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Characterizing the distribution and rates of microbial sulfate reduction at Middle Valley hydrothermal vents

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Running Title: Sulfate reduction rates at hydrothermal vents

32 **ABSTRACT**

33 Few studies have directly measured sulfate reduction at hydrothermal vents, and
34 relatively little is known about how environmental or ecological factors influence rates of
35 sulfate reduction in vent environments. A better understanding of microbially mediated sulfate
36 reduction in hydrothermal vent ecosystems may be achieved by integrating ecological and
37 geochemical data with metabolic rate measurements. Here we present rates of microbially
38 mediated sulfate reduction from three distinct hydrothermal vents in the Middle Valley vent
39 field along the Juan de Fuca Ridge, as well as assessments of bacterial and archaeal diversity,
40 estimates of total biomass and the abundance of functional genes related to sulfate reduction,
41 and *in situ* geochemistry. Maximum rates of sulfate reduction occurred at 90°C in all three
42 deposits. Pyrosequencing and functional gene abundance data reveal differences in both
43 biomass and community composition among sites, including differences in the abundance of
44 known sulfate reducing bacteria. The abundance of sequences for *Thermodesulfovibro*-like
45 organisms and higher sulfate reduction rates at elevated temperatures, suggests that
46 *Thermodesulfovibro*-like organisms may play a role in sulfate reduction in warmer
47 environments. The rates of sulfate reduction presented here suggest that - within anaerobic
48 niches of hydrothermal deposits - heterotrophic sulfate reduction may be quite common and
49 can contribute to secondary productivity, underscoring the potential role of this process in both
50 sulfur and carbon cycling at vents.

51 **Keywords:** Hydrothermal Vent/Microbial Ecology/Primary productivity/Sulfate Reduction
52 **Subject Category:** Geomicrobiology and microbial contributions to geochemical cycles

53

54 **INTRODUCTION**

55 Deep sea hydrothermal vent ecosystems are complex dynamic habitats characterized by
56 steep gradients in temperature and geochemistry (Jannasch & Mottl, 1985). In these habitats,
57 as hot hydrothermal fluid mixes with cold seawater, the precipitation of minerals creates large
58 and complex hydrothermal chimney deposits. Within these permeable mineral structures, the
59 continued mixing of chemically reduced, vent-derived fluids and oxidized seawater provides
60 favorable conditions that support the growth of endolithic microbial communities (Schrenk *et*
61 *al.* 2003)

62 Sulfide oxidation is considered to be one of the most important microbial
63 chemosynthetic pathways at ridge ecosystems, as evidenced by the ubiquity of sulfide oxidizing
64 *Epsilon-* and *Gammaproteobacteria* at ridge environments (Nakagawa *et al*. 2004; Huber *et al*.
65 2007; Nakagawa & Takai, 2008; Nakagawa *et al*. 2005; Campbell *et al.* 2006). To date,
66 significantly less attention has been paid to the distribution and magnitude of sulfate reduction
67 at vents, though sulfate reducing bacteria and archaea have frequently been isolated from deep
68 sea hydrothermal environments (Houghton *et al*. 2007; Audiffrin *et al*. 2003; Alazard *et al*.
69 2003; Jannasch *et al*. 1988; Blöchl *et al*. 1997). Moreover, analyses of functional genes that
70 express key proteins required for sulfate reduction suggest there is a high diversity of sulfate
71 reducing organisms at vents, higher than predicted via 16S rRNA gene analyses alone
72 (Nakagawa *et al*. 2004; Nercessian *et al*. 2005).

73 From a biogeochemical and bioenergetic perspective, both sulfide oxidation and sulfate
74 reduction would be favored at hydrothermal vents, though to varying degrees as a function of
75 environmental chemistry. Sulfide oxidation is most favorable when coupled to oxygen or nitrate

76 as an electron acceptor (Amend & Shock 2001). Around vents, sulfide is typically in µM to mM
77 concentrations (Butterfield, et al. 1994; Butterfield et al. 1994), while oxygen and nitrate are
78 around 110 and 40 µM respectively (Johnson *et al*. 1986). In contrast, sulfate reduction is highly
79 favored in anoxic niches at vents, as it is in other marine anaerobic environments (Muyzer &
80 Stams, 2008). Here, as in most marine systems, sulfate is abundant at 28 mM two to three
81 orders of magnitude higher than oxygen At vents, sulfate reduction would occur in regimes
82 where seawater-derived sulfate is still present but oxygen is absent, e.g. within hydrothermal
83 vent deposits. Sulfate reducing microorganisms commonly use hydrogen and/or dissolved
84 organic matter as electron donors, both of which are found within hydrothermal fluids (Lang *et*
85 *al*. 2006; Cruse & Seewald, 2006). Sulfate reduction –as a function of its extent and magnitude-
86 could readily influence the cycling of sulfur and sulfur isotopes, as well as carbon, within
87 hydrothermal environments.

