

# Historical factors associated with past environments influence the biogeography of thermophilic endospores in Arctic marine sediments

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40 **Abstract**

41

42 Selection by the local, contemporary environment plays a prominent role in shaping the biogeography  
43 of microbes. However, the importance of historical factors in microbial biogeography is more  
44 debatable. Historical factors include past ecological and evolutionary circumstances that may have  
45 influenced present-day microbial diversity, such as dispersal and past environmental conditions.

46 Diverse thermophilic sulphate-reducing *Desulfotomaculum* are present as dormant endospores in  
47 marine sediments worldwide where temperatures are too low to support their growth. Therefore, they  
48 are dispersed to here from elsewhere, presumably a hot, anoxic habitat. While dispersal through ocean  
49 currents must influence their distribution in cold marine sediments, it is not clear whether even earlier  
50 historical factors, related to the source habitat where these organisms were once active, also have an  
51 effect. We investigated whether these historical factors may have influenced the diversity and  
52 distribution of thermophilic endospores by comparing their diversity in 10 Arctic fjord surface  
53 sediments. Although community composition varied spatially, clear biogeographic patterns were only  
54 evident at a high level of taxonomic resolution (>97% sequence similarity of the 16S rRNA gene)  
55 achieved with oligotyping. In particular, the diversity and distribution of oligotypes differed for the  
56 two most prominent OTUs (defined using a standard 97% similarity cutoff). One OTU was dominated  
57 by a single ubiquitous oligotype, while the other OTU consisted of ten more spatially localised  
58 oligotypes that decreased in compositional similarity with geographic distance. These patterns are  
59 consistent with differences in historical factors that occurred when and where the taxa were once  
60 active, prior to sporulation. Further, the influence of history on biogeographic patterns was only  
61 revealed by analysing microdiversity within OTUs, suggesting that populations within standard OTU-  
62 level groupings do not necessarily share a common ecological and evolutionary history.

63

64 Keywords: biogeography, thermophile, endospore, marine sediment, *Desulfotomaculum*, sulphate-  
65 reducing bacteria, dispersal

## 66 **Introduction**

67

68 Some microbes have globally widespread distributions while others do not; yet pinpointing the  
69 mechanisms responsible for these biogeographic patterns remains challenging (Hanson et al., 2012). A  
70 classic perspective of biogeography divides the underlying mechanisms into two main categories:  
71 those that are driven by the contemporary, local environment versus those that rely on circumstances  
72 that have happened in the past (Martiny et al., 2006). These historical factors include all ecological and  
73 evolutionary events that previously influenced (either long ago or relatively recently) a present-day  
74 population or community, such as dispersal and past environmental conditions. The role of historical  
75 factors in shaping the diversity and biogeography of microbes has been hotly debated for decades  
76 because – it is contended – microbes’ enormous potential for rapid and widespread dispersal should  
77 erase any signature of past events. However, much recent evidence now suggests that at least one  
78 historical factor, dispersal limitation, indeed shapes the biogeography of many microbes (reviewed in  
79 Martiny et al. (2006) and Hanson et al. (2012)).

80

81 However, the degree to which current microbial biogeographic distributions are shaped by historical  
82 factors other than dispersal limitation remains largely untested, especially for marine microbes.  
83 Thermophilic, fermentative and sulphate-reducing endospore-forming bacteria within the phylum  
84 *Firmicutes*, including members of the genus *Desulfotomaculum*, are useful models for studying the  
85 role of historical factors in marine microbial biogeography. These thermophiles are abundant in cold  
86 marine sediments as dormant endospores (Hubert et al., 2009; de Rezende et al., 2013; Volpi et al.,  
87 2017) and have been detected in marine sediments worldwide (Müller et al., 2014). Because the  
88 ambient temperature in sediments and the overlying water column are far too low to support their  
89 activity (Hubert et al., 2009; Hubert et al., 2010; de Rezende et al., 2013), endospores of thermophiles  
90 in cold sediments must be derived from a different ecosystem or location (i.e., they are not  
91 autochthonously derived). Further, they remain as dormant endospores and do not undergo endogenous  
92 growth in these sediments and are therefore not subject to local selection by environmental factors.  
93 Thus, contemporary, local environmental factors have effectively no influence on the distribution of  
94 thermophilic spores in cold sediments. Instead, their distribution and diversity is the result of historical  
95 factors alone.

