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Fatty Acid Preservation in Modern and Relict Hot Spring Deposits in Iceland, with Implications for Organics Detection on Mars

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3 **1 Fatty Acid Preservation in Modern and Relict Hot Spring Deposits in Iceland, with**
4 **2 Implications for Organics Detection on Mars**

6
7 **3 *Running Title: Organics Preservation in Icelandic Hot Springs***

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49 22 Keywords: Hot springs, Sinter, Fatty acids, TMAH, SAM instrument

26 **Abstract**

27 Hydrothermal spring deposits host unique microbial ecosystems and have the capacity to preserve
28 microbial communities as biosignatures within siliceous sinter layers. This quality makes
29 terrestrial hot springs appealing natural laboratories to study the preservation of both organic and
30 morphologic biosignatures. The discovery of hydrothermal deposits on Mars has called attention
31 to these hot springs as Mars-analog environments, driving forward the study of biosignature
32 preservation in these settings to help prepare future missions targeting the recovery of
33 biosignatures from Martian hot spring deposits. This study quantifies the fatty acid load in three
34 Icelandic hot spring deposits ranging from modern and inactive to relict. Samples were collected
35 from both the surface and 2 to 18 cm in depth to approximate the drilling capabilities of current
36 and upcoming Mars rovers. To determine the preservation potential of organics in siliceous sinter
37 deposits, fatty acid analyses were performed with pyrolysis-GC-MS utilizing thermochemolysis
38 with tetramethylammonium hydroxide (TMAH). This technique is available on both current and
39 upcoming Mars rovers. Results reveal that fatty acids are often degraded in the subsurface relative
40 to surface samples but are preserved and detectable with the TMAH pyrolysis-GC-MS method.
41 Hot spring mid-to-distal aprons are often the only texturally and geomorphically definable feature
42 in older, degraded terrestrial sinter systems and are therefore most readily detectable on Mars from
43 orbital images. These findings have implications for the detection of organics in Martian
44 hydrothermal systems as they suggest that organics might be detectable on Mars in relatively
45 recent hot spring deposits, but preservation likely deteriorates over geologic time scales. Rovers
46 with thermochemolysis pyrolysis-GC-MS instrumentation may be able to detect fatty acids in hot
47 spring deposits if the organics are relatively young; therefore Martian landing site and sample
48 selection are of paramount importance in the search for organics on Mars.

49 **1. Introduction**

50 Siliceous sinter are terrestrial geothermal spring deposits often formed in volcanic settings from
51 silica-supersaturated fluids heated to high temperature by magmatic reservoirs. These fluids
52 rapidly ascend to the surface where the water discharges at geysers and hot spring vents,
53 precipitating silica as the pressure and temperature of the fluids rapidly decrease (Campbell et al.,
54 2015; Renaut and Jones, 2011). The precipitating silica can nucleate and coat all surfaces in the
55 spring discharge flow path, resulting in unique mineralogical textures that are dependent on

1
2
3 56 temperature and fluid composition when the silica precipitates. Microorganisms that thrive in these
4
5 57 extreme temperature, flow rate, and fluid compositions include bacteria, archaea, phages, and
6
7 58 eukaryotes (e.g. diatoms) (Breitbart et al., 2004; Leira et al., 2017; Pumas et al., 2018; Saini et al.,
8
9 59 2017; Skirnisdottir et al., 2000), and the cells of these organisms can also be entombed by silica
10
11 60 precipitation, preserving both the physical structure of the cell as well as cellular organic matter
12
13 61 (Cady and Farmer, 1996). These domains of life inhabit specific microenvironments at the hot
14
15 62 springs conducive to the organisms' ideal growth conditions. Hyperthermophiles and thermophiles
16
17 63 are found near the higher-temperature spring vent, mesophilic organisms such as cyanobacteria
18
19 64 inhabit fluids <73 °C between the vent, proximal slope, mid-apron, and distal-apron, and plants
20
21 65 and other mesophiles inhabit the lower temperature environments (commonly proximal slope,
22
23 66 mid-apron or distal-apron) (Figure 1).

24
25 67 The preservation of organic biosignatures in geothermal spring deposits is of particular interest to
26
27 68 the astrobiology community, as several siliceous hot spring deposits have been identified on Mars
28
29 69 with both remote sensing and *in situ* exploration. Hydrothermally-driven siliceous sinter deposits
30
31 70 have been identified on Mars from orbital observations in Nili Patera (Skok et al., 2010) and Arabia
32
33 71 Terra (Allen and Oehler, 2008; Rossi et al., 2008). Hot spring opal-A deposits with nodular and
34
35 72 digitate stromatolitic morphologies have been identified in Columbia Hills, Gusev crater, by the
36
37 73 *Spirit* rover (Ruff et al., 2011; Squyres et al., 2008), which are strikingly similar to digitate
38
39 74 stromatolites with putative biogenic origins found at the El Tatio geothermal field in Chile (Ruff
40
41 75 and Farmer, 2016). Although not geothermal in nature, diagenetic silica enrichment is also
42
43 76 observed in Marias Pass, Gale crater, by the *Curiosity* rover, which implies groundwater
44
45 77 circulation in Gale crater long after lacustrine activity ceased (Frydenvang et al., 2017).

46
47 78 The preservation of physical cells and cellular organic matter in hot springs both constitute
48
49 79 biosignatures. Biosignatures are defined as an object, pattern, or substance whose origin
50
51 80 specifically requires a biological agent (Hays et al., 2017). Extensive work has been performed
52
53 81 that documents the extraordinary preservation of microbial cell structure in sinter deposits by
54
55 82 entombment in a very fine matrix of silica particles (Brasier et al., 2011; Cady and Farmer, 1996;
56
57 83 Campbell et al., 2015; Guido et al., 2010; Jones et al., 2000; Konhauser et al., 2001; McCollom et
58
59 84 al., 2016; Munoz-Saez et al., 2016; Renaut et al., 1998; Tarhan et al., 2016; Walter, 1996; Walter
60
61 85 and Des Marais, 1993; Westall et al., 2015; Tobler et al., 2008). Few studies, however, have

86 focused on the preservation of organic matter in modern and relict (up to 900 year old) siliceous
87 sinter deposits (Chaves Torres et al., 2019; Gibson et al., 2014; Kaur et al., 2015, 2011, 2008;
88 Pancost et al., 2005; Pancost et al., 2006; Teece et al., 2019), and even fewer studies have explored
89 organic preservation potential in Mars-analog hot spring environments (e.g. Williams et al., 2019).

90 **1.1. Martian *In Situ* Organics Detection**

91 The *in situ* search for organic molecules on Mars has been a priority of the scientific community
92 for decades, and several missions have carried payloads with varying organics detection
93 capabilities. The approaches that have been deployed and/or planned to detect organics on Mars
94 missions include Raman spectroscopy (Hays et al., 2017 and references therein), deep UV laser
95 excitation (Hays et al., 2015 and references therein), and gas-chromatography mass-spectrometry
96 (GC-MS). GC-MS has the highest heritage, having flown in modified forms on the Mars Viking
97 landers (Biemann et al., 1977), the Mars Phoenix polar lander (Boynton et al., 2001), and the
98 Sample Analysis at Mars (SAM) instrument on the Mars *Curiosity* rover (Mahaffy et al., 2012). A
99 GC-MS is also included as part of the Mars Organic Molecule Analyzer (MOMA) instrument on
100 the upcoming European Space Agency's *Rosalind Franklin* (ExoMars) rover (Goesmann et al.,
101 2017; Goetz et al., 2016).

102 The fundamentals of the GC-MS system for all Mars flight instruments are essentially the same:
103 the goal of the approach is to detect and identify various molecular species and classes at low
104 concentrations with high analytic specificity. Volatile compounds thermally evolved from solid
105 samples in an oven may be directly heated (pyrolyzed) or subjected to a derivatization procedure.
106 The evolved species are separated by the GC into a time sequence, then separated by mass and
107 analyzed with the MS. Although pyrolysis GC-MS has the potential to liberate organics bound
108 within mineral matrices, the detection of those organics can be compromised by the presence of
109 perchlorate or other oxidizing salts. Perchlorate is present on the Martian surface and is known to
110 combust organics during pyrolysis due to the evolution of O₂ from the perchlorate, effectively
111 destroying the organic molecule in the process (Guzman et al., 2018). However, certain
112 derivatization or thermochemolysis reactions can liberate and volatilize select organics in the
113 presence of perchlorates without the organics combusting.

114 A derivatization or thermochemolysis reaction—referred to here, as in the literature, as a “wet
115 chemistry experiment”—is a “one-step” reaction with a reagent that improves organic matter yield

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3 116 and liberates organics bound within macromolecules. Wet chemistry experiments aim to transform
4
5 117 polar organic molecules (e.g. carboxylic acids) into volatile derivatives that are amenable and
6
7 118 detectable by GC-MS (del Rio et al., 1996; Metcalffe and Wang, 1981), as well as freeing bound
8
9 119 components of larger macromolecules otherwise undetectable with GC-MS (Grasset et al., 2002).

10
11 120 Different wet chemistry reagents can be used based on the chemistry of the targeted labile group
12
13 121 and the required yield of the reaction, such as *N,N*-methyltert-butyl-
14
15 122 dimethylsilyltrifluoroacetamide (MTBSTFA; (Goesmann et al., 2017; Mahaffy et al., 2012), *N,N*-
16
17 123 dimethylformamide dimethyl acetal (DMF-DMA; Goesmann et al., 2017), trimethylsulfonium
18
19 124 hydroxide (TMSH; Ishida et al., 1999), and tetramethylammonium hydroxide (TMAH; Grasset et
20
21 125 al., 2002; Mahaffy et al., 2012), to name just a few. Thermochemolysis with TMAH in methanol
22
23 126 (MeOH, a solvent) allows access to refractory or insoluble organic material, including
24
25 127 macromolecular organics such as kerogen-like compounds. Simply pyrolyzing a sample at high
26
27 128 temperature (>650°C) results in fragmentation of the parent molecule due to thermal stress, which
28
29 129 limits molecule identification. To cleave these refractory molecules at a milder temperature (e.g.
30
31 130 ≤600°C), thermochemolysis with TMAH allows the analysis of refractory matter in complex
32
33 131 matrices containing low amounts of organic material with minimal destruction of the organic
34
35 132 material (Geffroy-Rodier et al., 2009; He et al., 2019; Williams et al., 2019). TMAH
36
37 133 thermochemolysis selectively cleaves ester and ether bonds, replacing a labile hydrogen atom with
38
39 134 a methyl group (Figure 2), rather than random and uncontrolled thermal decomposition and
40
41 135 fragmentation of the organic material (as during pyrolysis). TMAH has been used for several
42
43 136 decades in a variety of terrestrial fields of study, including petroleum geochemistry (Larter and
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45 137 Horsfield, 1993), soil science (Chefetz et al., 2000; Deport et al., 2006; Schulten, 1996), and
46
47 138 sedimentology (Guignard et al., 2005; Pulchan et al., 1997). TMAH is currently available on the
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49 139 *Curiosity* rover SAM instrument (Mahaffy et al., 2012; Williams et al., 2019) and will also be used
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51 140 on the ExoMars rover MOMA instrument (Goesmann et al., 2017). Fortunately, perchlorate does
52
53 141 not substantially affect the recovery of fatty acids liberated with TMAH thermochemolysis (He et
54
55 142 al., *submitted*), making TMAH an ideal reagent to use in the search for select organics on Mars.

50 143 Although the SAM instrument TMAH experiment on the *Curiosity* rover has yet to be performed
51
52 144 (as of the time of this writing), several other lines of evidence already indicate that organics are
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54 145 present on the surface and near-surface (<6 cm) of Mars. Chlorinated hydrocarbons such as
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56 146 chlorobenzene and C₂ to C₄ dichloroalkanes were detected in the Sheepbed mudstone in

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3 147 Yellowknife Bay, Gale crater (Freissinet et al., 2015), and thiophenic, aromatic, and aliphatic
4
5 148 organic compounds were found in the 3.5 Gy Murray Formation lacustrine mudstones of Pahrump
6
7 149 Hills, Gale crater (Eigenbrode et al., 2018). In more recent SAM experiments, preliminary analyses
8
9 150 suggest the presence of select medium-chain alkanes (Freissinet et al., 2019) and medium to high
10
11 151 molecular masses, including derivatized molecules (Millan et al., 2019), in samples exposed to
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13 152 SAM's other wet chemistry experiment, derivatization with MTBSTFA (Buch et al., 2006).