88 To date, studies have quantified rates of sulfate reduction in hydrothermal-influenced
89 sediments (Weber & Jorgensen, 2002; Jorgensen *et al*. 1992; Elsgaard *et al.* 1994; Kallmeyer &
90 Boetius, 2004; Elsgaard *et al*. 1994; Elsgaard *et al*. 1995) and isolated vent microorganisms
91 (Hoek *et al*. 2003). In contrast to the numerous studies of sulfate reduction in marine sediments
92 (Canfield 1989), studies of sulfate reduction in hydrothermal deposits are few (Bonch-
93 Osmolovskaya *et al*. 2011), due in part to the challenges associated with sampling and studying
94 the heterogeneous and consolidated sulfide deposits typical of hydrothermal vent chimneys.

95 Here we present rates of microbially mediated sulfate reduction from three distinct,
96 active hydrothermal “chimneys” found in the Middle Valley field along the Juan de Fuca Ridge,
97 as well as assessments of bacterial and archaeal diversity, estimates of total biomass and the

98 abundance of functional genes related to sulfate reduction, and *in situ* geochemistry. These
99 analyses further our understanding of sulfate reduction (including rates, diversity and
100 distribution of known sulfate-reducing microbes) in vent ecosystems. Moreover, they
101 underscore the potential role of heterotrophic sulfate reduction in hydrothermal systems, and
102 constrain their potential influence on both sulfur and carbon cycling,
103
104 **METHODS**
105
106 **Geologic Setting and Sampling of hydrothermal deposits**
107 Middle Valley (48°27'N, 128°59' W) is an intermediate spreading, axial rift valley,
108 located along the Endeavor Segment of the Juan de Fuca Ridge in the Northwest Pacific ocean.
109 Layers of continental-derived sediments characteristically cover Middle Valley, though the
110 hydrothermal vents remain prominent above the sediments. Hydrothermal deposits were
111 collected from 3 active hydrothermal spires during dive 4625 with the *HOV Alvin* (*R/V Atlantis*
112 expedition AT15-67, July 2010) and brought to the surface in a sealed, temperature-insulated
113 polyethylene box. Samples were recovered from actively venting sulfide deposits at Needles
114 (48.45778, -128.709, 2412.212 m, T_{max}=123°C), Dead Dog (48.45603,-128.71, 2405.268 m,
115 T_{max}=261°C), and Chowder Hill (48.455543, -128.709, 2398.257 m, T_{max} =261°C) vents. Once on
116 board ship, samples were directly transferred to sterile anaerobic seawater and
117 handled/processed using appropriate sterile microbiological techniques. Subsamples were
118 immediately transferred to gastight jars (Freund Container Inc.), filled with sterile anaerobic
119 seawater containing 2 mM sodium sulfide at pH 6, and stored at 4°C. Upon return to the
120 laboratory, all samples were provided with fresh 2 mM sulfidic, anaerobic seawater every 8 to
121 12 weeks and were kept in the dark and 4°C prior to incubation.

122 **Vent fluid volatile geochemistry via *in situ* mass spectrometry**

123 *In situ* concentration of dissolved volatiles (H_2S, H_2, CO_2, O_2, and others.) were measured
124 at each site with an *in situ* mass spectrometer (ISMS) as previously described (Wankel *et al*.
125 2011). Briefly, dissolved volatiles were quantified *in situ* by sampling vent effluent for up to 10
126 minutes, until partial pressures reached steady state (data was monitored in real time within
127 the submersible). Concentrations were determined from empirically derived calibrations and
128 validated by comparison with discrete samples collected using titanium gastight samplers.

