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

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Abstract

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

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

1 Introduction

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

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

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

2 Methods

2.1 Sample collection and processing

Hydrothermal vent fluids were collected using either the ROV *Jason* or HOV *Alvin* deployed from the R/V *Atlantis* during two cruises to the basalt-hosted deep-sea hydrothermal vent field at 9º50'North on the East Pacific Rise in January and November 2014 (AT26-10 and AT26-23). During each cruise, vent fluid samples for chemical analysis were collected from the Crab Spa and Bio 9 vents, which are both located within the axial summit caldera at 9ºNorth (see map in Fornari et al., 2012). Crab Spa is a low temperature (25°C) diffuse-flow hydrothermal vent (McNichol et al., 2018; McNichol et al., 2016) and Bio 9 is a high temperature (366°C) focused-flow hydrothermal vent. Both sites are dominated by basalt. During the January 2014 cruise, a background seawater sample was collected 20 m above the seafloor, away from the hydrothermal vent sites, for comparison with the vent fluids. All water samples were collected

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

The fluid samples were returned to the ship-board laboratory for processing within 5 – 10 h of collection with the longer 10-hour delay being typical for the January 2014 samples due to the use of ROV *Jason* which operates for longer periods of time at the seafloor. Water samples were filtered with 0.2-µm Omnipore filters (Millipore) mounted in perfluoroalkoxy (PFA) filter holders (Advantec). A 40 ml aliquot of 0.2 µm-filtered water was acidified with concentrated hydrochloric acid and stored in combusted glass vials at 4°C for measurement of DOC and dissolved total nitrogen (TN) using a Shimadzu TOC-V$_{CSH}$ total organic carbon analyzer equipped with a TNM-1 nitrogen analyzer in a shore-based laboratory. Blanks (Milli-Q water from both the ship and the shore-based laboratory) and known concentrations of potassium hydrogen phthalate and potassium nitrate were interspersed with sample runs during the analysis. 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 (25ºC) 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

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

2.3 Targeted metabolomics

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

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

3 Results

3.1 Bulk chemical parameters in hydrothermal fluids

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

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

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

3.2 Direct infusion mass spectrometry

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

Elemental formulas were calculated from the *m/z* values. The resulting formulas can be grouped based on the elements present to provide an overview of the types of organic matter within the samples. For the three samples analyzed by direct infusion in this project, the 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,

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

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

4 Discussion

4.1 Dissolved organic carbon in hydrothermal vent fluids

Hydrothermal activity at oceanic spreading centers can be generally characterized as either high-temperature (250–400 ºC) focused flow or lower temperature diffuse-flow. Low 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 9º North EPR were elevated by ~2 μM 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 subseafloor microbial community
260 (Butterfield et al., 2004; McNichol et al., 2016), the degradation products generated during
261 microbial biomass heating in subseafloor 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.

4.2 Overall composition of dissolved organic matter

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

The *m/z* values generated by a mass spectrometry allow consideration of the diversity of organic matter across samples and they can be converted into elemental formulas that provide compositional information about the organic matter within a sample. Within our data, the low temperature vent fluid had the highest number of *m/z* values while the lowest number of *m/z* values were measured within the high temperature vent fluid. As dissolved organic matter is exposed to increasingly higher temperatures (up to 380ºC), decreasing numbers of *m/z* values are measured and the remaining dissolved organic matter has smaller oxygen:carbon molar ratios (Hawkes et al., 2016). Our field data are consistent with the experimental results of Hawkes et al. (2016) because we observed the lowest oxygen:carbon molar ratio in the sample from the high 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 ºC 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