14 153 These findings represent the first confirmed *in situ* organics detection on Mars, but the source of
15
16 154 these organic molecules is uncertain. Abiotic Martian sources (e.g., from atmospheric,
17
18 155 hydrothermal, or igneous processes), abiotic exogenous sources such as comets, interplanetary
19
20 156 dust particles, or meteorites (Eigenbrode et al., 2018; Freissinet et al., 2015), and life all produce
21
22 157 organic molecules. TMAH thermochemolysis, however, provides an unprecedented opportunity
23
24 158 to assess the biogenicity of Martian organics, specifically carboxylic and fatty acids. Fatty acids,
25
26 159 bound in phospholipids and glycolipids, are abundant and ubiquitous components of eukaryotic
27
28 160 and bacterial cellular membranes (Vestal and White, 1989). Carboxylic acids have also been
29
30 161 identified in exogenous carbonaceous material, such as in the Murchison meteorite (Cronin et al.,
31
32 162 1993). Biotic and abiotic-generated carboxylic acids produce distinctly different profiles in GC-
33
34 163 MS analyses. Carboxylic and fatty acids derived from metabolic processes can range from C₂ to
35
36 164 >C₃₀ in length have an even-over-odd carbon chain length preference due to enzymatically formed
37
38 165 acetyl (C₂) units derived from glucose (Volkman, 2006). Abiotic fatty acid patterns will favor
39
40 166 shorter carbon chain lengths with no carbon preference (Bray and Evans, 1961; McCollom et al.,
41
42 167 1999), although it is important to note that very short chain carboxylic acids can represent
43
44 168 metabolic byproducts that were not incorporated into cellular membranes. Therefore, within a
45
46 169 certain level of uncertainty, characteristics of a FAME profile could be used to deconvolve the
47
48 170 origin of fatty acids detected on Mars (Williams et al., 2019).

49 171 The work presented here investigates the preservation and detection of organic molecules,
50
51 172 specifically fatty acids, in modern inactive and relict Icelandic hydrothermal spring systems that
52
53 173 precipitate siliceous sinter and travertine. Organics preservation in these Mars-analog hot spring
54
55 174 environments is compared between mostly inactive and relict spring deposits and include samples
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57 175 from spring vent, mid-apron and distal apron facies, and within a depth range comparable to the
58
59 176 sampling depth of the *Curiosity* and future rover drill systems. We present data to assess the limits

177 of space-flight-like analyses and how returned *in situ* data from extraterrestrial hot springs may be
178 interpreted.

179 2. Methods

180 2.1. Sample Locations

181 Samples were collected at three Icelandic hydrothermal sinter sites, at the surface and subsurface
182 ranging from 2 to 18 cm in depth from spring vent to distal apron, to explore the coupling of system
183 activity with potential preservation of biosignatures. In hydrothermal hot-spring systems there are
184 three main regions: near-vent, the location of higher-temperature fluid discharge from the
185 subsurface; the middle-apron, a moderate temperature region often with pooling water; and the
186 distal-apron, areas of cooler fluid further from the vent (e.g. Figure 1). A currently active region,
187 Hveravellir, sits in the central highlands area of Iceland (Figure 3B) and contains many hot springs
188 and fumaroles. This area is protected as a monument in Iceland, with boardwalks and restricted
189 sites that preserve the formations and minimize human alteration. At Hveravellir, we collected
190 sample 160730.01 (mid-apron) near an active spring vent, but sampling site was only moist and
191 not within the main flow region (Figure 4). We also sampled at a recently inactive vent (160730.09)
192 and the distal-apron (160730.08) of an inactive vent. The pisolith sample (160730.04) had formed
193 in pools at an actively flowing vent. Given the variety of samples from active and recently inactive
194 sites, the Hveravellir system will be referred to as ‘inactive’ in this work. The geothermally active
195 Hveravellir region has been geochemically characterized, with the spring vents in the area
196 generally emerging at *ca.* 90 °C, with a pH of 8.60 and [SiO₂] of 599 ppm (Camacho, 2017). The
197 actively flowing spring vents proximal to Hveravellir vent sample “160730.09” ranged from 91.0
198 to 97.1°C, pH 8.15 to 8.65, [SiO₂] of 321 to 641 ppm, and [Cl] of 53.1 to 63.6 ppm. Hveravellir
199 spring waters are considered part of a population of high-temperature geothermal waters, along
200 with Geysir, Hveragerdi, and Reykjadalir, which contain elevated silica concentrations relative to
201 the lower-temperature geothermal regions on the island.

202 A second site, Gunnuhver, lies on the Reykjanes peninsula in SW Iceland (Figure 3C). The
203 sampling site is recently inactive (no spring activity), but with a large active fumarole ~10 m away
204 and geothermal heat still warming the surface. A downstream (~50 m) channel from the active
205 vent in the Gunnuhver region was 24.1°C with a pH = 7.34. Samples collected at Gunnuhver

206 included those near-vents, mid- (15 to 23 m) and distal-apron (33 to 49 m) locations from deduced
207 vent locations (Figure 4).

208 The third site visited was Lýsuhóll, a farmland area with relict sinter deposits and few active vent
209 systems (Figure 3D) with lower temperatures (*ca.* 41°C) and pH = 6.3. Other low temperature (*ca.*
210 50 °C) thermal springs are present nearby in the Snæfellsnes region (Camacho, 2017). Silica sinter
211 (e.g. Opal-A) deposits constitute the mineralogy of older regions in this deposit, overprinted by
212 younger travertine deposits (Jones and Renaut, 2017, Jones et al., 2005). Vent and near-vent
213 samples were collected at Lýsuhóll. The determination of vent locations was more difficult at this
214 relict site as erosion had disrupted many of the deposits; therefore only three well preserved sites
215 were sampled (Figure 4).

216 All samples were collected with the appropriate permissions. Hveravellir is a protected natural
217 monument for which we were permitted to sample by the Environment Agency of Iceland. We
218 were permitted to sample the Gunnuhver site by the National Energy Authority. Lýsuhóll is private
219 land for which we had owner permission to sample. Lastly, we also had permission from the
220 Icelandic Institute of Natural History to transport samples out of Iceland.

221 2.2. Sampling Procedure

222 To minimize contamination, all sinter samples were collected using solvent-washed and ashed
223 tools, while wearing gloves, facemasks and hairnets. Samples were collected using solvent cleaned
224 (MeOH – MeOH/DCM – DCM) and ashed (at 500°C) chisels, spoons, and tweezers. Rock
225 hammers were covered in a new piece of ashed (at 500°C) foil before use. Samples were placed in
226 solvent washed and ashed (at 500°C) glass jars and stored on ice for transport back to the
227 laboratory. Samples were collected both from the surface (denoted with an ‘S’) or from several
228 centimeters into the subsurface (denoted with an ‘I’). Fresh solvent-washed tools were used for
229 each surface and subsurface sample to limit cross-contamination between sample depths. Samples
230 were transported to the laboratory on ice and stored in a -20 °C freezer upon arrival. Samples for
231 SEM analysis were either collected in the field in sterile whirlpak bags with solvent washed tools,
232 or subsampled in the lab from the solvent washed and ashed glass jars with solvent washed tools.
233 In the laboratory, sinter from the glass jars was subsampled by breaking off smaller pieces with
234 solvent washed and ashed tweezers. These subsamples were powdered with a solvent washed and

235 ashed ceramic mortar and pestle and ground for 3 minutes to a homogenous powder. As samples
236 were carefully collected using organically-clean tools and techniques in the field, samples were
237 not treated to remove potential natural external contamination prior to powdering. This follows the
238 approach of Wilhelm et al. (2017).

2.3. Imaging and Mineralogy

240 The aerial drone maps for Figure 3 was acquired by DJI Mavic Pro with the onboard camera at
241 12MP resolution. Images were recorded at 2-second intervals to ensure that most surface points
242 would be captured in >5 images. The images were stitched together using the
243 commercially available Pix4D Mapping Software to combine the images into the mosaic. Due to
244 the size of the field sites, map images were acquired at different flight altitudes resulting in the
245 following pixel resolutions: Gunnuhver (5 m, 0.32 cm/pixel), Hveravellir (33 m, 1.41
246 cm/pixel), and Lýsuhóll (60 m, 2.76 cm /pixel).

247 Scanning electron microscope (SEM) images of sinter were collected with a Phenom ProX G5
248 tabletop environmental SEM. Qualitative analyses were conducted using a 3- μm spot size, a beam
249 current of 2.25–2.33 nA, and an accelerating voltage of 15 kV under low vacuum conditions
250 consistent with environment SEM operation.

251 Mineralogy was determined via X-ray diffraction with either a Bruker D8 Discover X-Ray
252 Diffractometer, (Cu K α radiation, $\lambda = 1.54059 \text{ \AA}$); patterns were acquired from 2-70° 2 θ , or an
253 Olympus Terra X-Ray Diffractometer, (Co K α radiation, $\lambda = 1.78897 \text{ \AA}$); patterns were acquired
254 from 5-55° 2 θ .

2.4. Pyrolysis GC-MS Conditions for Analog Samples

256 Aliquots of each powdered sample underwent pyrolysis (with a commercial Frontier pyrolyzer
257 3030D) GC-MS analysis to measure the distribution and abundance of FAMEs. Ground rock or
258 sediment samples were weighed into non-reactive metal cups (sample mass was ca. 5 to 10 mg,
259 depending on the sample). Just prior to sample analysis, TMAH in the ratio of 1 mg sample to 1
260 μL TMAH was added to the cup. The *n*-C_{19:0} internal standard was injected into each sample
261 immediately prior to analysis to determine the efficiency of FAME transfer. The sample was then
262 immediately loaded and dropped into the pyrolyzer oven to begin the run. The GC-MS analysis
263 was run on either an Agilent 7890A-5975C inertXL GC-MS or a Thermo Trace 1310 GC-MS
264 equipped with a 30 m Restek capillary column (MXT-5) with a 0.25 mm internal diameter and

1
2
3 265 0.25 μm thick Crossbond® 5% diphenyl/95% dimethyl polysiloxane stationary phase, and He as
4
5 266 the carrier gas at a 3 mL min^{-1} flow rate and 10:1 split. Blank (without TMAH) and “TMAH-
6
7 267 blank” (with TMAH) clean up analyses were run between each sample to determine and subtract
8
9 268 any background level of residual FAMES in the column.

10
11 269 Chromatograms and the mass spectra for FAMES were analyzed with ChemStation software
12
13 270 (Agilent Technologies). Identifications were based on comparison to known FAME retention
14
15 271 times of a Supelco 37 component FAME mixture that contains saturated FAMES, monounsaturated
16
17 272 fatty acid methyl esters (MUFAMES), and polyunsaturated fatty acid methyl esters (PUFAMES)
18
19 273 (Sigma-Aldrich). In addition, mass spectra were compared to the National Institute of Standards
20
21 274 and Technology (NIST) Spectral Library. Quantifications were based on integration under peaks
22
23 275 and scaled to the known amount of the n-C_{19:0} internal standard.

24
25 276 The pyrolyzer and GC programs used are detailed below: For the SAM-like pyrolysis ramp and
26
27 277 GC column method, the pyrolyzer program started at 50 °C and was ramped at 35 °C/min to 400
28
29 278 °C with no hold. The inlet program and Tenax ® TA trap started at 15 °C with a 2 minute hold.
30
31 279 The inlet was ramped at 68 °C/min to 150 °C and held for 2 minutes, then ramped at 80 °C/min to
32
33 280 305 °C and held for 3 minutes. The GC column program started at 35 °C and was held for 10.9
34
35 281 minutes, then ramped at 15 °C/min to 175 °C with no hold, then ramped at 10 °C/min to 305 °C
36
37 282 and held for 1 minute. The auxiliary transfer line was held at 135 °C.

38
39 283 For the 500 °C flash pyrolysis method, the sample was dropped into a 500 °C pyrolyzer oven and
40
41 284 held for 1 minute. The inlet program and Tenax ® TA trap started at 15 °C with a 2 minute hold.
42
43 285 The inlet was ramped at 900 °C/min to 300 °C and held for 2 minutes. The GC column program
44
45 286 started at 35 °C and was held for 2.5 minutes, then ramped at 5 °C/min to 300 °C and held for 5
46
47 287 minutes. The auxiliary transfer line was held at 270 °C.

48 288 **3. Results**

49 289 **3.1. Mineralogy**

50
51 290 The mineralogy of the three sample locations was generally dominated by opal-A, opal-CT, and
52
53 291 amorphous silica (Table 1). The modern inactive hot spring vent at Hveravellir was dominantly
54
55 292 opal-A with lesser percentages of clinopyroxene, plagioclase, gypsum, and magnesite. Several
56
57 293 Hveravellir locations were composed entirely of amorphous silica. The modern inactive hot spring

1
2
3 294 at Gunnuhver was predominantly opal-CT, with the distal apron being dominantly amorphous
4
5 295 silica. This system also contained lesser percentages of halite and quartz. The older relict hot spring
6
7 296 at Lýsuhóll is composed almost exclusively of amorphous silica with some minor calcite, and one
8
9 297 sample site was composed entirely of calcite.

10 11 298 **3.2. FAMES Detected with 500 °C Instantaneous Flash Pyrolysis**

12
13 299 Samples were initially analyzed using a 500 °C flash pyrolysis technique to enable
14
15 300 transesterification of fatty acids with TMAH thermochemolysis. Using this technique there was
16
17 301 relatively high diversity and abundance of FAMES in the Hveravellir spring site, a lower diversity
18
19 302 and abundance of FAMES at the inactive Gunnuhver spring site, and diminished diversity and
20
21 303 abundance of FAMES at the Lýsuhóll relict site (Table 2).