129 **Measuring sulfate reduction rates**

130 Hydrothermal deposits were homogenized in a commercial blender (Xtreme™ blender,
131 Waring Inc.) under a nitrogen atmosphere. Anaerobic homogenization was designed to
132 minimize fine-scale geochemical and microbial heterogeneity and facilitate more accurate
133 experimental replication. Hydrothermal homogenate (made up of both mineral deposit and
134 interstitial fluid)) was aliquoted volumetrically (7.5 mL, ca. 29 g wet weight and ca. 20 g dry
135 weight) into Balch tubes in an anaerobic chamber. The tubes were supplemented with 15 mL of
136 sterile artificial vent fluid media designed to mimic the geochemical conditions within a sulfide
137 deposit (pH 6, 14 mM SO_4^{2-}, 2.3 mM $NaHCO_3$, 1 mM H_2S, and 10 µM each of pyruvate, citrate,
138 formate, acetate, lactate). Organic acid concentrations are comparable to those measured *in*
139 *situ* (Lang *et al*. 2006). Sufficient $^{35}SO_4^{2-}$ was added to achieve 555 kBq (15 µCi) of activity. Due
140 to technical difficulties with post processing methodology, shipboard incubations using fresh
141 material were not successful. The data presented here were generated using samples that had
142 been kept at 4 °C and refreshed with vent-like effluent (as described above) for one year.
143 Samples were incubated anaerobically for 7 days at 4, 30, 40, 50, 60, 80 and 90°C. Controls for

144 sulfate reduction consisted of samples amended with 28 mM molybdate, a competitive
145 inhibitor of sulfate reduction (Saleh *et al.* 1964; Newport & Nedwell, 1988). Six biological
146 replicates were run for each treatment, and three biological replicates for each control. Upon
147 completion, reactions were quenched with the injection of 5 mL 25% zinc acetate (which is ~20-
148 fold more Zinc than the maximum sulfide concentration), and all samples were frozen at -20° C
149 for further analysis.

150 To determine sulfate reduction rates, samples were thawed and the supernatant was
151 removed and filtered through a 0.2 µm syringe filter. The crushed deposits that remained in the
152 tube were washed three times with deionized water to remove any remaining sulfate. One
153 gram (wet weight) of crushed deposit was analyzed via chromium distillation (see Supplemental
154 Methods) and sulfate reduction rates (SRR) were calculated as in (Fossing & Jorgensen,
155 1989)using the following calculation.

156 $$SRR = \frac{nSO_4^{2-} \cdot a \cdot 1.06}{(a+A) \cdot t} \quad \text{Eq.1}$$

157 Where nSO_4^{2-} is the quantity (in moles) of sulfate added to each incubation (14 mM * 15 mL =
158 210 µmol), *a* is the activity (dpm) of the trapped sulfide, 1.06 is the fractionation factor
159 between the sulfide and sulfate pools, *A* is the activity of the sulfate pool at the completion of
160 the incubation and t is the incubation time (days). The rates are presented in units of nmol S g^{-1}
161 day^{-1}.

162 **DNA Extraction**

163 Immediately prior to conducting the rate experiments, a subsample of homogenized
164 hydrothermal deposit was removed and frozen at -80° C for molecular analysis. DNA was

165 extracted from this crushed deposit sample with a protocol modified from (Santelli *et al*. 2008).
166 Subsamples were washed with 0.1 N HCl, followed by two rinses with a sterile solution
167 containing 10 mM Tris (pH 8.0) and 50 mM EDTA. A known mass of material was added to
168 PowerSoil beadbeating tubes(MoBio Laboratories, Carlsbad CA), incubated at 70°C for 10
169 minutes, and then amended with 200 ng of poly-A. Subsamples were subjected to beadbeating,
170 followed by three cycles of freeze-thaw steps to further lyse cells. Nucleic acids were extracted
171 using hot phenol (60°C for 3 min.), followed by two chloroform:isoamyl separations and
172 precipitated with ethanol. DNA was resuspended in TE (pH 8.0) and quantified using the Qubit™
173 fluorometer (Life Technologies, Grand Island, NY).