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

4.4 Sulfur-containing organic compounds

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

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

4.5 Vitamins and amino acids in hydrothermal fluids

Low temperature diffuse flow fluids are a potential source of vitamins to the deep ocean. In general, water-soluble vitamins are thermally unstable and would not persist under extended periods of exposure to high temperatures. We suggest that the measureable quantities of vitamins in the Crab Spa fluids reflects production by microbial communities in the subseafloor at levels in excess of their metabolic needs. While we are not aware of research that quantifies the production of vitamins in the deep sea, the process might be similar to the accumulation of vitamins such as B_2 in the surface ocean (Heal et al., 2014). In the surface ocean, many phytoplankton depend on an external supply of vitamins for growth (Croft et al., 2006), and changes in the concentration of vitamins in the surface ocean have been attributed to the extracellular release of B vitamins by picocyanobacteria (Bonnet et al., 2010). Less is known about the vitamin requirements of chemoautotrophic microorganisms, although cultured chemoautotrophs for which vitamin dependency has been tested are able to grow without externally supplied vitamins (e.g., Sievert et al., 2000; Takai et al., 2004a; Takai et al., 2004b). The B vitamins are required for enzymatic reactions and all known carbon fixation pathways (Monteverde et al., 2017), including pathways that have been described for deep-sea hydrothermal vent microorganisms (Hügler and Sievert, 2011). Given these examples, it is likely 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-Garcia 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 4ºS

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

5 Conclusions

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

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

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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 9º50'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.

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

659

Station	**# of *m/z* values**	**# of elemental formulas**	**Mean molecular weight**	**$H:C_w$**	**$O:C_w$**
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

660

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

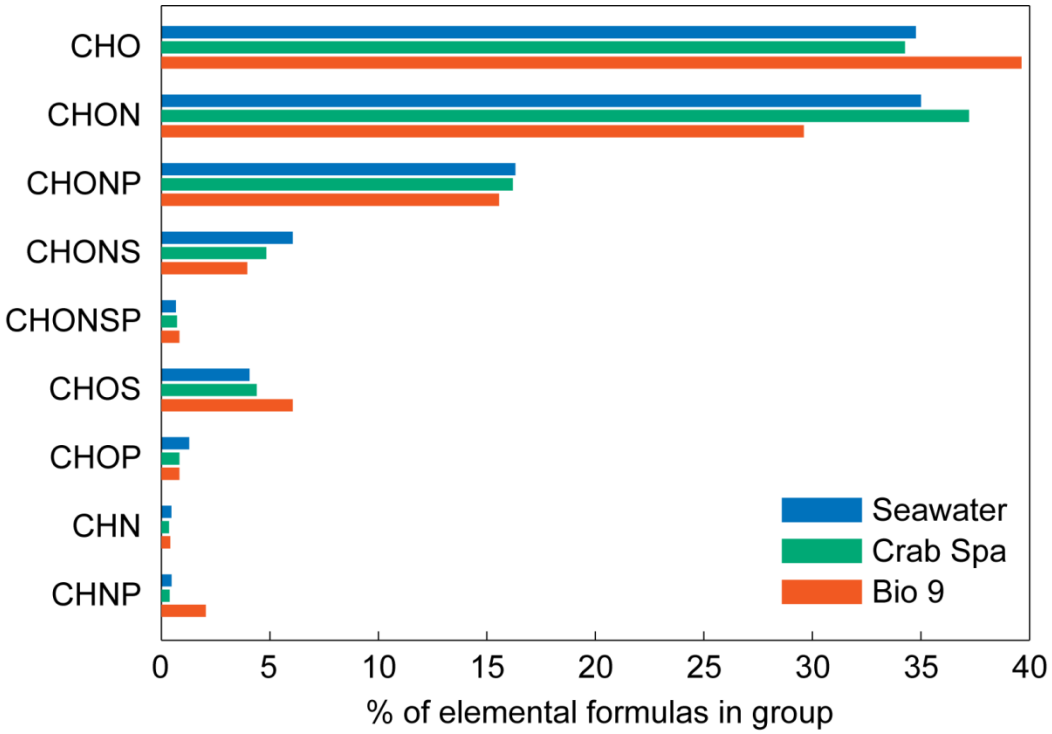
Figure legends

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

Figure 2. Concentrations of vitamin B_2, B_5, B_7, and vitamin B_7's precursor, desthiobiotin in seawater and hydrothermal fluids from Crab Spa and Bio 9 vents.