22
23 304 At the Hveravellir site, samples were collected from the spring vent, mid-apron, and distal-apron,
24
25 305 as well as a pisolith-bearing layer near an active vent. FAMES detected ranged from *n*-C_{6:0} to *n*-
26
27 306 C_{27:0} and included a few monounsaturated fatty acids (MUFAs) and branched *iso*- and *antiso*-C_{15:0}
28
29 307 (Figure 5, Table 2). *n*-C_{16:0} and *n*-C_{18:0} were the most abundant FAMES in all samples, and FAMES
30
31 308 longer than *n*-C_{18:0} were identified at all sites except the vent. In general, the abundance and
32
33 309 diversity of FAMES was increased in the surface samples relative to the subsurface samples, which
34
35 310 ranged from 3 to 18 cm in depth. The distal apron sample demonstrated an unexpected general
36
37 311 trend of greater FAME concentrations in the interior samples than the surface samples.

38
39 312 At the inactive Gunnuhver site, samples were collected from two spring vents, the mid-apron, and
40
41 313 the distal-apron. FAMES detected ranged from *n*-C_{4:0} to *n*-C_{27:0} and included few MUFAs (Figure
42
43 314 6, Table 2). No methyl-branched fatty acids were detected. Again *n*-C_{16:0} and *n*-C_{18:0} were the most
44
45 315 abundant FAMES in all samples. FAMES longer than *n*-C_{18:0} were only identified at the mid- and
46
47 316 distal-apron sites. In general, the abundance and diversity of FAMES was increased in the surface
48
49 317 samples relative to the subsurface samples, which ranged from 5 to 7 cm in depth.

50
51 318 At the relict Lýsuhóll site, much of the characteristic features of the proximal slope, mid- and
52
53 319 distal-aprons were too degraded to assess, so samples were collected from three (near-)vent sites
54
55 320 which could be identified in field observations. FAMES detected ranged from *n*-C_{6:0} to *n*-C_{27:0} and
56
57 321 included a few MUFAs and one polyunsaturated fatty acid (PUFA) (Figure 7, Table 2). Methyl-
58
59 322 branched *iso*- and *antiso*-C_{15:0} were also detected in the surface and subsurface. Again *n*-C_{16:0} and

1
2
3 323 n -C_{18:0} were the most abundant FAMES in most samples, although this is not the case for Vent #1
4 (160809.03). FAMES longer than n -C_{18:0} were identified at all sites. The abundance and diversity
5 324 of FAMES was increased in the surface samples relative to the subsurface samples, which ranged
6
7 325 from 2 to 3 cm in depth.
8
9 326

327 3.3. FAMES Detected with SAM-Instrument-like Pyrolysis Ramp

328 Samples were analyzed using a 35°C/min pyrolysis ramp to approximate the pyrolysis ramp used
329 by the SAM instrument on the *Curiosity* rover (Mahaffy et al., 2012; Williams et al., 2019). This
330 technique was utilized to explore how the FAME detection would change if sinter-bearing hot
331 springs were analyzed on Mars with a SAM comparable TMAH experiment. In general, with this
332 technique there was lower diversity and abundance of FAMES in all three spring systems (Table
333 3).

334 At the Hveravellir site, FAMES detected ranged from n -C_{6:0} to n -C_{20:0} and included two MUFAs.
335 n -C_{16:0} and n -C_{18:0} were the most abundant FAMES in all samples, and only one FAME longer
336 than n -C_{18:0} was identified at the surface mid-apron site. In general, the abundance and diversity
337 of FAMES was equal to or increased in the surface samples relative to the subsurface samples.

338 At the inactive Gunnuhver site, FAMES detected ranged from n -C_{4:0} to n -C_{27:0} and included
339 several MUFAs and PUFAs. n -C_{16:0} and n -C_{18:0} were generally the most abundant FAMES in
340 the mid- and distal-apron samples, and ~~no~~ FAMES longer than n -C_{18:0} were detected in all
341 Gunnuhver samples. ~~No FAMES larger than n -C_{16:0} were detected in the Vent 2 site.~~ The
342 abundance and diversity of FAMES was uniformly higher in the surface vent samples relative to
343 the subsurface samples, but lower than the subsurface samples from the mid- and distal apron
344 environments, with no FAMES detected in the subsurface Vent 2 sample.

345 At the relict Lýsuhóll site, FAMES detected ranged from n -C_{4:0} to n -C_{18:0} and included three
346 MUFAs and one PUFA. n -C_{16:0} and n -C_{18:0} were the most abundant FAMES in all samples, and no
347 FAMES longer than n -C_{18:0} were detected. The only site with both a surface and subsurface sample
348 was the 160807.04 Vent #2 location, and the abundance and diversity of FAMES was higher in the
349 surface sample relative to the subsurface sample.

350 4. Discussion

351 The samples studied here comprise silica sinter and calcite hot spring deposits ranging from
352 modern and partially inactive to older, relict spring systems. Thus, they represent a range in
353 geochemistry, mineralogy, age, and diagenetic history, as well as spanning a diversity of
354 subenvironments present in hot spring deposits: vents, mid-aprons, and distal aprons. Despite these
355 differences, several trends in FAME abundance and diversity are shared between the hot spring
356 locations.

357 A high abundance of $n\text{-C}_{16:0}$ and $n\text{-C}_{18:0}$ is observed in all of the surface and subsurface samples
358 relative to other FAMES in all three locations, which is consistent with these two fatty acids being
359 the most common in both bacteria and eukarya (Kaur et al., 2015; Johnson et al., 2019; Wilhelm
360 et al., 2017; Williams et al., 2019). Elevated $n\text{-C}_{16:0}$ and $n\text{-C}_{18:0}$ FAME abundances relative to other
361 FAMES are expected in younger sinters versus much older, inactive sinters (Kaur et al., 2011),
362 reflecting more extant diverse microbial communities.

363 ~~Most-All~~ samples contain FAMES longer than $n\text{-C}_{18:0}$, which most often represent a terrigenous
364 plant or algal origin (Volkman et al., 1989; Kaur et al., 2011; Wilhelm et al., 2017; Johnson et al.,
365 2020), although these fatty acids may be derived from bacterial free, long chain fatty acids
366 (Summons et al., 2013) or intact polar lipids. Based on the abundance of surface vegetation near
367 the spring sites, the FAMES here are assumed to represent waxy longer chain FAMES that are often
368 more stable than short chain FAMES and may be more resistant to diagenetic processes and
369 preserved longer after entombment (Wilhelm et al., 2017). Long chain FAMES were identified in
370 ~~nearly~~ all surface and subsurface samples. At Hveravellir, they are present in greater abundance at
371 the mid- and distal apron sites than the vent site. At Gunnuhver, long chain FAMES are ~~also~~ present
372 in the mid- and distal-apron sites, ~~and present at a lower abundance with a noticeable absence~~ in
373 the vent sites. This increase in abundance is consistent with models of lower temperatures ($<40^{\circ}\text{C}$)
374 downstream from vents, which support plant growth and the potential for their entombment in the
375 sinter (Walter, 1976). At Lýsuhóll these FAMES are present in the relict vent sites both on the
376 surface and in the subsurface. Much higher concentrations of long chain FAMES in the surface
377 samples suggest a more modern source. Observation of the long chain FAMES at depth suggests
378 that plant matter grew and was entrained in the sinter as flow decreased and the spring system
379 cooled and became dormant. The lower concentrations in the subsurface samples indicate some

380 higher plants were also incorporated into the rock record, with some degradation of the FAMES
381 due to burial processes.

382 A strong even-over-odd carbon number preference is consistent with the production of the fatty
383 acids due to enzymatic effects (e.g. the monomer addition of a 2-C sub-unit, (Georgiou and
384 Deamer, 2014; Summons et al., 2008) and of microbial cellular metabolic processes (Elias et al.,
385 1997; Grimalt and Albaiges, 1987; Nishimura and Baker, 1986; Volkman, 2006). All surface and
386 subsurface samples from the Hveravellir site exhibit this trend, as do all surface samples from
387 Gunnuhver.

388 4.1. Organics Load in the Hveravellir Hot Spring System

389 The surface FAME load in the Hveravellir spring system varied greatly between the vent, mid-
390 apron, and distal apron facies. The abundance of FAMES detected with flash pyrolysis at the opal-
391 A vent facies is an order of magnitude (>35000 ng total FAME/ mg TOC) greater than the FAME
392 abundance at the amorphous silica distal apron (ca. 1875 ng total FAME/ mg TOC). The
393 amorphous silica mid-apron FAME abundance was lower still, at ca. 600ng total FAME/ mg TOC.
394 The elevated FAME abundance at the Hveravellir vent sample indicates that the change in water
395 flow and/or temperature at this recently ceased vent has likely led to colonization by bacteria with
396 a lower temperature tolerance. Water flow and temperature are major factors in determining
397 microbial diversity and biofilm texture in hydrothermal systems. When flow shifts, as it has at this
398 vent and outflow system, change to abundance and diversity of the fatty acids should be expected.
399 At the vent, the presence of the C₂₀ and C₂₂ fatty acids indicate that thermophilic bacterial growth
400 has occurred, however, the relatively high FA abundance, together with an increase in the
401 cyanobacterial C_{18:09} indicates that a reduction in water temperature probably resulted in increased
402 growth of a thermotolerant cyanobacterium such as the high-temperature form of *Mastigocladus*
403 isolated from an Iceland hot spring (Castenholz, 1969) or perhaps diatoms (e.g. Figure 4), or both.
404 This is consistent with the relatively low abundance and lack of C_{18:09} FA in the subsurface sample.
405 As we report on and discuss our results for the sinter samples below it is important to keep in mind
406 that these hydrothermal siliceous springs are dynamic systems suited for the production and
407 preservation of fatty acid, and that under conditions ranging from recent to ancient, fatty acids
408 survive for extended time entombed in siliceous sinter.

~~409 indicates that lower thermal tolerance bacteria have recolonized this recently ceased vent (i.e. no
410 water flow). The expected trend for an active high temperature spring system would include a
411 higher fatty acid abundance further from the high temperature vent (Kaur et al., 2011), as a greater
412 diversity of organisms with higher growth rates tend to colonize these regions.~~

413 The opal-A pisolith layer was collected further away from the other three Hveravellir samples but
414 exhibits a similar trend in even-over-odd carbon number predominance, with $n\text{-C}_{16:0}$ and $n\text{-C}_{18:0}$
415 dominating the FAME profile. Pisoliths are composed of >2 mm diameter spherical concretionary
416 grains called pisolites which are often formed from calcite or amorphous silica. Due to the
417 concretionary nature of the grains, pisolites and related ooids often contain bound organic matter
418 between cortices or submillimeter concretion layers (Davies et al., 1978; Folk and Lynch, 2001;
419 O'Reilly et al., 2017). Therefore, the trends in presence and preservation of FAMES in the pisolith
420 layer is expected.

421 The FAME load at Hveravellir changed with depth between the vent, mid-apron, and distal apron
422 facies as well. Most subsurface FAMES decreased in abundance or were not detected relative to
423 their complementary surface samples (e.g. Figure 8, where the total organic carbon load is
424 generally lower in subsurface samples relative to surface samples). A few exceptions exist in this
425 data set, in particular in the mid-apron sample, in which all of the subsurface FAMES were elevated
426 in concentration relative to the surface (Figure 5). In the distal-apron samples, several individual
427 subsurface FAMES were elevated relative to their complementary surface samples. This trend was
428 distinct at the distal apron site, where FAME concentration often increased stepwise from the
429 surface to progressively deeper sampling depths (e.g. Figure 5). We interpret this trend to represent
430 either a temporary increase in organic preservation potential at spring sites that experience lower
431 temperatures and diagenetic alteration, or a modern and robust subsurface microbial community
432 that is generating the elevated FAME abundance. This trend could also be an indication of
433 preservational bias related to differences in mineralization rates, where early mineralization
434 minimizes the degradation of organics and body fossils (Alleon et al., 2016). If a diversion of water
435 flow precipitated a localized temperature shift, the supply of dissolved silica would also decrease
436 leaving the microbial community more vulnerable to biotic and abiotic degradation processes since
437 it was not mineralizing. These observations reinforce the complexity of these spring systems.
438 While general trends can be preserved, changes in the local physical conditions, (like diverted

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3 439 water flow) can have cascading impacts on the microbial composition and their ecology, that
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5 440 together end up biasing their fossil record.
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7 441 Although our sampling techniques were optimized to decrease any chance of anthropogenic
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9 442 contamination, we cannot rule out the possibility that some of these samples reflect an
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11 443 anthropogenic influence, as this hot spring is a tourist destination and the mid-apron sample was
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13 444 collected below a visitor boardwalk. Elevated FAMES from eukaryotic (e.g. human) sources may
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15 445 have been entrained in the sinter. Contamination of this type should yield a much higher FAME
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17 446 concentration, especially in the C_{16:0} and C_{18:0} FAMES, in the surface relative to the subsurface
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19 447 samples. Surface samples did contain higher concentrations of FAMES than the subsurface, but
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21 448 C_{16:0} and C_{18:0} FAMES were not uniformly elevated. Therefore, we interpret the Hveravellir FAME
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23 449 concentration trends as reflecting a diverse and active microbial community.