174 **Enumeration of gene abundance via quantitative PCR**

175 Quantitative PCR (qPCR) was used to determine the abundance of bacterial and archaeal
176 16S rRNA genes. In addition, qPCR was used to enumerate the abundance of sulfate reducing
177 prokaryotes by amplifying the adenosine 5'-phosphosulfate reductase (*aprA*) gene with primers
178 targeting sulfate reducing bacteria and archaea (Christophersen et al. 2011). Primers specific to
179 bacterial dissimilatory sulfite reductase (*dsrA*) (Kondo et al. 2004) and *Deltaproteobacteria* 16S
180 rRNA genes (Stults et al. 2001) provide alternate estimates of sulfate reducing bacteria
181 populations. Quantification was performed in triplicate with the Stratagene MX3005p qPCR
182 System (Agilent Technologies) using the Perfecta SYBR FastMix with low ROX (20 µL reactions,
183 Quanta Biosciences, Gaitherburg, MD), specific primers and annealing temperatures (Table 1)
184 and 10 ng of template gDNA. The temperature program for all assays was 94°C for 10 minutes,
185 35 cycles of 94°C for 1 minute, the annealing temperature for 1 minute (Table 1), extension at
186 72°C for 30 seconds and fluorescence read after 10 seconds at 80°C. Following amplification,

187 dissociation curves were determined across a temperature range of 55°C to 95°C. Ct values for
188 each well were calculated using the manufacturer's software. Plasmids containing bacterial and
189 archaeal 16S rRNA and functional gene inserts (amplified from *Arcobacter nitrofigulis* (ATCC
190 33309), *Methanosarcina acidovorans* and *Desulfovibrio vulgaris* Hildenborough (ATCC 29579/
191 NCIMB 8303/ AE017285) respectively) were used as standards for calibration (see
192 Supplemental Methods for more detail).

193 **Sequencing and Phylogenetic Analysis via 454 pyrosequencing**

194 DNA samples were sequenced by Research and Testing Laboratory (Lubbock, TX) using 454
195 pyrotag methods similar to those described previously (Dowd *et al*. 2008). All samples were
196 sequenced using a 454FLX instrument (Roche Inc.) with Titanium™ reagents. The resulting
197 bacterial and archaeal 16S rRNA and *drsB* genes (primers in Table 1) datasets were analyzed via
198 Mothur (Schloss *et al*. 2009). Sequences were trimmed, quality checked, aligned to the SILVA-
199 compatible alignment database reference alignment (*dsrB* gene datasets were aligned to a *dsrB*
200 gene database generated from the Ribosomal Database Project (RDP)), analyzed for chimeras,
201 classified against the Greengenes99 database and clustered in to OTUs (see Supplemental
202 Methods for more detail). Rarefaction curves were used to examine the number of OTUs as a
203 function of sampling depth. Alpha diversity was assessed by generating values from the Chao1
204 richness estimators and the inverse Simpson diversity index.

205 **Sequence Accession numbers**

206 The 16S rRNA and *dsrB* gene sequences reported in this study have been submitted to
207 Sequence Read Archive under the accession numbers SRX154520 through SRX154528.

208

209 **RESULTS**

210

211 ***Physical and geochemical characteristics of the study sites***

212 The hydrothermal deposits sampled from Middle Valley were all relatively friable and

213 were composed predominantly of anhydrite ($CaSO_4$, M. Tivey, pers. comm). Chowder Hill and

214 Dead Dog had the highest observed venting fluid temperatures (measured *in situ* at 261°C),

215 followed by Needles (123°C). *In situ* measurements of dissolved hydrogen sulfide (H_2S) revealed

216 significant differences in hydrothermal fluid composition among hydrothermal deposits.

217 Unfortunately the inline pH probe with the ISMS malfunctioned during the dive. Using

218 previously reported pH values (Butterfield *et al*. 1994), Chowder Hill would have the highest *in*

219 *situ* measurement of total sulfide (3.9 mM), followed by Dead Dog (2.2 mM), and Needles (0.59

220 mM) (Table 2). These concentrations are within the same magnitude of previously reported H_2S

221 in focused vent fluids at Middle Valley (Butterfield *et al*. 1994). Chowder Hill did exhibit the

222 highest *in situ* concentration of hydrogen (1.86 mM) followed by Dead Dog (1.66 mM) and

223 Needles (~1.42 mM). These values are also consistent with previous studies (Cruse & Seewald

224 2006), as well as gastight samples collected and analyzed shipboard (M. Lilley, pers. comm).

