

1 **Dissolved organic carbon compounds in deep-sea hydrothermal vent fluids**  
2 **from the East Pacific Rise at 9°50'N**

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11 Declarations of interest: none

12 Revised for submission to: *Organic Geochemistry*

13

14 **Abstract**

15           Deep-sea hydrothermal vents are unique ecosystems that may release chemically distinct  
16 dissolved organic matter to the deep ocean. Here, we describe the composition and  
17 concentrations of polar dissolved organic compounds observed in low and high temperature  
18 hydrothermal vent fluids at 9°50'N on the East Pacific Rise. The concentration of dissolved  
19 organic carbon was 46  $\mu\text{M}$  in the low temperature hydrothermal fluids and 14  $\mu\text{M}$  in the high  
20 temperature hydrothermal fluids. In the low temperature vent fluids, quantifiable dissolved  
21 organic compounds were dominated by water-soluble vitamins and amino acids. Derivatives of  
22 benzoic acid and the organic sulfur compound 2,3-dihydroxypropane-1-sulfonate (DHPS) were  
23 also present in low and high temperature hydrothermal fluids. The low temperature vent fluids  
24 contain organic compounds that are central to biological processes, suggesting that they are a by-  
25 product of biological activity in the seafloor. These compounds may fuel heterotrophic and  
26 other metabolic processes at deep-sea hydrothermal vents and beyond.

27

28 *Keywords:* metabolomics; hydrothermal vents; deep-sea; dissolved organic matter; vitamins

## 29 **1 Introduction**

30           Dissolved organic matter in seawater is a heterogeneous mixture of compounds, each  
31 with different physical and chemical properties. While cycling of seawater through the  
32 seafloor during hydrothermal circulation can be a source of dissolved organic carbon (DOC)  
33 to the deep ocean (Druffel and Griffin, 2015; Hedges, 1992; McCarthy et al., 2011), the chemical  
34 composition and lability of this organic matter may be distinct from organic matter produced in  
35 the non-vent deep ocean. These potential differences in chemical composition can have a  
36 substantial impact on carbon cycling. Within hydrothermal systems, microbial communities may  
37 play a fundamental role in regulating the composition of dissolved organic matter released to the  
38 water column. For example, metabolites formed as by-products of chemoautotrophic activity  
39 (Brault et al., 1988) represent a source of dissolved organic matter in hydrothermal fluids  
40 <121°C. Hydrothermal fluids also contain hydrocarbons generated during thermal alteration of  
41 microbial biomass (Dittmar, 2008; Konn et al., 2011; Reeves et al., 2014). In addition to  
42 biogenic sources, dissolved organic compounds may be derived from thermal alteration of  
43 dissolved organic matter initially present in circulating seawater (Hawkes et al., 2016; Rossel et  
44 al., 2017) and abiotic processes that occur during hydrothermal circulation (Lang et al., 2010;  
45 Lin et al., 2017; McCollom and Seewald, 2007; McDermott et al., 2015a; Proskurowski et al.,  
46 2008). We posit that information on the composition of organic compounds delivered to the  
47 water column by hydrothermal fluids can help identify the source of these compounds, and the  
48 roles that they play in deep-sea carbon cycling.

49           Here we use a combination of direct infusion and liquid chromatography (LC)-based  
50 mass spectrometry methods to analyze polar organic compounds found in hydrothermal vent

51 fluids. Direct infusion coupled to ultrahigh resolution mass spectrometers has been used in a  
52 variety of ecosystems to consider large-scale differences in the composition of dissolved organic  
53 matter (e.g., Kujawinski et al., 2009; Medeiros et al., 2015; Ohno et al., 2010). To complement  
54 these data, we rely on targeted analytical methods that allow us to identify and quantify known  
55 organic compounds (Kido Soule et al., 2015). LC-based mass spectrometry methods have proven  
56 valuable in characterizing the composition of metabolites produced by marine microorganisms  
57 (Fiore et al., 2015; Johnson et al., 2016). These methods can characterize biologically relevant  
58 organic molecules found within seafloor hydrothermal vent ecosystems. Our results allow us  
59 to compare the composition and concentration of these compounds in hydrothermal fluids  
60 compared to conditions in non-vent deep seawater.

## 61 **2 Methods**

### 62 **2.1 Sample collection and processing**

63 Hydrothermal vent fluids were collected using either the ROV *Jason* or HOV *Alvin*  
64 deployed from the R/V *Atlantis* during two cruises to the basalt-hosted deep-sea hydrothermal  
65 vent field at 9°50'North on the East Pacific Rise in January and November 2014 (AT26-10 and  
66 AT26-23). During each cruise, vent fluid samples for chemical analysis were collected from the  
67 Crab Spa and Bio 9 vents, which are both located within the axial summit caldera at 9°North (see  
68 map in Fornari et al., 2012). Crab Spa is a low temperature (25°C) diffuse-flow hydrothermal  
69 vent (McNichol et al., 2018; McNichol et al., 2016) and Bio 9 is a high temperature (366°C)  
70 focused-flow hydrothermal vent. Both sites are dominated by basalt. During the January 2014  
71 cruise, a background seawater sample was collected 20 m above the seafloor, away from the  
72 hydrothermal vent sites, for comparison with the vent fluids. All water samples were collected

73 using a titanium inlet snorkel consisting of a coil of narrow titanium tubing (1/8" O.D. x 0.085"  
74 I.D.) connected with Teflon tubing to a FlexFoil Plus sample bag (SKC, Pennsylvania, US). The  
75 titanium coil allowed high temperature fluids to cool before entering the Teflon tubing. Prior to  
76 deployment, the 86 ml of dead volume in the titanium and Teflon tubing was pre-filled with  
77 Milli-Q water. The FlexFoil Plus bags have been previously tested to confirm the absence of  
78 contaminants that would interfere with our analyses. Water samples were pumped into the bag  
79 using a peristaltic pump with PharMed BPT tubing (Masterflex) at the pump head at  
80 approximately 25-40 mL minute<sup>-1</sup>. Two liters of fluid were collected for all samples except for  
81 the November 2014 Bio 9 sample that had 1.4 L of fluid. Vent fluid temperature was monitored  
82 continuously during sample collection using a type J thermocouple attached to the inlet of the  
83 sampling snorkel.