24 450 In general, branched and unsaturated fatty acids were either not detected in subsurface samples or
25
26 451 were decreased in abundance relative to the surface samples. This degradation was likely as the
27
28 452 unsaturation in the fatty acid is a more reactive location for the molecule to be cleaved by microbial
29
30 453 heterotrophy or diagenesis (Killops and Killops, 2005; Sun et al., 1997). A decrease in unsaturated
31
32 454 fatty acids and branching is documented in older sinter samples (Kaur et al., 2011) and is consistent
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34 455 with the expectation for preferential degradation of unsaturated fatty acids. However, this trend
35
36 456 was reverse in the mid-apron sample, in which all of the subsurface FAMES, including branched
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38 457 and MUFAs were elevated in concentration relative to the surface.

38 458 **4.2. Organics Load in the Gunnuhver Hot Spring System**

39
40 459 The surface FAME load in the inactive Gunnuhver spring system also varied between the vent,
41
42 460 mid-apron, and distal apron facies, although to a lesser degree than in the Hveravellir spring
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44 461 system. The abundance of FAMES detected with flash pyrolysis is comparable between the opal-
45
46 462 CT vent and opal-CT mid-apron facies (ca. 18,000 to 20,000 ng total FAME/ mg TOC) and is
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48 463 elevated in the amorphous silica distal apron facies (ca. 37000 ng total FAME/ mg TOC). This
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50 464 trend in abundance is expected, as a greater diversity of organisms with higher growth rates tend
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52 465 to colonize lower temperature geothermal regions such as the distal apron.

53 466 The abundance and diversity of lower molecular weight FAMES increases to the mid-apron and
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55 467 distal apron facies relative to the vent facies, suggesting that higher molecular weight (longer)

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3 468 FAMES are not less well preserved further from the vent and are likely degraded to shorter chains,
4 469 potentially due to microbial heterotrophy.

7 470 The FAME load in this system changed with depth more dramatically than in the Hveravellir
8 471 system. Subsurface FAME abundance decreases substantially or to no detection relative to their
9 472 complementary surface samples, indicating continued degradation of the FAMES in the subsurface
10 473 due to diagenesis and microbial recycling. Only four FAMES (*n*-C_{8:0}, *n*-C_{10:0}, *n*-C_{15:0} and *trans*-
11 474 C_{18:1}) are detected in the subsurface distal apron site with flash pyrolysis(~~*n*-C_{8:0}, *n*-C_{10:0}, *n*-C_{15:0}~~
12 475 ~~and *trans*-C_{18:1}~~). The subsurface FAME profiles exhibit strong to weak even-over-odd trends in
13 476 carbon number. The subsurface distal apron sample does not exhibit any trends in carbon number
14 477 or molecular weight, which likely reflect whole-scale degradation of all FAMES in the subsurface.
15 478 Mono- and polyunsaturated fatty acids are already limited in detection at the surface, a dearth that
16 479 continues into the subsurface and indicates preferential degradation of the unsaturated FAMES.
17 480 Branched fatty acids are not detected in ~~neither the surface nor~~ subsurface samples with flash
18 481 pyrolysis.

29 482 **4.3. Organics Load in the Lýsuhóll Hot Spring System**

30 483 In contrast with the opal and amorphous silica-dominated Hveravellir and Gunnhver sites, the
31 484 Lýsuhóll relict spring system is composed of both amorphous silica and calcite, with Vent #3
32 485 composed entirely of calcite. The spring waters that precipitate these deposits, and the subsequent
33 486 microbial communities that colonize the springs, may be different from those present in the
34 487 siliceous sinter deposits (Zhang et al., 2004). Additionally, several samples from the relict Lýsuhóll
35 488 system were destroyed and/or contaminated during transport back to the United States, reducing
36 489 the completeness of the surface versus subsurface samples originally collected at these sites.
37 490 Regardless, several conclusions can be drawn from the remaining samples about the organics load
38 491 in this relict system. All sample sites at Lýsuhóll represented near-vent environments as these were
39 492 the most readily identified in older, degraded hot spring deposits. The surface FAME load in the
40 493 relict Lýsuhóll system also varied between the vent facies, ranging from *ca.* 3,700 ng total FAME/
41 494 mg TOC in the amorphous silica and calcite dominated Vent #2, to *ca.* 350 ng total FAME/ mg
42 495 TOC in the amorphous silica Vent #1, to *ca.* 83 ng total FAME/ mg TOC in the calcite Vent #3
43 496 (Table 2). As in the other spring systems, the surface FAME concentrations are higher than in
44 497 subsurface samples. The total FAME abundance ranges from *ca.* 3700 ng total FAME/ mg TOC

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3 498 in the Vent 160807.04 (Vent #2) surface sample to ca. 1500 ng total FAME / mg TOC in the
4
5 499 complementary subsurface sample. This indicates continued degradation of the FAMES in the
6
7 500 subsurface due to diagenesis and microbial recycling. The Lýsuhóll samples exhibit a moderate to
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9 501 weak even-over-odd preference, which may indicate degradation of fatty acids from the original
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11 502 microbial community, and limited input of organics from a modern community. The relict
12
13 503 Lýsuhóll system is the only site where the PUFA *trans*-C_{18:2} algal biomarker is detected (Campbell
14
15 504 et al., 2015), and then only in two subsurface samples. Branched and unsaturated fatty acids are
16
17 505 already limited in detection at the surface, a dearth that continues into the subsurface and indicates
18
19 506 preferential degradation of these fatty acids.

20 507 4.4. Comparison between Flash Pyrolysis and Flight-like Ramp Pyrolysis

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22 508 The 500 °C flash pyrolysis method, combined with TMAH thermochemolysis, represents a
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24 509 laboratory-optimum method to release organics bound within macromolecules and sorbed to
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26 510 minerals. The SAM instrument on the *Curiosity* rover, while able to perform similar experiments,
27
28 511 is limited to a 35°C/ min pyrolysis ramp. To assess how this method variation might affect the
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30 512 detection of FAMES in these Mars-analog siliceous sinters, samples were analyzed with the SAM-
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32 513 like 35°C/ min pyrolysis ramp.

33 514 The flash pyrolysis method improved FAME detection uniformly across the sample suite (Figure
34
35 515 8), and with ~~few one~~ exceptions, FAMES >*n*-C_{18:0} were only detected with the flash method. The
36
37 516 ramp method released between 30 and 86% of the FAMES detected in the flash method. ~~Two~~
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39 517 ~~outliers to this data set include sample 160727.03.I (200% improvement in the ramp versus flash~~
40
41 518 ~~method) and 160727.02.I (no fatty acids liberated using the ramp method).~~ Although the flash
42
43 519 method is more efficient at FAME release, it is promising for the search for organics on Mars that
44
45 520 FAMES are also detectable with the SAM instrument pyrolysis ramp rate.

46 521 Correlations vary between the number of FAMES detected, the concentration of FAMES in the
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48 522 sample suite. In the Hveravellir system, the greatest diversity of FAMES detected was generally
49
50 523 correlated with low % TOC. This diversity is invaluable in assessing microbial community
51
52 524 composition, even at low concentrations. In the inactive spring system, the greatest diversity of
53
54 525 FAMES correlates with higher % TOC, which represents an anticipated trend with regards to
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56 526 FAME preservation (and degradation), FAME diversity and concentration, and the total organic
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58 527 carbon load in the sample (Table 1). The greatest diversity of FAMES was detected in the relict

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3 528 spring system, as were some of the highest % TOC values. In particular, *n*-C_{16:0} concentrations
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5 529 were predictably low in the subsurface samples and high in the surface samples. These lines of
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7 530 evidence indicate the relict system is experiencing higher delivery of organic load now than it
8
9 531 perhaps did in the past. The organics that constitute these higher % TOC values may represent a
10
11 532 far greater diversity of organic molecules than just fatty acids, which are preserved but in low
12
13 533 concentrations in the relict system.

14 534 **4.5. Mineralogic Evolution and the Preservation of Organic Matter**

15
16 535 It is important to note that the primary controls on lipid preservation in siliceous sinter deposits
17
18 536 are thermal alteration, high temperatures, and the pH of each spring (Kaur et al., 2015; Pancost et
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20 537 al., 2006), which can vary greatly between spring systems. A great variety of lipid biomarkers have
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22 538 been identified in modern to *ca.* 900 year old geothermal environments (Jahnke et al., 2001; Shiea
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24 539 et al., 1991; Pancost et al., 2005, 2006; Zhang et al., 2007, Kaur et al., 2011). Often branched or
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26 540 unsaturated fatty acids are prevalent in younger deposits (Kaur et al., 2011), as was also
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28 541 demonstrated in the work presented here. In an actively flowing spring system, temperature in
29
30 542 particular tends to ~~drive~~ strongly influence the length and therefore abundance of ~~fatty~~
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32 543 ~~acid~~ hydrocarbons, such as bacterial diethers in active geothermal systems (Kaur et al., 2015).
33
34 544 Thermophilic organisms tend to have ~~longer~~ higher molecular weight diethers with higher
35
36 545 thermostabilities, longer fatty acid chains (Oshima and Miyagawa, 1974; Russell, 1984;
37
38 546 Weerkamp and Heinen, 1972), increased ~~the ratios~~ of *iso*- to *anteiso*-branched fatty acids (Oshima
39
40 547 and Miyagawa, 1974; Shen et al., 1970), and fewer unsaturations and branches (Daron, 1970; Ray
41
42 548 et al., 1971). All of these adaptations serve to maintain optimal membrane fluidity at higher
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44 549 temperatures.

45
46 550 Although the Hveravellir vent sampled here was no longer actively flowing, several of these trends
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48 551 were observed (Figure 9), consistent with the recent cessation of flowing spring water. The average
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50 552 chain length ranges from 15.1 to 16.4 for all surface and subsurface Hveravellir samples, with the
51
52 553 exception of the surface vent sample with an ACL of 13.6. These values suggest that the surface
53
54 554 vent microbial community and/or FAME preservation is different from the other surface and
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56 555 subsurface samples at Hveravellir. The low ACL value of the surface vent suggests that this spring
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58 556 vent never experienced very high fluid temperatures and even if thermophilic organisms were
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60 557 present, their organic biosignatures are not well preserved. An increase in heterotrophy may have

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2
3 558 occurred concurrent with the cessation of water activity that hosted the thermophiles. The ratio of
4 559 *i-* to *a-C*₁₅ is elevated near the vent where elevated temperatures affect community composition
5
6 560 and the *iso* to *anteiso* ratio trend was consistent at this site between the surface and subsurface
7
8 561 samples. Additionally, the ratio of branched to total fatty acids increases with distance from the
9
10 562 higher temperature vent, consistent with a decrease in the dominance of any thermophilic
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12 563 organisms.

13
14 564 In contrast, the inactive Gunnuhver site had a relatively consistent, elevated total FAME
15
16 565 concentration at the vent and mid-apron facies, and a much higher FAME concentration at the
17
18 566 distal apron facies, consistent with secondary recolonization of the sinter by more diverse, lower
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20 567 temperature mesophiles after spring flow ceased. Average chain length also decreases from the
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22 568 vent facies (ACL = 10.7 to 13.4 at vents) to the distal apron facies (ACL = 0 to 8.9), suggesting
23
24 569 the degradation of fatty acids to shorter chains due to heterotrophy or diagenesis.

25
26 570 Only relict inactive vents were sampled at the Lýsuhóll spring site, which allows a comparison
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28 571 with the modern inactive vent systems. The total FAME concentrations at the relict site was within
29
30 572 the range of the inactive spring sites, with higher abundances in the surface samples and lower
31
32 573 abundances in the subsurface samples. Average chain length at the relict site (ACL = 14.9 to 17.4)
33
34 574 is within the range of the Hveravellir spring site, suggesting that these Lýsuhóll vents were higher
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36 575 temperature when flowing and the thermophile FAMES are well preserved. The *iso* to *anteiso* ratio
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38 576 was lower than at the modern inactive spring sites, suggesting degradation of the branched fatty
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40 577 acids from an original, higher temperature population. The ratio of branched to total fatty acids,
41
42 578 however, was comparable to or higher than the Hveravellir vent site, indicating that more branched
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44 579 fatty acids relative to the total fatty acid load are preserved. This trend may be due to a greater
45
46 580 initial branched fatty acid load that, along with the total fatty acids, has experienced degradation.
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48 581 The branched to total fatty acid ratio at Vent #3 (160807.12) is higher than all other samples and
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50 582 contains the longest ACL of all samples from this study. This vent is composed solely of travertine,
51
52 583 which may introduce a preservation bias between this younger calcite deposit and the other vents
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54 584 that are older and silica-dominated. The high branched to total fatty acid ratio and ACL value of
55
56 585 Vent #3 may also correspond to an active mesophilic microbial community at this site.