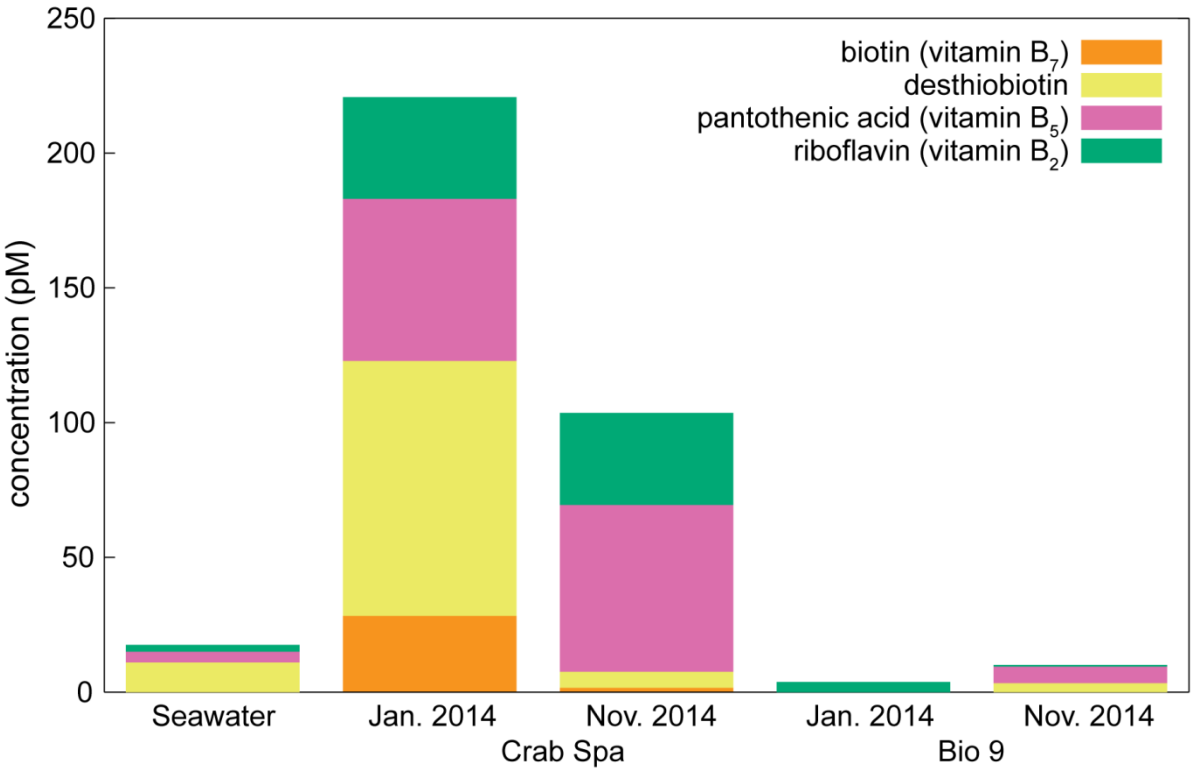
Figure 3. Concentrations of tryptophan, phenylalanine, and leucine/isoleucine in seawater and hydrothermal fluids from Crab Spa and Bio 9 vents.. Using the targeted metabolomics method described in section 2.3, leucine and isoleucine cannot be quantified separately.

681 Figure 1



682

684 Figure 2


250
200
150
100
50
0
concentration (pM)
biotin (vitamin B7)
desthiobiotin
pantothenic acid (vitamin B5)
riboflavin (vitamin B2)
Seawater
Jan. 2014
Nov. 2014
Jan. 2014
Nov. 2014
Crab Spa
Bio 9