84         The fluid samples were returned to the ship-board laboratory for processing within 5 – 10  
85 h of collection with the longer 10-hour delay being typical for the January 2014 samples due to  
86 the use of ROV *Jason* which operates for longer periods of time at the seafloor. Water samples  
87 were filtered with 0.2-µm Omnipore filters (Millipore) mounted in perfluoroalkoxy (PFA) filter  
88 holders (Advantec). A 40 ml aliquot of 0.2 µm-filtered water was acidified with concentrated  
89 hydrochloric acid and stored in combusted glass vials at 4°C for measurement of DOC and  
90 dissolved total nitrogen (TN) using a Shimadzu TOC-V<sub>CSH</sub> total organic carbon analyzer  
91 equipped with a TNM-1 nitrogen analyzer in a shore-based laboratory. Blanks (Milli-Q water  
92 from both the ship and the shore-based laboratory) and known concentrations of potassium  
93 hydrogen phthalate and potassium nitrate were interspersed with sample runs during the analysis.  
94 Comparisons to standards provided by Prof. D. Hansell (University of Miami) were made daily.

95 The coefficient of variability between replicate injections averaged <1%. Dissolved Mg  
96 concentrations were analyzed by ion chromatography with suppressed conductivity detection  
97 using a DIONEX DX500 system. pH measurements (25°C) were done using an Accumet  
98 Ag/AgCl combination reference electrode.

99 The remaining filtrate was acidified and dissolved organic compounds were extracted  
100 using Bond Elut PPL cartridges (1 g/6 ml sized cartridges, Agilent) following the protocol of  
101 Dittmar et al. (2008) as modified by Longnecker (2015). DOM was eluted from the cartridges  
102 using 100% methanol and stored at -20°C. The amount of DOC extracted was determined by  
103 evaporating the methanol solution to dryness using a Vacufuge (Eppendorf), re-dissolving the  
104 residue in Milli-Q water, and analyzing for DOC as described above. The DOC concentration in  
105 the filtrate was also measured, allowing for calculation of the PPL cartridge extraction  
106 efficiency.

107 In addition to the seawater and vent fluids, we processed and analyzed a Milli-Q water  
108 sample in the same manner as the fluid samples. The Milli-Q water came from the Milli-Q water  
109 system on board the R/V *Atlantis*, and is the same water that was used to pre-fill the sampling  
110 apparatus before each deployment. Data on the concentration of compounds in the Milli-Q water  
111 are provided in Table S1. These data were used to correct the concentration of each organic  
112 compound present in the samples that originated from the Milli-Q water.

## 113 **2.2 Ultrahigh resolution mass spectrometry – direct infusion**

114 The January 2014 samples were analyzed using direct infusion with a syringe pump in  
115 negative ion mode on a 7 Tesla Fourier-transform ion cyclotron resonance mass spectrometer  
116 (FT-ICR-MS, Thermo Fisher Scientific, Waltham, MA) using electrospray ionization (ESI). The

117 dried organic matter extracts were reconstituted in 50:50 methanol:water and infused into the  
118 ESI interface at 4  $\mu\text{L min}^{-1}$ . The capillary temperature was set to 250 °C and the spray voltage  
119 was between 3.7 and 4 kV. At least 200 scans were collected for each sample which is a  
120 sufficient number of scans for good peak reproducibility (Kido Soule et al., 2010). The resulting  
121 data are measured mass-to-charge ( $m/z$ ) values and peak heights for organic compounds within  
122 the extracted organic matter. Elemental formulas were assigned using the algorithm developed  
123 by Kujawinski and Behn (2006). Magnitude-averaged elemental ratios were calculated following  
124 the formulas provided in Sleighter and Hatcher (2008). Values for the number of condensed  
125 aromatic compounds were calculated using the corrected version of the aromaticity index (Koch  
126 and Dittmar, 2016).

### 127 **2.3 Targeted metabolomics**

128 The concentrations of 92 organic compounds were determined using targeted  
129 metabolomics methods outlined by Kido Soule et al. (2015). Dried sample extracts were re-  
130 dissolved in 95:5 (v/v) water:acetonitrile with deuterated biotin (final concentration 0.05  $\mu\text{g ml}^{-1}$ )  
131 as an internal standard. Organic compounds were chromatographically separated using a Synergi  
132 4u Fusion – RP 80A 150  $\times$  2.00 mm column (Phenomenex, Torrance, CA) before being  
133 introduced to a Thermo Scientific TSQ Vantage Triple Stage Quadrupole Mass Spectrometer via  
134 a heated electrospray ionization source for mass spectrometric analysis. The chromatographic  
135 separation used a binary gradient with eluent A being water with 0.1% formic acid and eluent B  
136 being acetonitrile with 0.1% formic acid. Samples run at 250  $\mu\text{L min}^{-1}$  with 5% B for 0–2  
137 minutes, ramp to 65% B from 2 to 20 minutes, ramp to 100% B from 20 to 25 min, and hold  
138 until 32.5 minutes. The column was re-equilibrated for 7 min between samples with 95% A. The

139 mass spectrometer was operated in selected reaction monitoring (SRM) mode; optimal SRM  
140 parameters (s-lens, collision energy) for each target compound were optimized individually using  
141 an authentic standard. Two SRM transitions per compound were monitored for quantification  
142 and confirmation. Eight-point external calibration curves based on peak area were generated for  
143 each compound. The resulting data were converted to mzML files using the msConvert tool  
144 (Chambers et al., 2012) and processed with MAVEN (Melamud et al., 2010). The targeted  
145 metabolomics data are available from MetaboLights under study accession number MTBLS428.

### 146 **3 Results**

#### 147 **3.1 Bulk chemical parameters in hydrothermal fluids**

148 The Crab Spa fluid samples contained 49 mmol/kg Mg in both January and November  
149 2014. Based on this concentration, Crab Spa vent fluid contained 90% seawater and is consistent  
150 with extensive seafloor mixing of cold seawater and a higher temperature zero-Mg  
151 hydrothermal fluid (McNichol et al., 2016). In contrast, the Bio 9 fluids samples contained 6.7  
152 and 9.4 mmol/kg Mg in January 2014 and November 2014, respectively. Because high  
153 temperature vent fluids in basalt-hosted hydrothermal systems are typically characterized by  
154 near-zero dissolved Mg concentrations (German and Von Damm, 2003), the measured levels of  
155 dissolved Mg in the Bio 9 samples suggest entrainment of 13% and 18% ambient bottom  
156 seawater during sample collection in January and November, respectively.