225 ***Sulfate Reduction Rates***

226 Among all samples, sulfate reduction was observed at temperatures between 4°C to

227 90°C (Figure 1). Maximal rates of sulfate reduction were observed between 88-90°C (2670 nmol

228 g^{-1} day^{-1} at Needles, 1090 nmol g^{-1} day^{-1} at Chowder Hill, and 142 nmol g^{-1} day^{-1} at Dead Dog;

229 Figure 1). Notably, the highest sulfate reduction rates were observed from Needles samples,

230 which were ~20-fold higher than those observed at Dead Dog, and ~2-fold greater than at

231 Chowder Hill. Many of the rates exhibit large deviations due to the high variability among the

232 biological replicates, most likely due to persistent mineralogical and microbiological

233 heterogeneity across incubations, even after homogenization. Sulfate reduction was also
234 observed in molybdate amended experiments, though we suspect that molybdate was
235 scavenged by minerals that attenuated the effect of the inhibitor as has been previously
236 observed in metal-rich environments (Bostick *et al*. 2003; Xu *et al*. 2006).

237 ***Quantification of Taxonomic and Functional Genes***

238 The abundance of total bacteria, archaea (16S rRNA genes), sulfate reducing
239 prokaryotes (*aprA* gene*)* and sulfate reducing bacteria (*dsrA* and *Deltaproteobacteria* specific
240 16S rRNA genes) were investigated in each deposit by quantitative PCR (Figure 2). Microbial
241 density (as estimated by 16S rRNA gene copies g^{-1} mineral) was greatest at Needles and lowest
242 at Dead Dog. Microbial communities at each site were dominated by archaea (Figure 2A), with
243 Needles showing the highest ratio of archaea to bacteria (227:1 as compared to 14:1 at Dead
244 Dog or 17.5:1 at Chowder Hill). Assuming an average of 4.19 copies of 16S rRNA gene per
245 bacterium and 1.71 copies of 16S rRNA gene per archaeon genome (Lee *et al*. 2009;
246 Klappenbach *et al*. 2001), Needles hosts a microbial community of 4.12×10^8 cells g^{-1} sample, 3
247 orders of magnitude higher than Chowder Hill (8.96×10^5 cells g^{-1} sample) and Dead Dog ($5.65 \times$
248 10^5 cells g^{-1} sample).

249 16S rRNA gene primers specifically targeting ribotypes allied to *Desulfovibrio,*
250 *Desulfomicrobium, Desulfuromusa*, and *Desulfuromonas* were used to enumerate
251 *Deltaproteobacteria* known to mediate sulfate reduction in many marine systems (Stults *et al.*
252 2001). However, given the difficulty in amplifying 16S rRNA genes from deep-sea thermophiles
253 with typical primer sets - due to mismatches with limited sequence representation in GenBank -
254 it is possible that these assays similarly underestimate abundances in these environments

255 (Teske & Sorensen, 2008). *Deltaproteobacterial* abundance at Needles was approximately 4.48
256 x 10^5 copies g^{-1} sample (approximately 26% of the entire bacterial population), though none
257 were detected at Chowder Hill or Dead Dog (data not shown). The abundance of both
258 functional genes for sulfate reduction, *dsrA* and *aprA*, was greatest at Needles and lowest at
259 Dead Dog (a pattern similar to that seen in the 16S rRNA gene abundance estimates; Figure 2B).
260 If we assume an average of 1 *dsrA* gene copy per genome (Klein *et al*. 2001; Kondo *et al*. 2004),
261 the proportion of sulfate reducing bacteria in the bacterial populations is only 2.7% in Needles
262 as compared with 28% in Dead Dog and 53% at Chowder Hill.

263 ***Microbial Diversity***

264 454 pyrotag sequencing (bacterial V1-V3 and archaeal V3-V4 of the 16S rRNA gene),
265 rarefaction analyses, and diversity metrics all revealed measureable differences in microbial
266 community composition among the three hydrothermal deposits (Figure 3, Figure 4, Table 3).
267 Via these assessments, Needles hosts the least diverse assemblage of bacteria and archaea,
268 while Chowder Hill and Dead Dog host communities of comparable diversity. Examination of
269 OTUs at 97%, 95% and 92% sequence similarity further reveal differences in microbial
270 community membership among the three sites. Among archaea at the 97% level, only two
271 archaeal OTUs (1% of all archaeal OTUs classified) are shared among the hydrothermal
272 deposits. The sequences classified to these OTUs represent 69%, 48% and 18% of all the library
273 sequences from Needles, Dead Dog and Chowder Hill respectively. One of these OTUs is allied
274 to the ammonium oxidizing archaeal Candidatus *Cenarchaeum* in the phylum *Thaumarchaeota*,
275 and accounts for 35% of Needles and less than 5.0% of Dead Dog or Chowder Hill library
276 sequences. The other OTU is allied to a thermophilic sulfur respiring archaeon within the class