57
58 586 Low temperature degradative processes such as heterotrophy and diagenesis control the fatty acid
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60 587 distribution observed in this study. The primary fatty acid distribution from thermophilic versus
588
589 mesophilic communities is discernable in some environments (e.g. Hveravellir vent) but not

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3 589 diagnostic of more specific temperature and pH conditions of each subenvironment at each spring
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5 590 system. The vents from the three systems studied here are compared to assess the preservation of
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7 591 organics as a concentration of FAMES in the surface and subsurface (Figure 10). Additional work
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9 592 can be completed to assess the preserved lipids separate from modern lipids in all three systems,
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11 593 including solvent washing sinter samples to remove surface lipids prior to analysis. At the recently
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13 594 inactive Hveravellir vent (sample 09), the C₁₆ and C₁₈ FAMES are likely elevated due to these being
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15 595 the dominant FAMES in both bacteria and eukarya, although anthropogenic influences, especially
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17 596 in the surface sample, cannot be completely dismissed. The lower concentrations of the other
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19 597 FAMES in the surface and subsurface reflect a nominal FAME profile from an active microbial
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21 598 community, with the expected even-over-odd carbon number preference. The inactive Gunnuhver
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23 599 vent generally had the lowest FAME concentrations relative to the Hveravellir and Lýsuhóll
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25 600 systems, but still exhibited the even-over-odd carbon number preference characteristic of an active
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27 601 microbial community. The low FAME concentrations reflects FAME degradation leading to low
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29 602 preservation potential, and a lack of extensive recolonization of the sinter by secondary microbial
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31 603 communities. The higher FAME concentrations at the relict Lýsuhóll Vent #2 (especially relative
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33 604 to the inactive Gunnuhver site) likely reflect microbial (re)colonization by a community distinct
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35 605 from that found in active/recent silica-precipitating hot springs. The mixed amorphous silica and
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37 606 calcite mineralogy of this vent likely also contributes to this marked difference in FAME
38
39 607 abundance.

40
41 608 The mineralogic evolution and diagenesis of siliceous hot spring sinters is well studied (Herdianita
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43 609 et al., 2000). Initial silica precipitates are composed of amorphous silica or opal-A (Tobler et al.,
44
45 610 2017), and the very fine size of these precipitates are documented to preserve microbial cells in
46
47 611 extraordinary physical detail (Cady and Farmer, 1996; Jones et al., 2000; Renaut et al., 1998),
48
49 612 although progressive silicification and preservation biases over long time scales can also destroy
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51 613 these physical biosignatures (Jones et al., 1997; Jones and Renaut, 1996). As siliceous sinter
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53 614 deposits experience diagenesis, opal-A incrementally recrystallizes to Opal-C (containing
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55 615 disordered α -cristobalite) or opal-CT (containing cristobalite and tridymite). This transition may
56
57 616 occur on the order of *ca.* 50 years up to tens of thousands of years. Over longer time scales (*ca.*
58
59 617 10,000 to 50,000 years), opal-C/ -CT will reorder to microcrystalline quartz. Although sinter age
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618 can sometimes be used as an estimate for crystallinity, variables such as fluid mobilization and
619
620 composition can also greatly affect crystallinity (Herdianita et al., 2000). This recrystallization

620 process, and specifically the transition to microquartz, reduces porosity and decreases the
621 preservation of organic matter in siliceous sinter (Teece et al., 2020).

622 These trends in mineralogy and organics preservation were upheld in the current study. In the
623 Hveravellir system, which is composed of amorphous silica and opal-A, the diversity and
624 abundance of FAMEs was elevated in both the surface and subsurface. In contrast, the inactive
625 Gunnuhver system, which is composed primarily of opal-CT, had a much lower diversity and
626 abundance of preserved FAMEs. Lynne et al. (2006) suggest that silica diagenesis is accelerated
627 in hot spring systems that have transformed from flowing water to steam dominated. The
628 transformation to opal-CT in this relatively young system was likely controlled by fluid
629 composition and fluid or steam mobilization. This mobilization led to accelerated diagenesis and
630 fumarolic degradation of organics in the Gunnuhver system. The surprisingly elevated diversity
631 and abundance of FAMEs in the relict Lýsuhóll system was controlled in part by the mineralogy,
632 which is dominated by calcite and amorphous silica, as well as a modern microbial community
633 that had colonized the surface of this deposit. These results further reinforce the importance of
634 ascertaining sample mineralogy prior to sampling for organics in hydrothermal systems, especially
635 on life detection missions beyond Earth.

636 **4.6. Biomarker Preservation and Implications for Organics Detection on Mars**

637 The size and distribution of hot spring facies varies across systems, yet trends in preservation
638 styles, from the outcrop- to the submicrometer scale, are present and can be used to provide
639 depositional context as we continue to explore life in other planets. On Earth, preservation at the
640 vent is often less favorable with microfossils usually in the form of smaller diameter molds that
641 can be readily infilled and obscured (Cady and Farmer, 1996). Vents may be identified by finely
642 laminated deposits in a circular form that are often associated with geyserite. The directed water
643 flow in channel facies produces bacterial streamer fabrics, long filaments parallel to water
644 direction, sometimes composed of cyanobacterial filaments (Parenteau et al., 2014). This streamer
645 morphology is readily identified in outcrop (e.g., Parenteau et al, 2014, Walter et al., 1998). The
646 lower temperatures of the distal apron are usually accompanied by thick microbial mats creating
647 palisade fabrics with abundant microfossils and organics that can be preserved (Cady and Farmer,
648 1996; Campbell et al., 2015). Apron facies are one of the better-preserved lithofacies in degraded
649 spring systems, with the mid-to-distal apron composing the largest aerial extent, followed by the

650 channel facies and then vent facies. Based solely on aerial extent, Martian hot spring apron facies
651 may be most easily detected from orbital imaging and targeted for *in situ* exploration.

652 Biosignature preservation within all facies is complicated **by** preservation bias and diagenesis over
653 geologic time scales. Even the proximal slopes and aerially extensive mid- and distal-aprons may
654 become challenging to identify as the fine scale micro-textures that define these spring sub-
655 environments can be lost to diagenesis and preservation biases (Cady and Farmer, 1996). Although
656 diagenesis can and does obscure some features, the apron facies contain structures such as pools
657 and terracettes that are identifiable in surface deposits and layered deposits with palisade fabric
658 identifiable in outcrop cross-section. Vents are just as prone to erosion and infilling (this was
659 observed at Lýsuhóll and Gunnuhver), and with a much smaller footprint, can be challenging to
660 identify from orbital images. However, the primary macro-scale textural characteristics of spring
661 vents can enable their identification after some geologic reworking (Cady and Farmer, 1996, and
662 references therein).

663 Given these complexities, it is therefore important to develop a facies-oriented understanding of
664 biosignature preservation potential in terrestrial hot springs to better predict the ideal locations to
665 search for organics in Martian relict hydrothermal spring systems. The temperature of fluids at
666 subaerial hydrothermal vent systems can vary greatly, up to or above surface boiling temperatures.
667 Non-photosynthetic microbes can live in these temperatures, with hyperthermophiles preferring
668 >80 °C up to a maximum of 110 °C where elevated hydrostatic pressure allows waters to reach
669 higher temperature without boiling (Seeger et al., 1993). For reference, the upper temperature
670 limit for archaeal life is 121 °C in submarine black smoker environments (Kashefi and Lovley,
671 2003); in contrast, photosynthetic microbes are present in waters below 73 °C (Ward et al., 2012).
672 Although these organisms do not directly contribute to silica precipitation in these spring systems,
673 they can be entombed and preserved in the rapidly precipitating silica, contributing to the organic
674 load and microstructures found in near-vent environments (Cady and Farmer, 1996). In our study,
675 silicified traces of microorganisms are readily identified in SEM images from the Hveravellir and
676 Lýsuhóll sample sites, but no distinct biogenic features were identified from the Gunnuhver sinter
677 samples (Figure 4). These observations appear to correlate with the relative abundances of organics
678 detected at these three locations.

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3 679 The search for (biogenic) organic molecules on Mars' near-surface requires new and novel
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5 680 techniques to detect these molecules with miniaturized space-flight payloads. In the terrestrial
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7 681 realm, many biomarkers are preserved and detectable in siliceous sinter environments with
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9 682 laboratory instrumentation such as off-line lipid fraction extraction, derivatization and/or
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11 683 saponification, followed by multiple GC-MS and/or liquid chromatography-mass spectrometry
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13 684 (LC-MS) analyses. However, suites of these laboratory techniques are often too complex to be
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15 685 successfully miniaturized for space-flight and remote operation. Therefore, "one-step"
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17 686 thermochemolysis or derivatization pyrolysis GC-MS is an optimized technique that is
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19 687 miniaturized to work on space-flight missions such as with the SAM and MOMA instruments. To
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21 688 be more "SAM-like" in this study, samples that may contain low-temperature surface biofilms
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23 689 were collected and ground as whole rock samples in a manner similar to *Curiosity's* sample
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25 690 acquisition system. Therefore, the results from this study likely reflect a greater abundance of
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27 691 organics than may be expected to be preserved in a Martian hot spring deposit with a hypothetical
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29 692 microbial community. Although more diagnostic biomolecules are present in Mars-analog
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31 693 hydrothermal spring environments, this work demonstrates that FAMEs are preserved and
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33 694 detectable with space-flight-like GC-MS techniques in modern inactive and relict siliceous sinter
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35 695 and calcite deposits from Iceland. The MOMA instrument on the *Rosalind Franklin* rover will be
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37 696 able to ramp the pyrolysis oven at >200 °C/min (from thermal vacuum), achieving a much faster
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39 697 ramp than the SAM instrument and perhaps increasing the likelihood of detecting Martian FAMEs,
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41 698 if present.

37
38 699 The rate of siliceous sinter formation, hydrothermal conditions, and those effects on biomass
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40 700 growth and potential for silicification in each individual Icelandic sinter deposit reflect differences
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42 701 in the diversity and abundance of biomolecules detected in this work. The geologic process of a
43
44 702 hot spring becoming quiescent should be similar on Earth and Mars. The evolution of a putative
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46 703 microbial community within that hot spring can be approximated in specific ways by assessing
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48 704 microbial community evolution in hot springs on Earth. The approximations ~~by definition~~
49
50 705 ~~require~~ require assumptions that do not account for Mars-specific conditions such as atmospheric
51
52 706 composition, radiation flux, energy sources, etc. By assessing the microbial communities and
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54 707 preserved organics from lower temperature inactive and relict spring systems, this work
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56 708 approximates the organics load in Martian hot springs with a hypothetical microbial community
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58 709 that would have evolved as the spring system cooled to dormancy. Understanding the distribution

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3 710 of organics in terrestrial systems is vital for choosing an ideal Martian hydrothermal target for
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5 711 organic biosignature exploration.

6
7 712 A final consideration in the search for organics on Mars is the deleterious effects of oxidants and
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9 713 radiation on organics. Although organic matter has been identified in Mars near-surface substrates
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11 714 with the SAM instrument (Eigenbrode et al., 2018; Freissinet et al., 2015), fewer molecules
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13 715 detected by SAM represent reduced carbon, with many of these organics being oxidized. For
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15 716 example, polycyclic aromatic hydrocarbons (PAHs) and kerogen from exogenous delivery via
16
17 717 meteoritic infall may undergo several oxidation reactions to form stable simple organics such as
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19 718 phthalic, mellitic, or oxalic acids (Benner et al., 2000; Freissinet et al., 2015) on the Martian
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21 719 surface, which are then detectable by the SAM instrument (e.g. possible source organics for
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23 720 benzene in the Sheepbed mudstone, Freissinet et al. (2015)). These oxidation processes are
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25 721 expected to occur both on the surface and in the subsurface of Mars.

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27 722 Ionizing radiation is another serious concern for the long-term preservation of Martian organics.
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29 723 The ionizing radiation dose on the surface of Mars is estimated at 0.54–0.85 Gray/year (Dartnell
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31 724 et al., 2007). Ionizing radiation destroys biomolecules (e.g. proteins or DNA), and most bacteria
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33 725 cannot survive doses greater than 200 Gray (Daly, 2009). Recent studies indicate that within 300
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35 726 million years, preservation of functionalized organic molecules greater than 500 amu in the top ~5
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37 727 cm of rock drops 1000x due to ionizing radiation (Pavlov et al., 2012). There is some indication
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39 728 that rapid burial of organics, short exposure ages due to relatively slow Martian weathering rates,
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41 729 and the characteristics of some entombing mineralogies may assist in organics preservation in the
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43 730 Martian near-surface (Hays et al., 2017 and references therein), but the need remains to sample the
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45 731 Martian subsurface to reduce the impact of degradation via radiation effects.

46
47 732 The *Curiosity* rover can drill to a maximum of six centimeters and the *Rosalind Franklin* rover
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49 733 will be able to drill to two meters depth. The work presented here indicates that even with
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51 734 diagenetic effects and heterotrophic cycling of organics, organic molecules are preserved and
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53 735 detectable over short timescales in the near-subsurface (2 to 18 cm in depth) of modern and relict
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55 736 terrestrial hot springs. Organics therefore may be detectable on Mars in relatively recent hot spring
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57 737 deposits, but preservation likely deteriorates over geologic time scales. These depths are within
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59 738 the drill sampling range of the *Curiosity* and *Rosalind Franklin* rovers (partial to complete depths,
60

739 respectively), and support the search for near-surface organics preserved in Martian hydrothermal
740 systems.