157 The DOC concentration in the low temperature Crab Spa fluids was slightly elevated  
158 compared to the background seawater, while the high temperature Bio 9 fluids were  
159 characterized by DOC concentrations substantially below seawater values (Table 1).  
160 Extrapolating the DOC concentrations at Bio 9 to a fluid with zero Mg results in an endmember



161 fluid with 8.5  $\mu\text{M}$  DOC. The ship-board Milli-Q system contained 16  $\mu\text{M}$  of DOC. Between 22  
162 and 38% of the organic carbon was extracted from the hydrothermal fluids using solid phase  
163 extraction (Table 1) and thus our assessment of the composition of organic matter from the vent  
164 fluids is restricted to approximately one-third of the DOM present in these fluids. The high  
165 temperature Bio 9 fluids and the low temperature Crab Spa fluids both had lower TN  
166 concentrations compared to background seawater. Extrapolating the Bio 9 fluids to zero Mg  
167 results in an endmember fluid with 1.1  $\mu\text{M}$  TN. The concentration of TN and DOC in  
168 background seawater is within the range of concentrations previously measured in the deep sea  
169 (Hansell et al., 2009; Ogawa et al., 1999). The pH of the fluids from Crab Spa and Bio 9 at 25°C  
170 is 5.7 and 3.3, respectively.

### 171 **3.2 Direct infusion mass spectrometry**

172 The negative ion mode spectra from the direct infusion mass spectrometry analysis of the  
173 January 2014 samples did not reveal large differences across the three samples (Supplemental  
174 Figure S1). The measured  $m/z$  values provide an overview of the similarities and differences in  
175 the chemical composition of organic compounds within a sample. The Crab Spa sample had  
176 slightly more  $m/z$  values and a higher mean molecular weight compared to the seawater sample,  
177 while the extractable organic matter in the Bio 9 fluids had a lower number of  $m/z$  values and  
178 lower mean molecular weight (Table 2).

179 Elemental formulas were calculated from the  $m/z$  values. The resulting formulas can be  
180 grouped based on the elements present to provide an overview of the types of organic matter  
181 within the samples. For the three samples analyzed by direct infusion in this project, the  
182 elemental formulas primarily contained CHO and CHON, regardless of the source of the organic

183 matter extract (Figure 1). The elemental formulas can also be considered as molar ratios of  
184 hydrogen:carbon or oxygen:carbon. The weighted H:C and O:C molar ratios were highest for the  
185 Crab Spa sample and lowest for the Bio 9 fluids (Table 2). The elemental ratios can also be used  
186 to assess the number of condensed aromatic compounds found in hydrothermal vent fluids  
187 compared to background seawater. The total number of condensed aromatic compounds was  
188 highest in background seawater which had 1228 features that would correspond to individual  
189 aromatic compounds based on the corrected aromaticity index (Koch and Dittmar, 2016). In  
190 contrast, 894 and 1155 condensed aromatic compounds were found in the high and low  
191 temperature vent fluids, respectively.

### 192 **3.3 Concentrations of selected metabolites in hydrothermal fluids**

193 The extraction of dissolved organic carbon from fluids by solid phase extraction is a  
194 widely-used technique in aquatic sciences. By extracting our samples using the solid phase Bond  
195 Elut PPL resin, we can measure a range of organic compounds and we can compare our results to  
196 existing data on the organic matter found at hydrothermal vents and in seawater. However, there  
197 are known issues with this method and some compounds are not well-retained by PPL (Johnson  
198 et al., 2017). To account for varying levels of retention on the extraction cartridges, we assumed  
199 that extraction efficiencies are not influenced by minor differences between the composition of  
200 seawater and the vent fluids and corrected the measured aqueous concentrations using extraction  
201 efficiencies determined by Johnson et al. (2017). We are actively working on methods to  
202 improve the extraction of dissolved organic matter from aqueous solutions; in the interim,  
203 caution is warranted when considering the absolute values for those compounds with low  
204 extraction efficiencies. To limit errors, the correction for extraction efficiency was applied only

205 to compounds with extraction efficiencies greater than 1% due to larger errors associated with  
206 correcting concentrations for compounds with lower extraction efficiencies.

207 In general, the concentrations of quantifiable organic compounds were highest in the low  
208 temperature vent fluids from Crab Spa (Table S1). Furthermore, the concentrations of  
209 quantifiable organic compounds are in the picomolar ( $10^{-12}$ ) range, which is well below the  
210 micromolar ( $10^{-6}$ ) concentrations obtained for the bulk organic carbon concentrations. The  
211 difference between the low temperature and high temperature vent fluids was greatest for the  
212 vitamins (Figure 2). Riboflavin and pantothenic acid were present at relatively high  
213 concentrations in the Crab Spa fluids in both January and November 2014. Biotin and its  
214 precursor, desthiobiotin, were also prevalent in January 2014, but were present at low levels in  
215 November 2014. By contrast, in the background seawater and high temperature vent fluid, the  
216 total amount of water-soluble vitamins was less than 20 pM.

217 Within the hydrothermal fluid samples, amino acids formed a substantial fraction of the  
218 quantifiable organic compounds. The data have been corrected for the amino acids present in the  
219 Milli-Q water used to pre-fill the dead volume in the tubing prior to deploying the sampling  
220 apparatus. This adjustment represents a minor fraction of the total amino acid concentrations in  
221 the fluid samples with the measured concentrations in the vent fluids ranging from 4 pM to 300  
222 pM while the values in MilliQ water contributed less than 6 pM. Phenylalanine concentrations in  
223 seawater were intermediate to the values obtained for the high temperature Bio 9 and low  
224 temperature Crab Spa fluids (Figure 3). Tryptophan was also present in most of the samples  
225 with a mean value of 41 pM (range = 0 to 113 pM, n=5). In the January 2014 samples,

226 leucine/isoleucine was present at low concentrations and showed no differences across the three  
227 types of samples; no leucine/isoleucine was detected in the samples collected in November 2014.

228         Dissolved organic sulfur compounds and benzoic acid derivatives were present in notable  
229 amounts in the vent fluids. In particular, the organic sulfur compound 2,3-dihydroxypropane-1-  
230 sulfonate (DHPS) was the most abundant organic sulfur compound present. Three additional  
231 organic sulfur compounds were measured (Supplemental Table S1), but represented a total of  
232 less than 5 pM of organic matter. Measureable amounts of DHPS were found in the background  
233 seawater sample and in both hydrothermal fluids, reaching the highest values in the high  
234 temperature Bio 9 vent fluid (Table 3). DHPS was absent from the MilliQ water. The DHPS  
235 concentrations presented here substantially underestimate the actual concentration because  
236 DHPS has an extraction efficiency below 1% with our methods (Johnson et al., 2017). DHPS  
237 concentrations in these samples are likely more than 100x higher than presented, thus  
238 approaching nM levels. However, we opted not to extrapolate to concentrations in the fluids for  
239 compounds with the lowest extraction efficiencies to limit errors in quantification. Three benzoic  
240 acid derivatives (2,3-dihydroxybenzoic acid, 4-aminobenzoic acid, and 4-hydroxybenzoic acid)  
241 were also present in vent fluids, and were not detected in MilliQ water or ambient seawater  
242 (Table 3).