277 *Thermoplasmata*. Nearly 40% of the archaeal sequences from Dead Dog were allied to this
278 archaeon. Methanogens allied to *Methanocaldococus* comprised about 1.0% of the total
279 archaeal sequences from Dead Dog, and were not represented in the libraries from Chowder
280 Hill or Needles. Most of the archaeal diversity at Chowder Hill (80% of sequences) and Dead
281 Dog (50% of sequences) was unclassified. No sequences allied to true sulfate reducing archaeal
282 lineages such as *Archaeoglobus fugilis or Aciduliprofundus boonei* were recovered. However,
283 the potential diversity of thermophilic sulfate reducing archaea in these samples is likely much
284 greater than suggested here. This may be explained in part by biases underlying DNA
285 extractions, primer binding and sequencing. For example the archaeal sequencing primers used
286 in this study only target about one third (34%, as assayed by Probe Match; Cole et al. 2005) of
287 the *Archaeoglous*-like sequences contained in the RDP database. Furthermore, the primers may
288 miss members of the dominant *Thermoplasmatales* as *in silico* analysis only returns 48%
289 (1715/3558 sequences) of the RDP reported sequences. In total, these archaeal sequencing
290 primers (349F-806R) miss 42% of the total archaeal sequences (67713/117373 sequences) in
291 the RDP database. Similar bias has been reported in other studies in the deep sea and deep
292 subsurface biotopes (e.g. Dhillion et al., 2003, 2005, Teske et al. 2007).

293 Among bacteria at the 97% similarity level, 54 of the bacterial OTUs classified (7.0%)
294 were shared among all hydrothermal deposits, and account for 84%, 80%, 71% of the
295 sequences from Chowder Hill, Needles and Dead Dog respectively (Figure4). One of these OTUs
296 accounted for 44%, 36% and 25% of the sequences from Chowder Hill, Dead Dog and Needles
297 respectively. Aligning representative sequences from this OTU via Blast-n (Altschul *et al.* 1997)
298 reveals a best match to *Thermodesulfovibrio*, an anaerobic, thermophilic, sulfate-reducing

299 bacterium, from the phylum *Nitrospira* (81% identity). Given its abundance, we postulate that it

300 likely contributes substantially to the high thermophilic sulfate reduction rates. Furthermore,

301 the *dsrB* gene library was dominated by sequences phylogenetically allied to

302 *Thermodesulfovibrio* (Supplemental Table S1). Other dominant groups of bacteria include

303 members of the *Gammaproteobacteria, Bacteroidetes, Deltaproteobacteria* and *α-*

304 *proteobacteria*. Via Blast-n, most of the unclassified sequences matched to partial 16S rRNA

305 gene sequences from hydrothermal vent fluid communities (Nunoura *et al*. 2010; Sylvan *et al*.

306 2012). Sequences classified as *Deltaproteobacteria* comprised 5.5%, 8.2%, or 14%, of the total

307 population of Chowder Hill, Needles, or Dead Dog respectively. While Dead Dog may have had

308 the highest proportion of its amplicons classified as *Deltaproteobacteria*, sequences related to

309 known sulfate reducers within the *Deltaproteobacteria (Desulfobacteraceae, Desulfobulbus*

310 *rhabdoformis, Desulfovibrio)* were only found at Needles and comprised 1.1% of the 16S rRNA

311 gene library. The majority of the sequences classified as *Deltaproteobacteria* in each of the

312 three sites were from one unclassified *Deltaproteobacterial* OTU consisting of 4.4, 5.3, and 13%

313 of the sequences from Chowder Hill, Needles and Dead Dog respectively.

314 **DISCUSSION**

315 Sulfate reduction rates measured in deposits recovered from the Middle Valley vent

316 field reveal the potential for active sulfate reduction within hydrothermal deposits. The

317 magnitude of all measured rates (from 15.7 nmol g^{-1} day^{-1} at Dead Dog at 60°C to 2670 nmol g^{-1}

318 day^{-1} at Needles at 90°C) under experimental conditions were markedly higher than those

319 typically observed in non hydrothermal deep sea sediments (0.1-10 nmol g^{-1} day^{-1}, converted

320 here for comparison assuming an average sediment density of 2 g cm^{-3}; Elsgaard, Isaksen, *et al*.