741 **5. Conclusions**

742 Hydrothermal spring deposits host unique microbial ecosystems and have the capacity to
743 physically preserve both microbial communities and their associated organic matter as
744 biosignatures within siliceous sinter. These terrestrial geothermal springs serve as Mars analog
745 environments to prepare future Mars missions to target their search for biosignatures in Martian
746 hot spring deposits. This study quantified the preservation and detectability of fatty acids in three
747 Icelandic hot spring deposits ranging from modern and inactive to relict, and samples were
748 collected both at the surface and at depth (ranging from 2 to 18 cm in depth). Analyses performed
749 with TMAH thermochemolysis pyrolysis-GC-MS indicate that fatty acids were preserved and
750 detectable in all three spring deposits, with the subsurface samples degraded relative to the surface
751 samples. TMAH thermochemolysis pyrolysis-GC-MS analyses using a SAM-instrument-like
752 procedure were also successful in liberating preserved fatty acids from these sinter samples, albeit
753 generally in lower abundances and with less diversity. These results build confidence in our ability
754 to detect fatty acids and distinguish biogenicity using the GC-MS instrumentation available on
755 current and future Mars missions.

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765 No competing financial interests exist.

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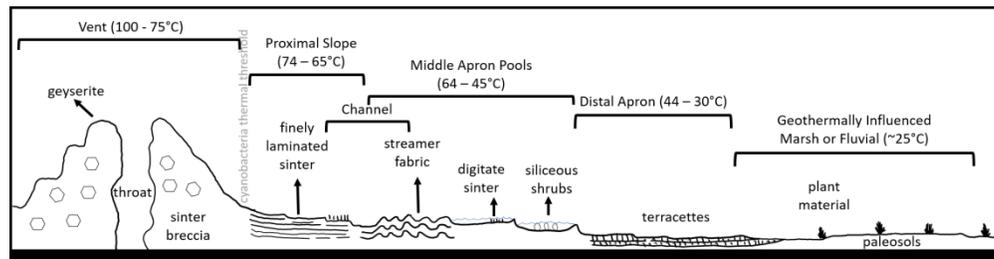


Figure 1. Environmental settings and facies of a generalized geothermal spring system. Modified from Campbell et al. (2015).

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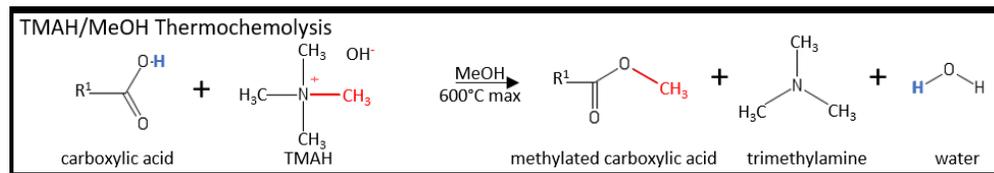


Figure 2. Example TMAH thermochemolysis reaction with a carboxylic acid to form a methylated carboxylic acid. After the reaction, the molecule is more volatile and detectable to GC-MS. TMAH in methanol is available on the SAM and MOMA instruments.

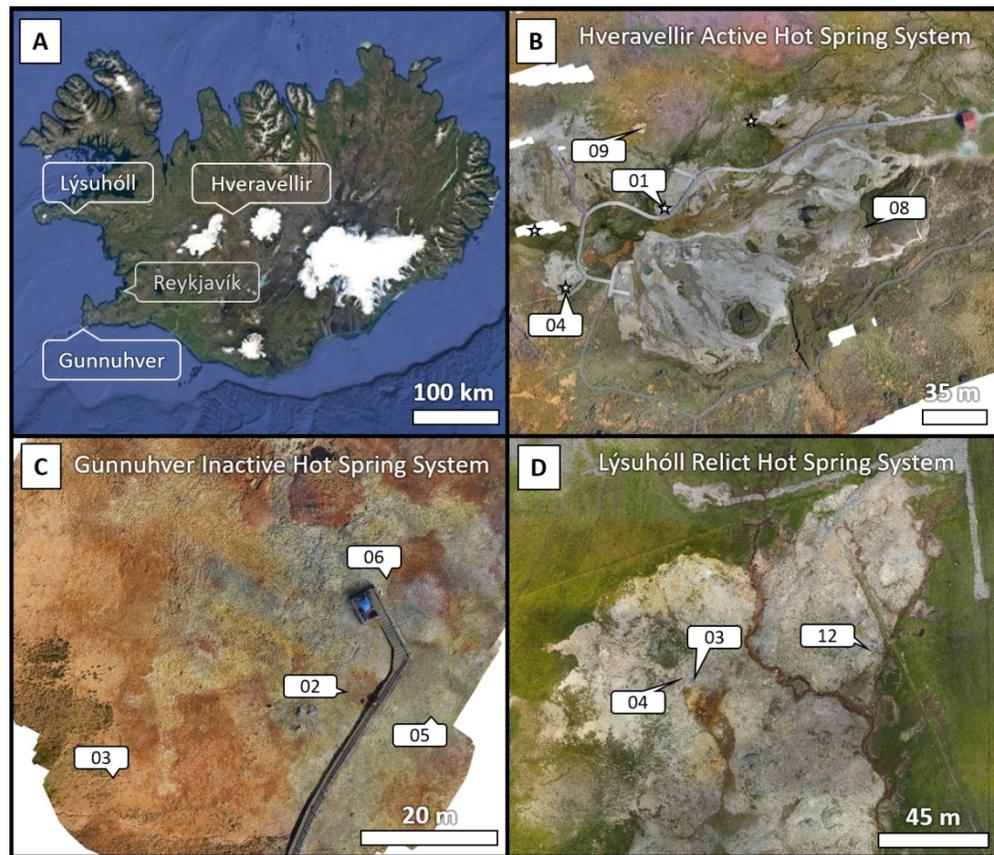


Figure 3. A) Map of field locations in Iceland, with the capitol of Reykjavík for reference. B-D) Field site numbers at the Hveravellir, Gunnuhver, and Lysuhöll spring systems. Stars at Hveravellir indicate active flowing spring vents where geochemical measurements were taken.

209x180mm (150 x 150 DPI)

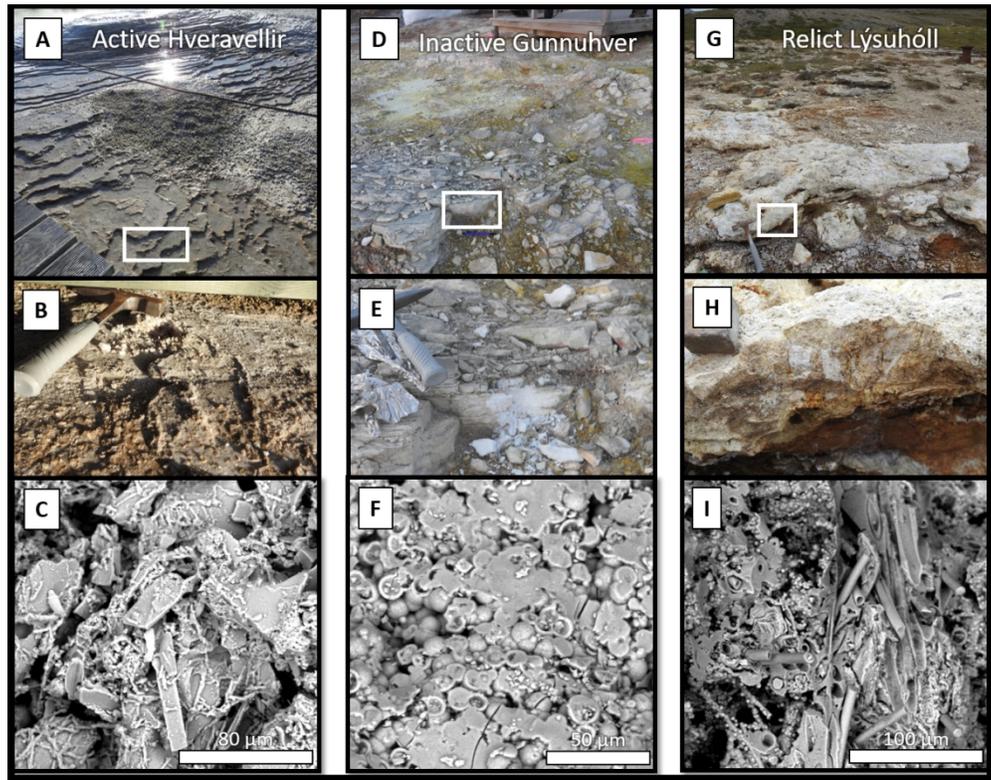


Figure 4. Field sample site images and corresponding SEM images of sinter from A-C) Hveravellir (active vent in top left of panel A), D-F) Gunnuhver, and G-I) Lýsuhóll. White box indicates zoom image B, E, or H. Rock hammer for scale in surface images. C) Silicified diatoms, microbial filaments, and putative plant matter present in the Hveravellir site. F) Opal-CT accretionary spheres with no confirmed biogenic features present in the Gunnuhver site. I) Abundant silicified casts of microbial filaments present in the Lýsuhóll site.

229x179mm (150 x 150 DPI)

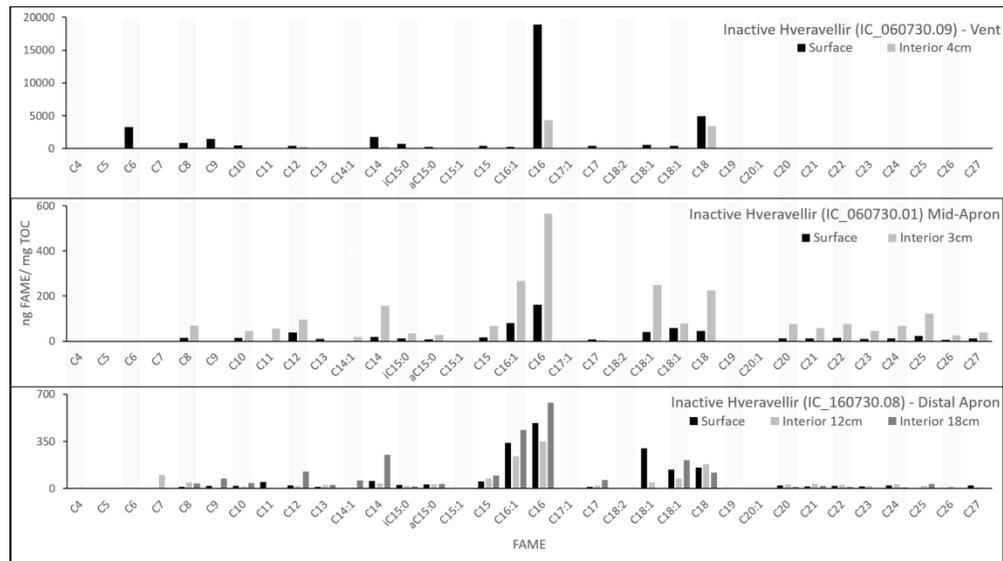


Figure 5. FAME profile for the active Hveravellir Hot Spring system analyzed with a 500 °C flash pyrolysis step.

339x190mm (150 x 150 DPI)

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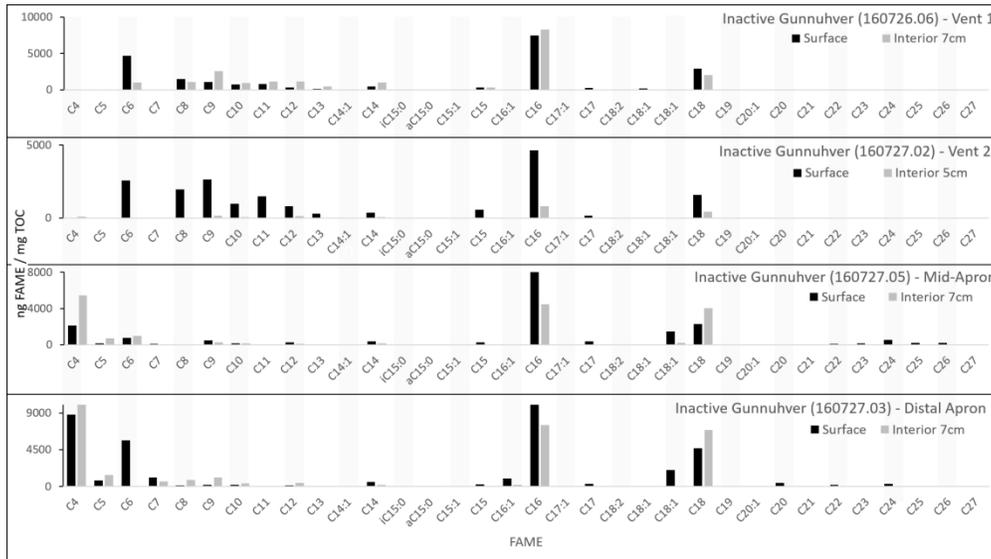


Figure 6. FAME profile for the inactive Gunnuhver Hot Spring system analyzed with a 500 °C flash pyrolysis step.