## 243 **4 Discussion**

### 244 **4.1 Dissolved organic carbon in hydrothermal vent fluids**

245         Hydrothermal activity at oceanic spreading centers can be generally characterized as  
246 either high-temperature (250–400 °C) focused flow or lower temperature diffuse-flow. Low  
247 temperature diffuse venting reflects subsurface mixing of high-temperature hydrothermal fluids

248 with cool geochemically unmodified seawater. In this study, we measured DOC concentrations  
249 as non-purgeable organic carbon and thus volatile organic compounds within hydrothermal vent  
250 fluids will not be included in our DOC measurements. The DOC concentration in the high  
251 temperature Bio 9 fluids was substantially less than the DOC concentration in bottom seawater  
252 that represents the source of the hydrothermal fluids. Lower DOC concentrations compared to  
253 ambient deep-sea water have previously been measured in high-temperature vent fluids (Hawkes  
254 et al., 2015; Lang et al., 2006) and ridge flank fluids (Lin et al., 2012), indicating that these  
255 systems act as sinks for DOC in the ocean. In contrast, the DOC levels in the low temperature  
256 vent fluid from Crab Spa at 9° North EPR were elevated by ~2  $\mu\text{M}$  above ambient seawater,  
257 consistent with observations at other hydrothermal systems (Brault et al., 1988; Lang et al., 2006;  
258 Lang et al., 2010). Processes known to occur in hydrothermal ecosystems that could result in  
259 elevated DOC levels include the metabolic activity of the seafloor microbial community  
260 (Butterfield et al., 2004; McNichol et al., 2016), the degradation products generated during  
261 microbial biomass heating in seafloor environments (Reeves et al., 2014), or abiotic  
262 processes such as reduction of dissolved inorganic carbon to form aqueous organic compounds  
263 (Lang et al., 2010; McDermott et al., 2015b). Regardless of its source, DOC can be consumed by  
264 the resident microbial community (Rossel et al., 2015) thereby fueling heterotrophic processes in  
265 the deep ocean (Meier et al., 2016). Consumption of hydrothermally-derived organic carbon can  
266 also be traced to higher trophic levels (Pearson et al., 2005), and we posit that the lability of  
267 DOC released at the seafloor is directly linked to its chemical composition.

## 268 **4.2 Overall composition of dissolved organic matter**

269 To obtain compositional information about organic compounds in vent fluids, we used  
270 solid phase extraction to concentrate the organic compounds and remove salt that interferes with  
271 the mass spectrometry-based methods. The extraction process biases measured concentrations  
272 due to variable extraction efficiencies for individual compounds. Using the Bond Elut PPL resin,  
273 we were able to extract 22-38% of the organic carbon in the samples from the vents we studied, a  
274 value that is generally higher than the extraction efficiencies obtained in oceanic basement fluids  
275 (LaRowe et al., 2017), but is lower than seawater extracts from the Arctic (Longnecker, 2015)  
276 and temperate coastal regions (Dittmar et al., 2008). The extraction of organic compounds from  
277 aqueous fluids sampled in hydrothermal environments, and elsewhere, is an on-going challenge  
278 in marine science. In the sections that follow we consider our organic matter compositional data  
279 obtained using solid phase extraction in the context of previous research.

280 The  $m/z$  values generated by a mass spectrometry allow consideration of the diversity of  
281 organic matter across samples and they can be converted into elemental formulas that provide  
282 compositional information about the organic matter within a sample. Within our data, the low  
283 temperature vent fluid had the highest number of  $m/z$  values while the lowest number of  $m/z$   
284 values were measured within the high temperature vent fluid. As dissolved organic matter is  
285 exposed to increasingly higher temperatures (up to 380°C), decreasing numbers of  $m/z$  values are  
286 measured and the remaining dissolved organic matter has smaller oxygen:carbon molar ratios  
287 (Hawkes et al., 2016). Our field data are consistent with the experimental results of Hawkes et al.  
288 (2016) because we observed the lowest oxygen:carbon molar ratio in the sample from the high  
289 temperature vent fluids at Bio 9. Yet, the observations average the set of  $m/z$  values for bulk

290 organic matter within our samples and obscure many of the details about the composition of  
291 organic compounds from hydrothermal ecosystems. In the sections that follow, we focus on  
292 specific groups of organic compounds that we characterized using direct infusion mass  
293 spectrometry and targeted mass spectrometry.

### 294 **4.3 Aromatic compounds at hydrothermal vents**

295 Mass spectrometry assessments of aromatic organic compounds provide an opportunity  
296 to compare the results of direct infusion mass spectrometry with the results of targeted mass  
297 spectrometry. Aromatic organic matter ranges from compounds containing a single benzene ring  
298 to condensed compounds with multiple fused benzene rings. The direct infusion data presents an  
299 overview of the number of compounds classified as aromatics or condensed aromatics, but does  
300 not provide concentration data. On the other hand, the targeted mass spectrometry approach  
301 provides concentration information for known compounds, but requires advance decisions as to  
302 which compounds will be analyzed. These are fundamentally different data sets. Here, we  
303 present an analysis of both datasets in order to emphasize the value of considering a combination  
304 of data streams rather than relying on a single assessment of the composition of dissolved  
305 organic matter.

306 For the direct infusion mass spectrometry data, we used the aromaticity index (Koch and  
307 Dittmar, 2006) to estimate how many different condensed aromatic compounds were in ambient  
308 seawater compared to the low and high temperature vent fluids. Based on this index, we found  
309 the highest number of condensed aromatic compounds within the dissolved, solid-phase  
310 extractable organic matter in ambient seawater. In contrast, while Dittmar and Koch (2006)  
311 suggested that deep-sea hydrothermal vents could be a source of increased numbers of

312 condensed aromatic compounds to the deep sea, temperatures as low as 100 °C can result in  
313 decreases in the number of  $m/z$  values (Hawkes et al., 2016), consistent with our observations of  
314 fewer types of aromatic compounds in both the high and low temperature hydrothermal fluids  
315 relative to seawater. Thus, thermal heating of organic matter in the subseafloor reduces both the  
316 concentration of organic matter (Hawkes et al., 2015) and its complexity as observed by the  
317 reduced number of aromatic compounds measured by ultrahigh resolution mass spectrometry.