321 1994; Weber & Jorgensen, 2002; Joye *et al*. 2004). These rates are comparable in magnitude to
322 those previously observed in hydrothermally influenced sediments (eg. Guaymas basin or Lake
323 Tanganyika (Weber & Jorgensen, 2002; Kallmeyer & Boetius, 2004; Elsgaard, Isaksen, *et al*.
324 1994; Elsgaard, Prieur, *et al*. 1994), even though the availability of organic carbon is markedly
325 higher in these hydrothermal vent sediments, with Guaymas having up to 200-times greater
326 concentrations of organic carbon (Lang *et al*. 2006; Cruse & Seewald, 2006; Chen *et al*. 1993).
327 To date, the only other measurement of sulfate reduction from sulfide deposits along the East
328 Pacific Rise exhibited rates comparable to those reported here, (Bonch-Osmolovskaya *et al*.
329 2011), but it should be noted that their samples were incubated under pure H_2 atmosphere.

330 Notably, the maximum rates of sulfate reduction in Middle Valley sulfides occurred at
331 90°C in all three deposits. This is in contrast to measurements of sulfate reduction in
332 hydrothermal sediments, where the greatest rates are often observed between 40-70°C, and
333 more modest rates of sulfate reduction have been reported between 80-91°C (Weber &
334 Jorgensen, 2002; Elsgaard, Prieur, *et al*. 1994; Elsgaard, Isaksen, *et al*. 1994). The relatively low
335 or insignificant sulfate reduction rates between 4-80°C suggest Middle Valley deposits harbor a
336 high proportion of hyperthermophilic sulfate-reducing microbes.

337 The significant differences in the rates we observed among deposits (Kruskal-Wallis,
338 $p<0.0001$) are likely due to differences in biomass and the composition of microbial
339 communities that are influenced by the geochemistry of each deposit. Indeed, microbial
340 biomass (as estimated by 16S rRNA genes) directly correlates to rates of activity and is likely
341 one of the strongest factors affecting the observed rates of sulfate reduction (Pearson
342 correlation coefficient $r=0.879$, $p<0.0005$). Needles had both the highest observed rates as well

343 as the highest cell density (Figure 2 and 3). Of all the deposits sampled, Needles had the lowest
344 venting fluid temperature (123°C) resulting in the largest zone of microbial habitability.
345 Consistent with this, Needles also had the greatest abundance of *dsrA* and *aprA* genes per
346 gram, suggesting a larger potential sulfate reducing community. Here, *Deltaproteobacteria*
347 allied to *Desulfovibrio, Desulfobulbus, Desulfobacteria*, and *Desulfuromonas* account for 25.7%
348 of the bacterial community. These clades of *Deltaproteobacteria* were not observed at
349 Chowder Hill or Dead Dog by either qPCR enumeration or pyrosequencing. Cultured
350 representatives from some of these *Deltaproteobacterial* clades (*Desulfovibrio vulgaris* and
351 *Desulfovibrio desulfuricans)* have been shown to reduce sulfate at high rates (ranging from 10-
352 1340 nmol min^{-1} mg^{-1} protein) with varying electron donors (Fitz & Cypionka, 1991; Cypionka &
353 Konstanz, 1989).

354 *Thermodesulfovibrio*-like organisms dominated the bacterial communities within each
355 hydrothermal deposit (35-44%; Fig. 4). *Thermodesulfovibrio* sp. are considered obligately
356 anaerobic, thermophilic bacteria that can reduce sulfate and other sulfur compounds (Garrity &
357 Holt, 2001). In pure cultures, members of this genus are able to link growth with hydrogen and
358 a limited range of organic carbon molecules (formate, pyruvate and lactate), maintaining
359 optimal growth between 55-70°C (Sekiguchi *et al*., 2008). Needles has a greater proportion of
360 sequences (from pyrosequencing) related to *Thermodesulfovibrio-like* species than the other
361 two deposits. The combination of many sequences related to a thermophilic sulfate reducing
362 bacteria and higher rates of sulfate reduction at elevated temperatures and the, suggests that
363 *Thermodesulfovibrio*-like organisms may play a role in sulfate reduction in warmer
364 environments. However, constraining the relative proportion of sulfate reduction by

365 *Thermodesulfovibrio*-like organisms in these mixed communities was beyond the scope of this
366 study.