339x190mm (150 x 150 DPI)

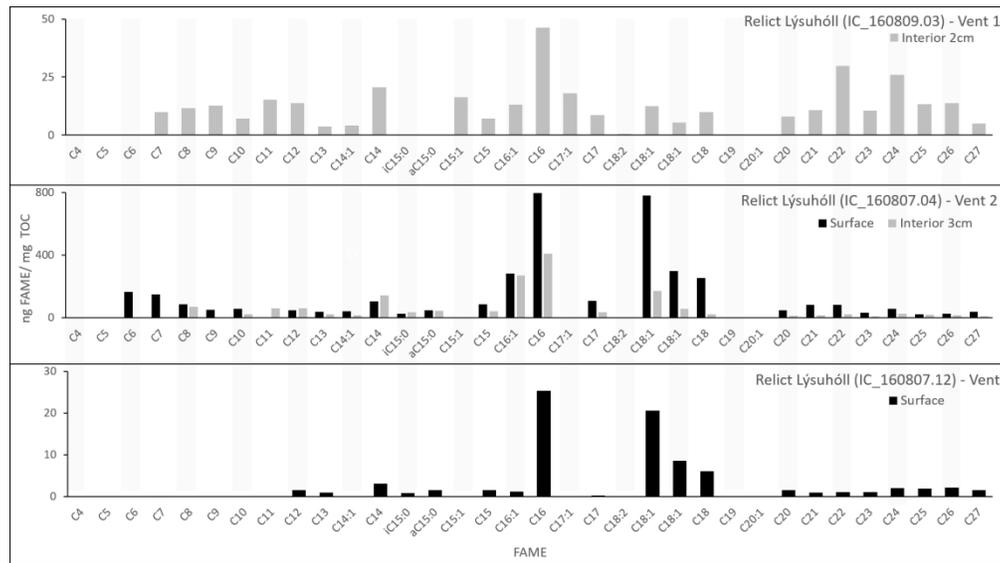


Figure 7. FAME profile for the relict Lýsuhóll Hot Spring system analyzed with a 500 °C flash pyrolysis step.

339x190mm (150 x 150 DPI)

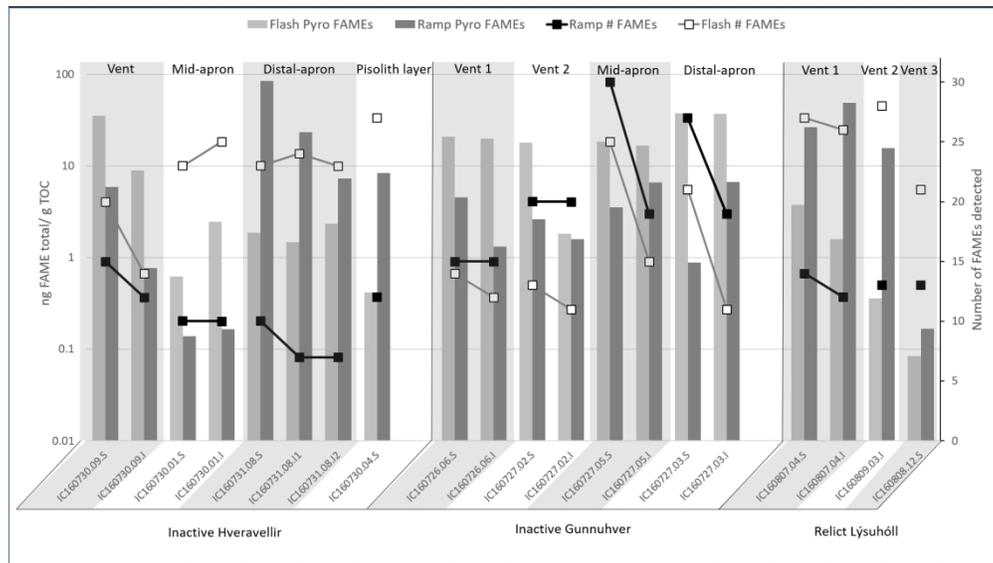


Figure 8. Comparison of total FAME abundance (in ng FAME/ g TOC) detected with the 500°C flash pyrolysis and the SAM-like 35°C/min ramp pyrolysis methods at each sinter sample location. The number of unique FAMES detected with each pyrolysis method is included on the secondary (right) y-axis. Flash pyrolysis nearly always yielded comparable numbers of FAMES relative to ramp pyrolysis.

339x191mm (150 x 150 DPI)

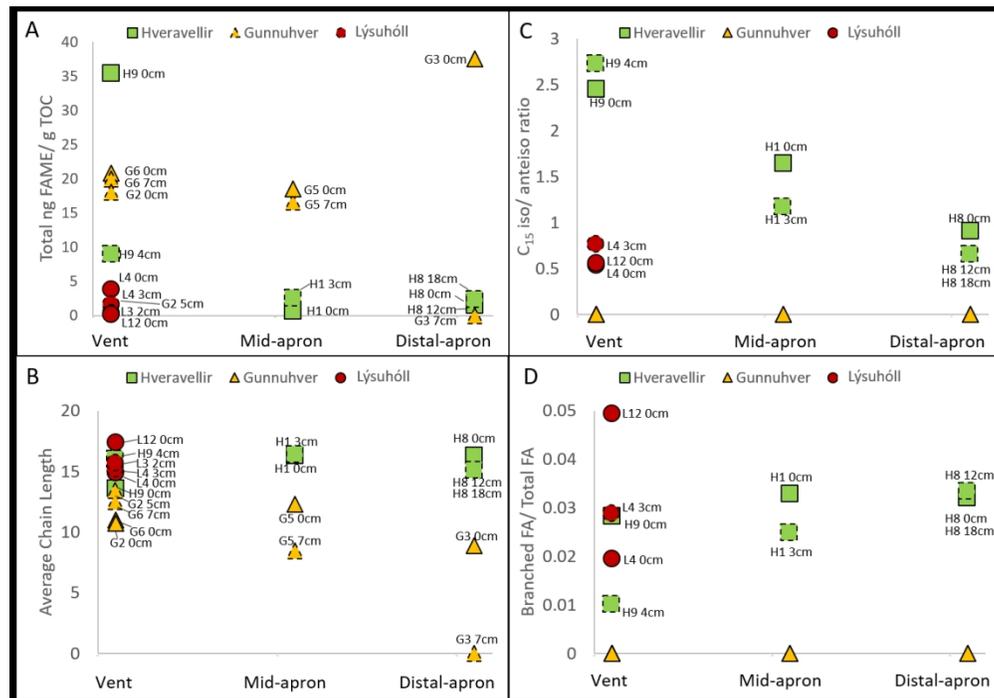


Figure 9. Fatty acid distribution across hot spring facies, with locations “vent”, “mid-apron” and “distal-apron” representing environmental facies as determined by textural characterization. A) Total ng of FAMES / g TOC. The total FAME concentration at the Hveravellir site is highest at the vent, whereas the concentration at the Gunnuhver site is highest at the distal apron. B) The average carbon chain length at the Hveravellir site slightly increases from vent to distal apron, whereas at the Gunnuhver site the average chain length dramatically decreases. C) The ratio of iso- to anteiso-C₁₅ is highest in the Hveravellir vent site and lowest in the distal apron facies. No br-FA were detected in the Gunnuhver site. The br-FA ratio was low for the Lýsuhóll vent sites. D) The ratio of branched fatty acids to total fatty acids was lowest at the Hveravellir vent site and highest at the distal apron site. No br-FA were detected in the Gunnuhver site. The ratio of br-FA to total FA was higher for the Lýsuhóll vent sites.

256x179mm (150 x 150 DPI)

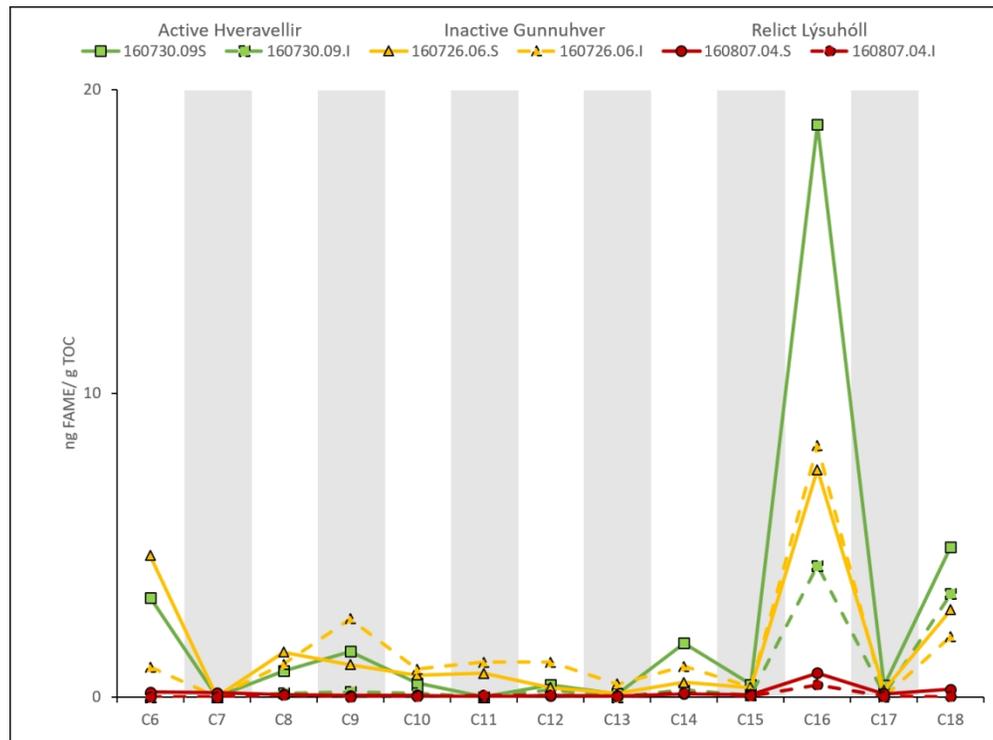


Figure 10. Comparison of FAME concentrations determined with flash pyrolysis experiments from the surface and subsurface vent sites from the Hveravellir, Gunnuhver, and Lýsuhóll spring systems. The even-over-odd carbon number preference is most apparent in the Hveravellir and Gunnuhver locations.

242x180mm (150 x 150 DPI)

Table 1. Sample location, ID, depth, mineralogy, latitude, longitude, percent total carbon and percent total organic carbon.								
<i>Group</i>	<i>Sample ID</i>	<i>Sample</i>	<i>Sample Depth</i>	<i>Mineralogy</i>	<i>Latitude</i>	<i>Longitude</i>	<i>% TC</i>	<i>% TOC ±0.06</i>
Hveravellir Modern Active Spring System	IC160730.09.S	Vent deposit	0 cm	Opal-A, clinopyroxenes (e.g. augite, diopside), plagioclase, gypsum	N64° 51.940'	W19° 33.513'	0.24	0.24
	IC160730.09.I	Vent deposit	4 cm	Opal-A, plagioclase, diopside, magnesite			0.08	0.08
	IC160730.01.S	Mid-apron deposit	0 cm	Amorphous	N64° 51.917'	W19° 33.437'	0.43	0.43
	IC160730.01.I	Mid-apron deposit	3 cm	Amorphous			0.10	0.10
	IC160731.08.S	Distal apron deposit	0 cm	Amorphous	N64° 51.911'	W19° 33.363'	0.08	0.08
	IC160731.08.I1	Distal apron deposit	12 cm	Amorphous			0.13	0.13
	IC160731.08.I2	Distal apron deposit	18 cm	Amorphous			0.15	0.15
	IC160730.04.S	Pisolith layer	0 cm	Opal-A, plagioclase, magnesite	N64° 51.893'	W19° 33.564'	0.30	0.30
Gunnahver Modern Inactive Spring System	IC160726.06.S	Vent deposit	0 cm	Opal-CT	N63° 49.148'	W022° 41.159'	0.02	0.02
	IC160726.06.I	Vent deposit	7 cm	Opal-CT			0.01	0.01
	IC160727.02.S	Vent deposit	0 cm	Opal-CT, halite, quartz	N63° 49.138'	W022° 41.168'	0.13	0.13
	IC160727.02.I	Vent deposit	5 cm	Opal-CT, halite, quartz			0.02	0.02
	IC160727.05.S	Mid-apron deposit	0 cm	Opal-CT	N63° 49.136'	W022° 41.149'	0.17	0.17
	IC160727.05.I	Mid-apron deposit	7 cm	Opal-CT			0.02	0.02
	IC160727.03.S	Distal apron deposit	0 cm	Amorphous	N63° 49.131'	W022° 41.205'	0.15	0.15
	IC160727.03.I	Distal apron deposit	7 cm	Amorphous			0.02	0.02
Lýsuhóll Relict Spring System	IC160807.04.S	Vent deposit	0 cm	Amorphous, calcite	N64° 50.651'	W023° 13.281'	1.83	0.61
	IC160807.04.I	Vent deposit	3 cm	Amorphous, calcite			0.85	0.18
	IC160809.03.I	Vent deposit	2 cm	Amorphous, minor calcite	N64° 50.650'	W023° 13.274'	0.61	0.43
	IC160808.12.S	Vent deposit	0 cm	Calcite	N64° 50.657'	W023° 13.177'	10.38	6.31

Table 1. Sample location, ID, depth, mineralogy, latitude, longitude, percent total carbon and percent total organic carbon.