318         Using the targeted mass spectrometry approach, we measured picomolar concentrations  
319 of three substituted benzoic acid compounds in the low and high temperature hydrothermal fluids  
320 while concentrations in the background seawater sample were below our detection limits (Table  
321 3). Bulk concentrations of organic carbon in our samples were in the micromolar range.  
322 Therefore, the aromatic compounds we quantify are only a small fraction of the organic  
323 compounds in vent fluids. Monocyclic aromatic hydrocarbons, including benzoic acid and its  
324 derivatives, have been previously documented in hydrothermal vent fluids (Konn et al., 2009;  
325 Simoneit et al., 1988). Although monocyclic aromatic hydrocarbons are reactive under  
326 hydrothermal conditions, the aromatic ring remains intact (McCollom et al., 2001). Rossel et al.  
327 (2015) observed a relative increase in aromatic molecular formulas during incubations with low  
328 temperature hydrothermal vent fluids, and their interpretation is that the biological community is  
329 not consuming aromatic compounds which then accumulate in the incubation. We did not  
330 observe consistent differences in the benzoic acid derivatives between the low- and high-  
331 temperature hydrothermal vent fluids, which is surprising considering the differences in how  
332 each fluid is formed. Additional research will be necessary to distinguish between microbial



333 activity and thermogenic production of benzoic acid derivatives and to constrain the short-term  
334 variability of these compounds in hydrothermal vent fluids.

#### 335 **4.4 Sulfur-containing organic compounds**

336 Hydrogen sulfide represents a substantial source of reduced sulfur within basalt-hosted  
337 hydrothermal systems. Vent fluids also contain organic sulfur compounds that can serve as an  
338 energy source in hydrothermal systems (Reeves et al., 2014; Rogers and Schulte, 2012). The  
339 organosulfur compound 2,3-dihydroxypropane-1-sulfonate (DHPS) has been observed in the  
340 surface ocean (Durham et al., 2015), but it has not previously been identified within  
341 hydrothermal fluids nor in the deep sea. In the surface ocean, the presumptive source of DHPS is  
342 biological degradation of sulfolipids followed by excretion of DHPS (Denger et al., 2014; Roy et  
343 al., 2003), although direct release of DHPS by diatoms has also been implicated (Durham et al.,  
344 2015). At present, the source of DHPS in the diffuse-flow vent fluids and seawater is not clear.  
345 However, *Sulfurimonas denitrificans*, a chemolithoautotrophic campylobacterium, has been  
346 found to produce elevated levels of DHPS in response to increased salt concentrations in its  
347 growth medium (Götz et al., 2018). *Campylobacteria*, previously classified as  
348 *Epsilonproteobacteria* (Waite et al., 2017), dominate the microbial community at Crab Spa and  
349 other diffuse-flow deep-sea vents (Huber et al., 2007; Longnecker and Reysenbach, 2001;  
350 McNichol et al., 2016), suggesting that *Campylobacteria* may be a source of DHPS at Crab Spa  
351 and possibly hydrothermal systems elsewhere. At the same time, thermal alteration of microbial  
352 biomass may release DHPS during a process that is analogous to that postulated for the  
353 production of methanethiol at hydrothermal vents (Reeves et al., 2014). However, there are  
354 substantial differences in DHPS concentrations between the January and November samples, and

355 measureable levels of DHPS within ambient seawater. The future development of a more  
356 efficient extraction protocol for DHPS will be critical because sulfur-based organic compounds  
357 such as DHPS may constitute an important carbon and energy source for the heterotrophic  
358 microorganisms found within and around hydrothermal ecosystems.

#### 359 **4.5 Vitamins and amino acids in hydrothermal fluids**

360 Low temperature diffuse flow fluids are a potential source of vitamins to the deep ocean.  
361 In general, water-soluble vitamins are thermally unstable and would not persist under extended  
362 periods of exposure to high temperatures. We suggest that the measureable quantities of vitamins  
363 in the Crab Spa fluids reflects production by microbial communities in the seafloor at levels  
364 in excess of their metabolic needs. While we are not aware of research that quantifies the  
365 production of vitamins in the deep sea, the process might be similar to the accumulation of  
366 vitamins such as B<sub>2</sub> in the surface ocean (Heal et al., 2014). In the surface ocean, many  
367 phytoplankton depend on an external supply of vitamins for growth (Croft et al., 2006), and  
368 changes in the concentration of vitamins in the surface ocean have been attributed to the  
369 extracellular release of B vitamins by picocyanobacteria (Bonnet et al., 2010). Less is known  
370 about the vitamin requirements of chemoautotrophic microorganisms, although cultured  
371 chemoautotrophs for which vitamin dependency has been tested are able to grow without  
372 externally supplied vitamins (e.g., Sievert et al., 2000; Takai et al., 2004a; Takai et al., 2004b).  
373 The B vitamins are required for enzymatic reactions and all known carbon fixation pathways  
374 (Monteverde et al., 2017), including pathways that have been described for deep-sea  
375 hydrothermal vent microorganisms (Hügler and Sievert, 2011). Given these examples, it is likely  
376 that chemoautotrophic microorganisms at deep-sea hydrothermal vents are a source of vitamins

377 to the deep sea. These vitamins could be consumed by organisms that cannot synthesize requisite  
378 vitamins and emphasizes the role that chemoautotrophic microorganisms may play in sustaining  
379 life in the deep-sea.

380         The processes responsible for the production and alteration of amino acids has long been  
381 a topic of interest at deep-sea hydrothermal vents (see review by Colín-García et al., 2016).  
382 Amino acid concentrations can vary in hydrothermal systems depending on the type of samples  
383 collected (Haberstroh and Karl, 1989) and the timing of sample collection (Klevenz et al., 2010).  
384 Our data are most comparable to measurements of dissolved free amino acids that show low  
385 concentrations or concentrations below detection in vent fluids and ridge flank basement fluids  
386 (Fuchida et al., 2014; Lin et al., 2015; McCollom et al., 2015). However, there are  
387 methodological differences that complicate direct comparisons between the data presented in this  
388 project and previous studies. While our extraction method is well suited to the analysis of  
389 aromatic amino acids including tryptophan and phenylalanine, it has lower extraction efficiencies  
390 for other amino acids (Johnson et al., 2017).

