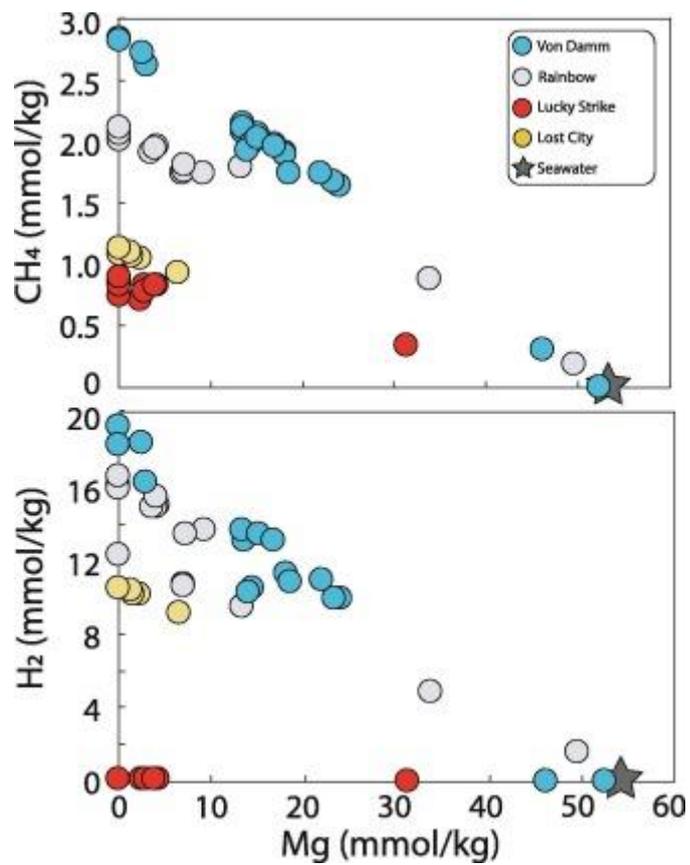


Figure 2

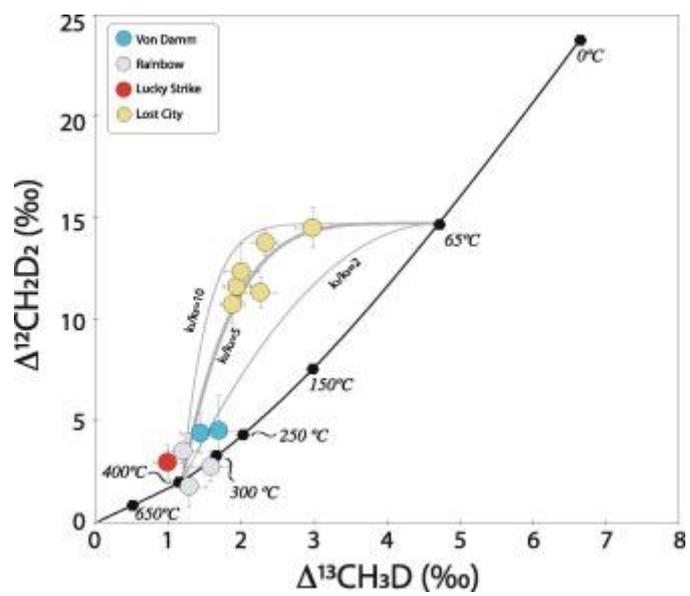


Figure 3

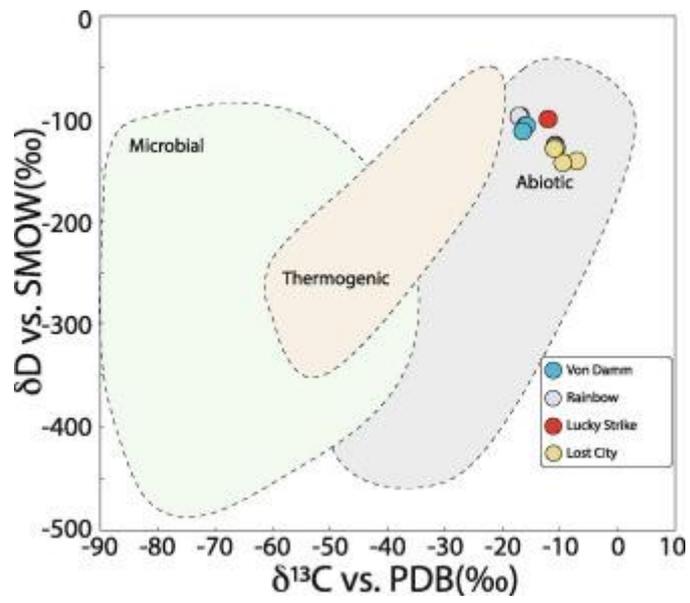


Figure 4

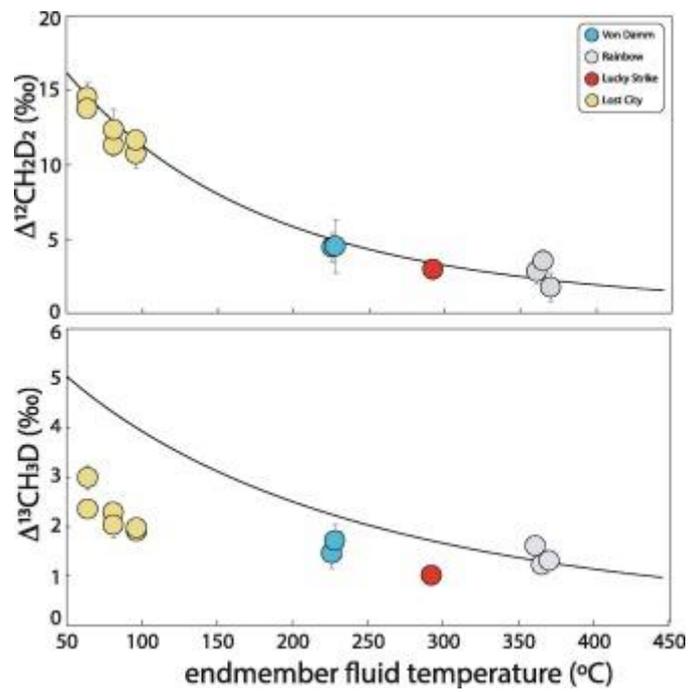


Figure 5