367 It is unclear why Chowder Hill and Dead Dog exhibit large differences in rates of sulfate
368 reduction despite other similarities in geochemistry and biomass. One plausible explanation
369 might be that different types of biological interactions (e.g. syntrophy or competition) occur
370 due to differences in the composition and distribution of microbial communities within the
371 mineral matrix of each deposit. Slight differences in community composition, like Dead Dog
372 having a higher representation of sequences related to sulfur respiring (*Thermoplasmata)* and
373 methanogenic (*Methanocaldococus-like*) archaea than Chowder Hill, may lead to biological
374 interactions that have different implications for rates of sulfate reduction in each deposit. Also,
375 substrate competition for H_2 or consumption of locally produced DOC (Oremland & Polcin,
376 1982; Lovley & Phillips, 1987) may be more prevalent in one deposit over another. Future
377 experiments should aim to better resolve how specific interaction between populations, for
378 example, syntrophy or competition for a common substrate, may influence sulfate reduction.

379 ***The relevance of heterotrophic sulfate reduction on hydrothermal vent biomass production***
380 ***and biogeochemistry***

381 Heterotrophic sulfate reduction is likely a prominent metabolic mode within Middle
382 Valley sulfides and sediments, and the sulfate reduction rate data herein (which solely measure
383 hetrotrophic sulfate reduction) support that supposition. Sedimented vent fields typically
384 contain allochthonous organic carbon that could readily support heterotrophy. Indeed, at
385 Middle Valley, bottom waters contain 3.5 mg DOC/L (about 7 fold higher than the overlying
386 surface seawater), while porewater concentrations range from 0.1 – 84.0 mg DOC/L at

387 sediment depths to 200 mbsf (Ran & Simoneit, 1994). Based on data from culture studies of

388 *Desulfovibrio* strains, including the H^+/H_2 ratio of 1.0 for *Desulfovibrio vulgarius* Marburg (Fitz &

389 Cypionka, 1991), the P/2e$^-$ ratio (number of ATPs produced for every 2 electrons transferred to

390 an electron acceptor) of 1/3 for *Desulfovibrio gigas* (Barton et al. 1983), and the assumption

391 that 10% of ATP production supports growth (20 mmol ATP per gram biomass), our estimates

392 suggest that - at our maximum empirically measured rates - heterotrophic sulfate reduction

393 could support 140 g biomass yr^{-1} (~1.5×10^{14} cells) at Chowder Hill (volume = 109,900 cm^3), 16 g

394 biomass yr^{-1} (~1.7×10^{13} cells) at Needles (volume = 5495 cm^3), and 2.1 g biomass yr^{-1} (~$2.2 \times$

395 10^{12} cells) at Dead Dog (volume=12560 cm^3). While these values may be small in comparison to

396 global estimates of chemoautotrophic biomass production on the global ridge system (10^{10} -

397 10^{13} g of biomass yr^{-1} ;McCollom & Shock 1997; Bach & Edwards 2003), the sulfide produced by

398 these heterotrophic sulfate reducers could represent up to 3% of the H_2S flux from Middle

399 Valley deposits (given previously published vent fluid flow rates from the Main Endeavor field,

400 (Wankel *et al*. 2011)). Additional rate measurements that represent the diversity of physico-

401 chemical conditions found within deposits or ridge systems are necessary to better constrain

402 the contribution of heterotrophic sulfate reducers to global vent biomass and geochemistry.

403 Hydrothermal vents are dynamic environments where carbon and sulfur cycling are

404 intimately linked. Both autotrophic and heterotrophic sulfate reducing microbes have been

405 isolated from vents, and the data shown here are among the first to constrain the potential for

406 heterotorophic sulfate reduction at vents (in particular those with higher organic carbon loads),

407 as well as the relationship between sulfate reduction rates, temperature, microbial biomass

408 and community density and composition. These data, as well as the vent field estimates of

409 sulfate reduction, , underscore the relevance of sulfate reduction in hydrothermal ecosystems
410 and further indicate the need for continued studies of sulfur cycling along ridge systems.
411

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422 **Supplementary information is available at ISME's website**

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