391         Amino acids represent a significant component of the organic compounds measured in  
392 vent fluids at 9° 50' N EPR. The amino acid concentrations are temporally variable at Bio 9 and  
393 Crab Spa and did not show the clear division between low temperature fluids and high  
394 temperatures fluids that was apparent in the vitamin data. This result contrasts with previous  
395 results for Mid-Atlantic Ridge fluids where Klevenz et al. (2010) measured higher dissolved free  
396 amino acid concentrations in the low temperature diffuse hydrothermal fluids compared to the  
397 high temperature fluids. The average dissolved free amino acid concentration in the samples  
398 from multiple sites on the Mid-Atlantic Ridge, including Logatchev and other sites between 4°S

399 and 9°S, was 143 nM (Klevenz et al., 2010), which is comparable to the amino acid levels we  
400 measured. In contrast, further north on the Mid-Atlantic Ridge (30°N to 37°N), amino acid  
401 concentrations were below detection in vent fluids that spanned a range of temperatures (51°C to  
402 > 360°C) and hydrothermal environments (McCollom et al., 2015). Furthermore, fluids from  
403 high temperature hydrothermal fluids from the sediment-covered Guaymas Basin also had amino  
404 acid levels below detection (Haberstroh and Karl, 1989). The challenges associated with  
405 sampling fluids at deep-sea hydrothermal vents did not allow us to collect replicate samples from  
406 each vent site and therefore we are hesitant to extrapolate past these data to consider what our  
407 data may reveal about factors controlling the composition of amino acids within vent fluids.  
408 Additional sampling campaigns will be necessary in order to fully quantify the temporal and  
409 spatial variability in the concentration of these organic compounds.

## 410 **5 Conclusions**

411 Dissolved organic carbon from deep-sea hydrothermal vents can be a source of  
412 chemically distinct compounds to the deep ocean. Here, we used a combination of direct infusion  
413 mass spectrometry and targeted mass spectrometry to expand our view into the composition of  
414 polar organic compounds that are present in deep-sea hydrothermal vent fluids. Water-soluble  
415 vitamins, amino acids, benzoic acid derivatives, and organic sulfur compounds were found to  
416 varying degrees within high and low temperature vent fluids. Yet, identifying the source of these  
417 compounds in hydrothermal systems is complicated by the interplay between biological  
418 processes and thermal heating of microbial biomass within the subsurface. The prevalence of  
419 vitamins in the low temperature vent fluid is strong evidence for an active seafloor biosphere

420 that is releasing compounds that can be used as energy, carbon, and organic nutrient sources for  
421 the natural microbial community in the vent fluids and the surrounding deep ocean.

## 422 **Acknowledgements**

423 We thank the captain and crew of the R/V *Atlantis*, the *JASON* group, and the *Alvin*  
424 group for assistance with sample collection. Winn Johnson, Bill Arnold, Cara Fiore, and Florian  
425 Götz provided assistance in the lab. Eugene Melamud and Jill McDermott provided useful  
426 computational tools and ideas. We thank Melissa C. Kido Soule for processing the mass  
427 spectrometry samples at the WHOI FT-MS Users' Facility funded by the National Science  
428 Foundation (grant OCE-0619608) and the Gordon and Betty Moore Foundation (GBMF1214).  
429 We appreciate editorial comments from the Kujawinski laboratory members on this manuscript.  
430 This project was funded by a grant from WHOI's Deep Ocean Exploration Institute and WHOI's  
431 Ocean Ridge Initiative (to EBK and SMS) and by NSF OCE-1154320 (to EBK and KL), OCE-  
432 1136727 (to SMS and JSS), and OCE 1131095 (to SMS).

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649

650 Table 1. Measured temperature, dissolved magnesium (Mg), dissolved organic carbon (DOC),  
651 and dissolved total nitrogen (TN) concentrations for water samples collected from 9°50'N, East  
652 Pacific Rise. Solid phase extraction was used to process fluids for mass spectrometry analysis  
653 and the extraction efficiency is the percent of organic carbon captured by the solid phase  
654 extraction method. 'n.a.' in the table indicates the value was not determined.

	<b>Date</b>	<b>Temperature (°C)</b>	<b>Mg (mmol/kg)</b>	<b>DOC (µM)</b>	<b>TN (µM)</b>	<b>Extraction efficiency</b>
Seawater	Jan. 2014	2 °C	53.5	44.3	43.2	31%
Crab Spa	Jan. 2014	25 °C	48.9	46.5	12.7	22 %
	Nov. 2014	24 °C	49.1	46.2	15.6	n.a.
Bio 9	Jan. 2014	366 °C	6.7	13.6	5.6	38%
	Nov. 2014	364 °C	9.4	14.2	9.4	n.a.

655 Table 2. Parameters for negative ion mode, direct infusion data collected from the January 2014  
 656 samples. Data are number of  $m/z$  values, the number of elemental ratios for all  $m/z$  values with  
 657 elemental formulas, mean molecular weight, and magnitude-averaged molar ratios calculated  
 658 following Sleighter and Hatcher (2008).

<b>Station</b>	<b># of <math>m/z</math> values</b>	<b># of elemental formulas</b>	<b>Mean molecular weight</b>	<b>H:C<sub>w</sub></b>	<b>O:C<sub>w</sub></b>
Seawater	6475	6226	458.61	1.13	0.42
Crab Spa	7363	7059	467.13	1.18	0.45
Bio 9	4644	4314	425.83	1.01	0.35

661  
 662 Table 3. Concentration of dissolved 2,3-dihydroxypropane-1-sulfonate (DHPS) and benzoic acid  
 663 derivatives in seawater, and high and low temperature hydrothermal vent fluids at 9°50'N East  
 664 Pacific Rise. The concentration data in the table have been corrected to consider the extraction  
 665 efficiency of each compound (Johnson et al., 2017), except for DHPS where uncorrected (\*)  
 666 measured data are provided. 'b.d.' are values below detection given the analytical approach.

667

Station	Date	DHPS (pM)	2,3-dihydroxybenzoic acid (pM)	4-aminobenzoic acid (pM)	4-hydroxybenzoic acid (pM)
Seawater	Jan. 2014	9.5 (*)	b.d.	b.d.	b.d.
Crab Spa	Jan. 2014	4.3 (*)	1.1	57.7	17.7
Crab Spa	Nov. 2014	17.3 (*)	2.5	4.0	52.9
Bio 9	Jan. 2014	8.7 (*)	1.3	b.d.	10.3
Bio 9	Nov. 2014	30.1 (*)	8.8	1.2	52.4

668

669

670 **Figure legends**

671 Figure 1. Percentage of elemental formulas assigned to each compositional group for seawater,  
672 Crab Spa, and Bio 9 organic matter extracts from the January 2014 samples. Only elemental  
673 formulas that represented more than 5% of the total elemental formulas are plotted.