Table 2. Flash pyrolysis FAME data in ng FAME/ mg TOC.

Group	Sample ID	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14:1	C14	iC15:0	aC15:0	C15:1	C15
Hveravellir Modern Active Spring System	IC160730.09.S	0.0	0.0	3281.4	0.0	862.1	1501.0	467.5	0.0	400.4	110.5	0.0	1780.5	711.3	290.4	0.0	419.3
	IC160730.09.I	0.0	0.0	0.0	0.0	136.2	171.8	127.7	0.0	247.3	0.0	0.0	231.9	66.4	24.4	0.0	68.2
	IC160730.01.S	0.0	0.0	0.0	0.0	13.8	0.0	13.9	0.0	38.9	9.2	0.0	19.5	12.8	7.8	0.0	17.3
	IC160730.01.I	0.0	0.0	0.0	0.0	68.5	0.0	45.0	57.0	95.0	1.6	18.5	157.3	33.1	28.2	0.0	67.4
	IC160731.08.S	0.0	0.0	0.0	0.0	11.0	18.5	18.4	49.8	22.3	14.1	0.0	57.2	28.5	31.4	0.0	53.2
	IC160731.08.I1	0.0	0.0	0.0	100.0	47.0	0.0	16.9	0.0	19.6	26.7	0.0	37.6	19.4	29.7	0.0	75.0
	IC160731.08.I2	0.0	0.0	0.0	0.0	38.7	74.3	41.1	0.0	126.4	25.5	61.8	252.4	14.7	36.0	0.0	98.5
Gunnuhver Modern Inactive Spring System	IC160726.06.S	0.0	0.0	4663.7	0.0	1478.2	1070.1	731.4	788.8	319.7	118.2	0.0	485.3	0.0	0.0	0.0	313.2
	IC160726.06.I	0.0	0.0	990.0	0.0	1085.4	2578.5	925.8	1147.4	1148.3	432.7	0.0	1017.4	0.0	0.0	0.0	329.4
	IC160727.02.S	0.0	0.0	2558.7	0.0	1960.6	2630.9	976.6	1494.4	807.0	310.5	0.0	377.5	0.0	0.0	0.0	554.9
	IC160727.02.I	94.7	0.0	0.0	0.0	0.0	163.1	72.9	0.0	120.3	14.8	0.0	59.1	0.0	0.0	0.0	18.9
	IC160727.05.S	2146.2	165.5	770.5	129.1	14.1	467.1	149.3	0.0	247.1	11.8	0.0	387.7	0.0	0.0	0.0	287.5
	IC160727.05.I	5466.7	683.5	1007.4	0.0	0.0	246.3	139.1	52.6	104.9	0.0	0.0	149.8	0.0	0.0	0.0	27.0
	IC160727.03.S	8777.9	715.6	5654.0	1077.1	141.7	181.6	188.0	0.0	123.7	0.0	0.0	554.0	0.0	0.0	0.0	282.1
IC160727.03.I	17486.5	1401.0	0.0	604.7	794.8	1075.5	366.6	0.0	440.7	0.0	0.0	219.2	0.0	0.0	0.0	0.0	
Lýsuhóll Relict Spring System	IC160807.04.S	0.0	0.0	163.4	147.0	84.3	49.0	57.3	0.0	46.9	36.8	39.7	104.8	25.8	48.0	0.0	83.2
	IC160807.04.I	0.0	0.0	0.0	0.0	69.1	0.0	22.3	60.2	58.6	21.8	13.7	142.7	33.8	44.4	0.0	39.5
	IC160809.03.I	0.0	0.0	0.0	9.9	11.5	12.6	7.2	15.3	13.7	3.8	4.0	20.5	0.0	0.0	16.3	7.1
	IC160808.12.S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.0	0.0	3.1	0.9	1.5	0.0	1.5

Table 2 con't. Flash pyrolysis FAME data in ng FAME/ mg TOC.

Group	Sample ID	C16:1	C16	C17:1	C17	trans-C18:2	cis-C18:1	trans-C18:1	C18	C20	C21	C22	C23	C24	C25	C26	C27
Hveravellir Modern Active Spring System	IC160730.09.S	244.0	18860.8	5.5	383.7	0.0	580.2	406.4	4953.4	133.6	0.0	10.5	0.0	0.0	0.0	0.0	0.0
	IC160730.09.I	0.0	4334.5	0.0	85.0	0.0	0.0	0.0	3405.9	49.5	0.0	6.2	0.0	0.0	0.0	0.0	0.0
	IC160730.01.S	79.4	160.6	0.0	7.5	0.0	40.1	59.2	44.5	11.4	11.3	13.5	10.8	11.9	22.9	6.0	11.8
	IC160730.01.I	266.7	565.5	0.0	4.5	0.0	249.3	77.2	224.9	75.1	57.8	75.8	46.0	66.5	122.8	24.9	39.5
	IC160731.08.S	340.9	488.0	0.0	13.7	0.0	300.6	141.3	156.8	25.3	15.8	20.5	17.8	24.6	0.0	0.0	25.3
	IC160731.08.I1	239.8	351.6	0.0	22.5	0.0	47.1	73.6	180.1	29.1	35.5	26.2	21.3	29.9	18.1	17.2	9.0
	IC160731.08.I2	433.4	638.5	0.0	64.6	0.0	0.0	211.3	119.3	11.3	20.5	11.1	5.7	9.9	36.1	4.4	0.0
Gunnuhver Modern Inactive Spring System	IC160726.06.S	0.0	7484.0	0.0	237.7	0.0	165.8	0.0	2881.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IC160726.06.I	0.0	8282.2	0.0	0.0	0.0	0.0	0.0	1993.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IC160727.02.S	0.0	4655.8	0.0	152.7	0.0	0.0	0.0	1571.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IC160727.02.I	0.0	820.1	0.0	17.6	0.0	0.0	0.0	439.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IC160727.05.S	62.2	8073.3	0.0	394.7	0.0	0.0	1469.2	2295.9	76.1	16.0	110.5	143.0	556.9	216.9	212.2	18.4
	IC160727.05.I	0.0	4444.4	0.0	32.1	0.0	0.0	195.7	4021.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IC160727.03.S	1010.7	10647.0	0.0	296.2	0.0	0.0	1984.8	4710.6	407.2	0.0	223.2	81.4	340.7	0.0	80.4	0.0
Lýsuhóll Relict Spring System	IC160807.04.S	282.3	796.2	0.0	108.5	0.0	780.4	296.8	254.0	46.0	80.2	82.6	29.0	57.2	20.5	22.7	35.4
	IC160807.04.I	268.0	409.4	0.0	33.5	0.5	171.4	54.5	21.4	10.6	14.9	20.7	8.8	22.8	16.7	13.4	9.8
	IC160809.03.I	13.2	46.2	18.1	8.7	0.5	12.5	5.4	9.8	8.1	10.8	29.9	10.5	25.9	13.2	13.8	5.0
	IC160808.12.S	1.2	25.3	0.0	0.3	0.0	20.6	8.6	6.0	1.5	1.0	1.0	1.1	2.0	1.9	2.1	1.5

Table 2. Flash pyrolysis FAME data in ng FAME/ mg TOC. Black cells = concentrations >100 ng/ mg TOC. Dark gray cells = Concentrations 10 to 100 ng/ mg TOC. Light gray cells = 0.01 to 10 ng/ mg TOC. White cells = No detection (0.0 ng / mg TOC).

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Group	Sample ID	F	R	F	R	F	R	F	R	F	R	F	R	F	R	F	R	F	R	F	R			
		C _{4:0}	C _{5:0}	C _{6:0}	C _{7:0}	C _{8:0}	C _{9:0}	C _{10:0}	C _{11:0}	C _{12:0}	C _{13:0}	C _{14:1}	C _{14:0}	iC _{15:0}	aC _{15:0}	C _{15:1}	C _{15:0}							
Modern Active Spring System at Hveravellir	IC160730.09.S			x	X	x	X	x	X	X	X		x	X	x	X				x	X			
	IC160730.09.I					x	X	x	X	x	X		X	x	X		X				x	X		
	IC160730.01.S				X	x	X		x		x		x	X	x	X					x	X		
	IC160730.01.I					x		X	x	X	x		x	X	x	X					x	X		
	IC160731.08.S					x	X	x	X	x	X	x		x	X	x	X					x	X	
	IC160731.08.11				x	x			x				x	X	x	X						x	X	
	IC160731.08.12					x	X	x		x	X		x	X	x		x					x		
	IC160730.04.S			x	X	x	X	x		x	X			x	X	x	X					x	X	
Modern Inactive Spring System at Gunnhver	IC160726.06.S			x		x	X	x	X	x	X	x		x	X							x	X	
	IC160726.06.I			x	X	x	X	x	X	x	X	x		x	X								x	X
	IC160727.02.S		X	X	x	X	X	x	X	x	X	x		x	X								x	X
	IC160727.02.I	x	X	X	X	X	X	X	X	X	X	X		x	X								x	X
	IC160727.05.S	x	X	x	X	x	X	x	X	x	X	x		x	X								x	X
	IC160727.05.I	x	X	x	X	x	X	X	X	x	X	x		x	X								x	X
	IC160727.03.S	x	X	x	X	x	X	x	X	x	X		X		x	X	X	X					x	X
	IC160727.03.I	x	X	x		x	X	x	X	x	X			x	X									X
Relict Spring System at Lýsuhóll	IC160807.04.S		X		x	X	x		x	X	x		x	X	x	X						x	X	
	IC160807.04.I				X		x	X	X	X	x	X	x	X	x	X							x	X
	IC160809.03.I				x		x	X	x	X	x	X	x	X							x		x	X
	IC160808.12.S					X			X			X	x	X	x	X	X	X					x	X

Table 3 con't. Comparison of flash and SAM-like ramp pyrolysis.

Group	Sample ID	F	R	F	R	F	R	F	R	F	R	F	R	F	R	F	R	F	R	F	R		
		C _{16:1}	C _{16:0}	C _{17:1}	C _{17:0}	trans-C _{18:2}	cis-C _{18:1}	trans-C _{18:1}	C _{18:0}	C _{19:0} std	C _{20:0}	C _{21:0}	C _{22:0}	C _{23:0}	C _{24:0}	C _{25:0}	C _{26:0}	C _{27:0}					
Modern Active Spring System at Hveravellir	IC160730.09.S	x	X	x	X	x		x	X	x	X	x		x									
	IC160730.09.I			x	X					x	X	x	X	x									
	IC160730.01.S	x		x	X			x	X	x	X	x	X	x	X	x	X	x	X	x	X	x	X
	IC160730.01.I	x	X	x	X			x		x	X	x	X	x		x	X	x	X	x	X	x	X
	IC160731.08.S	x		x	X			x		x	X	x	X	x		x	X	x	X	x	X	x	X
	IC160731.08.11	x		x	X			x		x	X	x	X	x		x	X	x	X	x	X	x	X
	IC160731.08.12	x		x	X			x		x	X	x	X	x		x	X	x	X	x	X	x	X
	IC160730.04.S	x	X	x	X	x		x	X	x	X	x	X	x	X	x	X	x	X	x	X	x	X
Modern Inactive Spring System at Gunnuhver	IC160726.06.S			x	X			x		x	X	x	X	x	X	x	X	x	X	x	X	x	X
	IC160726.06.I			x	X			x	X	x	X	x	X	x	X	x	X	x	X	x	X	x	X
	IC160727.02.S			x	X			x	X	x	X	x	X	x	X	x	X	x	X	x	X	x	X
	IC160727.02.I			x	X			x	X	x	X	x	X	x	X	x	X	x	X	x	X	x	X
	IC160727.05.S	x		x	X	x	X	x	X	x	X	x	X	x	X	x	X	x	X	x	X	x	X
	IC160727.05.I			x	X			x		x	X	x	X	x	X	x	X	x	X	x	X	x	X
	IC160727.03.S	x		x	X	x	X	x	X	x	X	x	X	x	X	x	X	x	X	x	X	x	X
	IC160727.03.I	x	X	x	X			x		x	X	x	X	x	X	x	X	x	X	x	X	x	X
Relict Spring System at Lysuhóll	IC160807.04.S	x	X	x	X			x	X	x	X	x	X	x	X	x	X	x	X	x	X	x	X
	IC160807.04.I	x		x	X			x		x	X	x	X	x	X	x	X	x	X	x	X	x	X
	IC160809.03.I	x	X	x	X	x		x		x	X	x	X	x	X	x	X	x	X	x	X	x	X
	IC160808.12.S	x	X	x	X			x	X	x	X	x	X	x	X	x	X	x	X	x	X	x	X

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Table 3. Comparison of flash pyrolysis and SAM-like ramp pyrolysis of sinter samples. Green = FAME detection in both experiments. Yellow = FAME detection in flash pyrolysis experiment only. Orange = FAME detection in ramp pyrolysis experiment only. Blank cells = no FAME detection. Detection of the C₁₉ standard is included for completeness.

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