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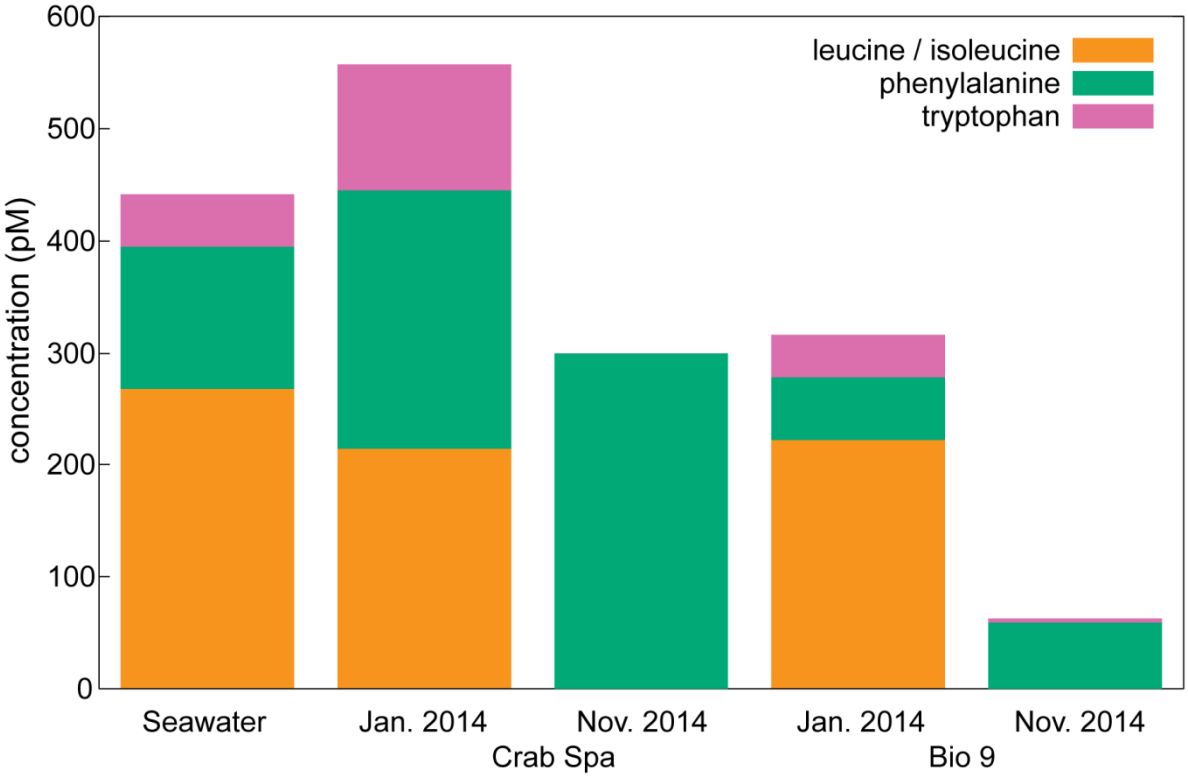
686 Figure 3



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.

	Milli-Q water Jan. 2014	Seawater Jan. 2014	Low T vent fluid Crab Spa Jan. 2014	Nov. 2014	High T vent fluid Bio 9 Jan. 2014	Nov. 2014
Vitamins						
biotin (vitamin B_7)	1		28	2		
cyanocobalamin (vitamin B_{12})			0	0		
desthiobiotin (precursor to biotin)		11	95	6		3
folic acid (vitamin B_9)		1	1	0		
pantothenic acid (vitamin B_5)	1	4	60	62		6
riboflavin (vitamin B_2)		2	38	34	4	1
Amino acids‡						
leucine / isoleucine	45	268	214		222	
phenylalanine	92	127	231	300	57	60
tryptophan	21	47	112		38	4
Dissolved organic sulfur compounds						
2,3-dihydroxypropane-1-sulfonate [*]		10	4	17	9	30
3-mercapto proprionate					4	
5'-deoxy-5'(methylthio)adenosine	0		1	0	1	
dimethylsulfoniopropionate (DMSP) [*]					1	
Dissolved organic phosphorus compounds						
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			

	Milli-Q water Jan. 2014	Seawater Jan. 2014	Low T vent fluid Crab Spa Jan. 2014	Nov. 2014	High T vent fluid Bio 9 Jan. 2014	Nov. 2014
Nucleic acid precursors						
adenosine		14	45	30	11	9
guanosine						10
inosine 5'-monophosphate [*]			1			
inosine				122		
uridine 5-monophosphate [*]			5			
xanthine [*]				1		1
xanthosine				3		3
Benzoic acid derivatives						
2,3-dihydroxybenzoic acid			1	3	1	9
4-aminobenzoic acid			58	4		1
4-hydroxybenzoic acid	1		18	53	10	52
Other metabolites						
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.