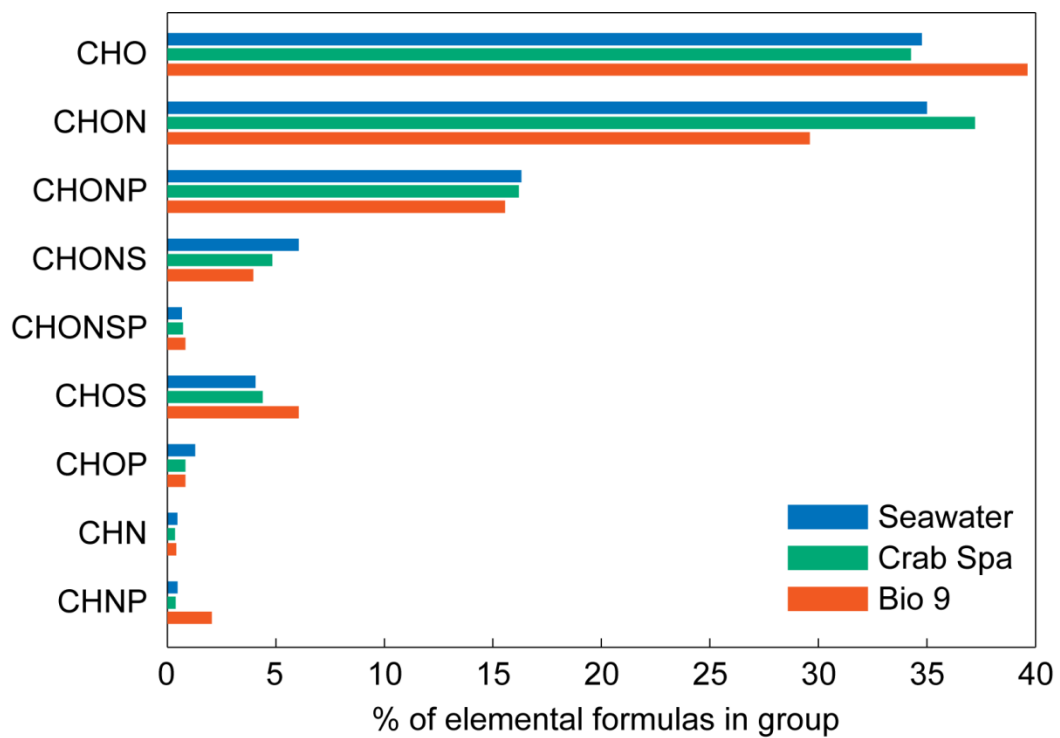
674 Figure 2. Concentrations of vitamin B<sub>2</sub>, B<sub>5</sub>, B<sub>7</sub>, and vitamin B<sub>7</sub>'s precursor, desthiobiotin in  
675 seawater and hydrothermal fluids from Crab Spa and Bio 9 vents.

676 Figure 3. Concentrations of tryptophan, phenylalanine, and leucine/isoleucine in seawater and  
677 hydrothermal fluids from Crab Spa and Bio 9 vents.. Using the targeted metabolomics method  
678 described in section 2.3, leucine and isoleucine cannot be quantified separately.

679

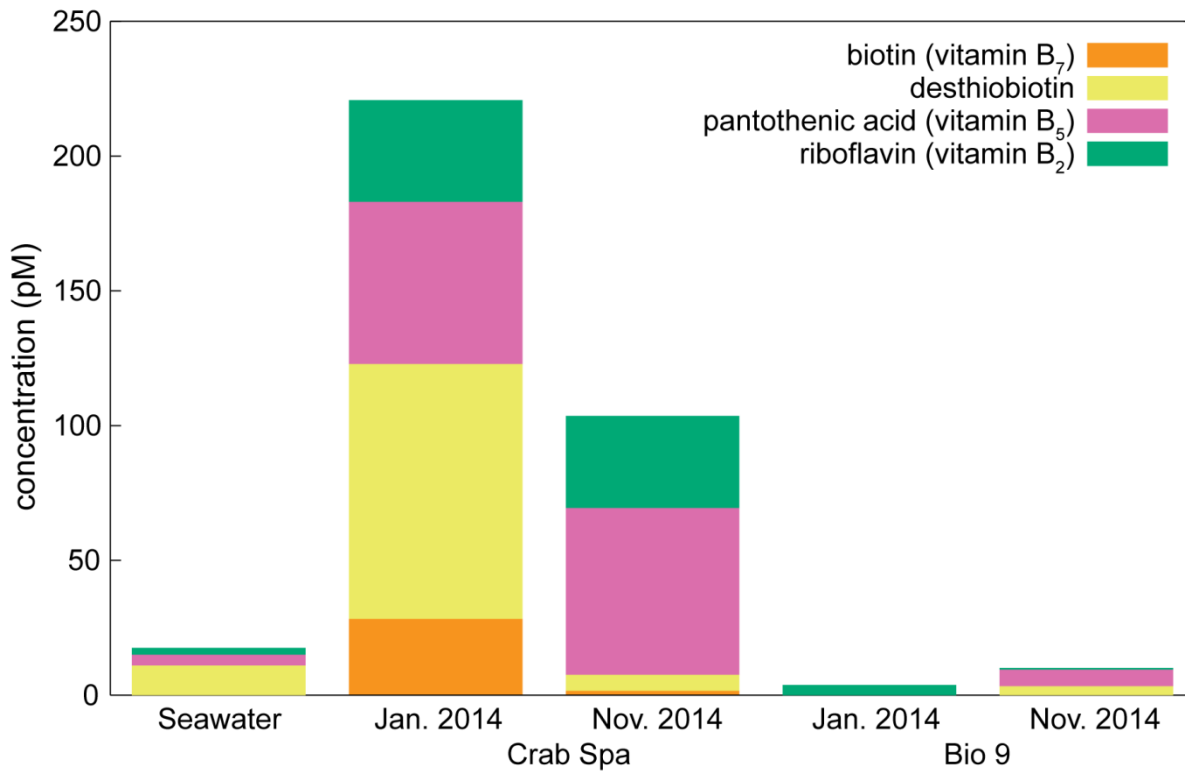


681 Figure 1

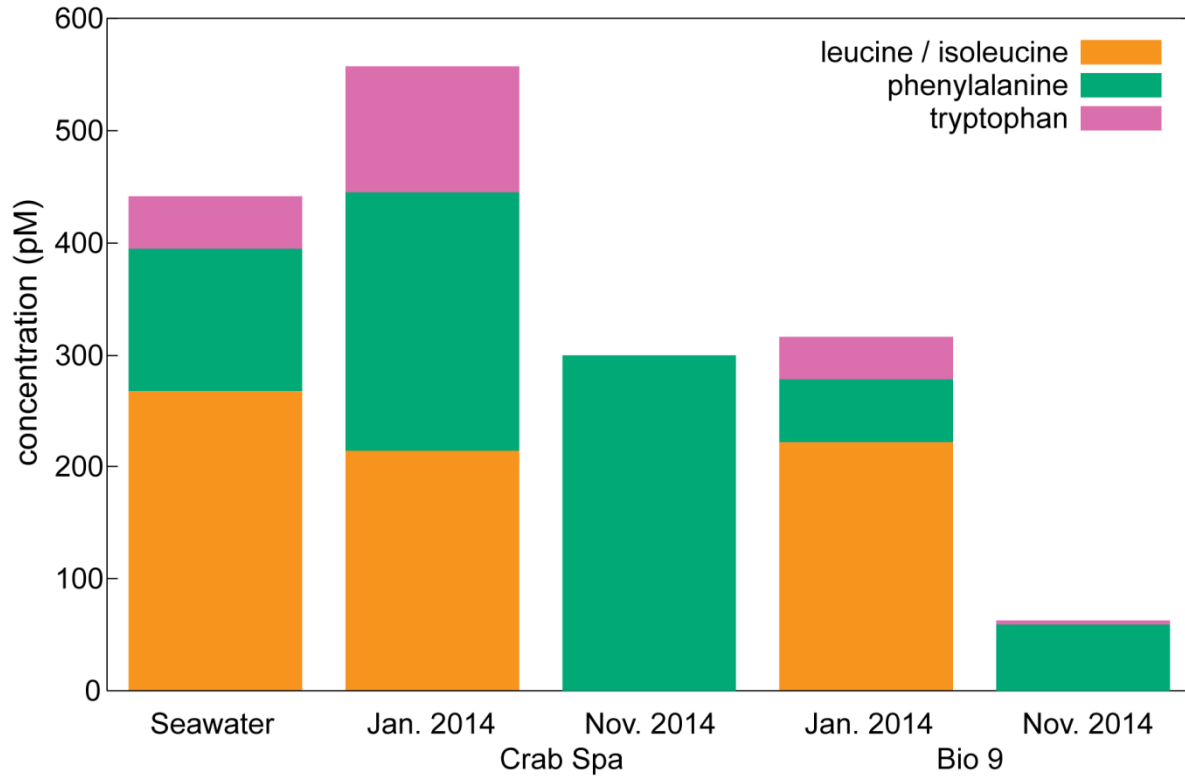


682

684 Figure 2



686 Figure 3



# **Dissolved organic carbon compounds in deep-sea hydrothermal vent fluids from the East Pacific Rise at 9°50'N**

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## Supplemental information

### Supplemental Table S1

List of metabolites detected in the extracellular fractions of dissolved organic matter. Four types of samples are given in the table: Milli-Q water, seawater, low temperature (low T) vent fluid from Crab Spa, and high temperature (high T) vent fluid from Bio 9. In each case, the fluids were filtered and the dissolved organic compounds extracted as described in the text.

Concentrations are given in picomolar, and are corrected for the extraction efficiency of each compound based on the values in Johnson et al. (2017). Compounds marked with [\*] have extraction efficiencies less than 1% and the data in the table are the measured values that have not been corrected for the extraction efficiency of the compound. If no value is given in the table, the sample was below the detection limit for the metabolite; limit of detection and limit of quantification for these compounds are provided in Johnson et al. (2017). ‡The following amino acids were below detection in this project: arginine, glutamine, proline, glutamate, cysteine, serine, and homoserine/threonine.

The fluid samples were 2 liters except for the November 2014 Bio 9 sample that was 1.4 L of fluid. Each sample included 86 ml of Milli-Q water because the titanium and Teflon tubing was filled with Milli-Q water before each deployment. We have corrected the seawater and vent fluid samples to account for the concentration of each metabolite in the Milli-Q water.

### References:

Johnson, W.M., Kido Soule, M.C., Kujawinski, E.B., 2017. Interpreting the impact of matrix on extraction efficiency and instrument response in a targeted metabolomics method. *Limnology and Oceanography Methods* 15, 417-428.

	Low T vent fluid		High T vent fluid		
	Milli-Q water	Seawater	Crab Spa	Bio 9	
	Jan. 2014	Jan. 2014	Jan. 2014	Nov. 2014	
				Jan. 2014	Nov. 2014
<i>Vitamins</i>					
biotin (vitamin B <sub>7</sub> )	1		28	2	
cyanocobalamin (vitamin B <sub>12</sub> )			0	0	
desthiobiotin (precursor to biotin)		11	95	6	3
folic acid (vitamin B <sub>9</sub> )		1	1	0	
pantothenic acid (vitamin B <sub>5</sub> )	1	4	60	62	6
riboflavin (vitamin B <sub>2</sub> )		2	38	34	4
<i>Amino acids</i> †					
leucine / isoleucine	45	268	214		222
phenylalanine	92	127	231	300	57
tryptophan	21	47	112		38
<i>Dissolved organic sulfur compounds</i>					
2,3-dihydroxypropane-1-sulfonate [*]		10	4	17	9
3-mercapto proprionate					4
5'-deoxy-5'(methylthio)adenosine	0		1	0	1
dimethylsulfoniopropionate (DMSP) [*]					1
<i>Dissolved organic phosphorus compounds</i>					
6-phosphogluconic acid [*]					3
D-glucosamine 6-phosphate [*]		4			
nicotinamide adenine dinucleotide (NAD)					1
nicotinamide adenine dinucleotide phosphate (NADP)		9			
D-glucose 6-phosphate [*]		0	2		
glyphosate			8		

			Low T vent fluid		High T vent fluid	
	Milli-Q water	Seawater	Crab Spa		Bio 9	
	Jan. 2014	Jan. 2014	Jan. 2014	Nov. 2014	Jan. 2014	Nov. 2014
<i>Nucleic acid precursors</i>						
adenosine		14	45	30	11	9
guanosine						10
inosine 5'-monophosphate [*]			1			
inosine				122		
uridine 5-monophosphate [*]			5			
xanthine [*]				1		1
xanthosine				3		3
<i>Benzoic acid derivatives</i>						
2,3-dihydroxybenzoic acid			1	3	1	9
4-aminobenzoic acid			58	4		1
4-hydroxybenzoic acid	1		18	53	10	52
<i>Other metabolites</i>						
chitotriose						16
choline [*]				7		7
indole 3-acetic acid	1		50	1		1
kynurenine						6
n-acetyl glutamic acid			237		96	
n-acetyl muramic acid				383		
sodium taurocholate			3	5		
tryptamine				5		5

**Supplemental Figure S1.** Negative ion mode spectra from DOM extracted from seawater, low temperature vent fluids from Crab Spa, and high temperature vent fluids from Bio 9.