96

97 Several studies have specifically investigated dispersal of thermophilic endospores by characterizing  
98 their biogeographic distribution in marine sediments at regional scales (de Rezende et al., 2013;  
99 Chakraborty et al., 2018) and global scales (Müller et al., 2014) as well as in estuarine systems (Bell et  
100 al., 2018). These studies have provided growing evidence in support of the hypothesis that many  
101 thermophilic endospores are derived from fluid flow expelled from marine deep biosphere ecosystems  
102 such as mid-ocean ridge venting systems and deeply buried hydrocarbon reservoirs, and are  
103 subsequently deposited to marine sediments via passive dispersal. Many thermophilic endospores  
104 identified in these studies are phylogenetically related to other organisms found at hydrothermal vents  
105 and/or hydrocarbon reservoirs (Hubert et al., 2009; Hubert et al., 2010; Nielsen et al., 2017;  
106 Chakraborty et al., 2018). Additionally, the diversity of thermophilic endospores in a single cold  
107 sediment can be considerable (de Rezende et al., 2013; Müller et al., 2014; Chakraborty et al., 2018)  
108 and may be explained by different taxa originating from different warm sources. These biogeographic  
109 studies also show that, despite being well-equipped for long-distance dispersal as spores, dispersal  
110 limitation influences the biogeography of some thermophilic endospores, while others are more  
111 cosmopolitan in distribution (Müller et al., 2014; Bell et al., 2018; Chakraborty et al., 2018). For  
112 example, Müller et al. (2014) detected 146 unique phylotypes (i.e., OTUs with  $\geq 97\%$  16S rRNA  
113 sequence identity) in their global survey of over 80 sediments, with some phylotypes present across  
114 many geographically distant locations while others detected in only a few samples. Moreover,

115 similarity in the composition of thermophilic endospore communities significantly decreased with  
116 increasing geographic distance (Müller et al. 204), a relationship that, in the absence of environmental  
117 selection, can only be caused by historical factors (Hanson et al., 2012), namely, in this case, dispersal  
118 limitation. For thermophilic endospores, we consider this dispersal to be inclusive of dissemination  
119 from a warm, allochthonous source environment followed by passive transport in oceanic currents and  
120 finally deposition to the seafloor via sedimentation.

121  
122 But do circumstances prior to this dispersal process, specifically the biological history of thermophilic  
123 endospore forming bacteria in the warm habitat(s) from which they are derived, influence their later  
124 distribution and diversity in cold sediments? In this case, history includes two components, which we  
125 refer to collectively as “historical factors”: 1) the ecological (both deterministic and stochastic) and/or  
126 evolutionary events associated with the former warm source habitat where thermophilic endospore  
127 forming populations were once active and 2) the location of those habitats, assuming they are fixed.  
128 These factors will have shaped the abundance, community composition, and genetic diversity of active  
129 microbes in those locations, which likely include warm habitats in the geosphere (Hubert et al. 2009).  
130 When endogenous microbes get expelled into the cold ocean as dormant spores, historical factors  
131 associated with their source habitat may therefore leave an imprint on their eventual distribution and  
132 diversity, e.g., in cold seabed surface sediments. Some examples of past ecological and evolutionary  
133 mechanisms that shape microbial diversity include but are not limited to: physical connectivity or  
134 habitat isolation that influenced immigration and gene flow (past dispersal), stochastic demographic  
135 changes (past ecological drift), and adaptation to environmental conditions (past selection) (Hanson et  
136 al., 2012).

137  
138 The west coast of Spitsbergen, Svalbard in the Arctic Ocean is an ideal location to address the role of  
139 historical factors in shaping the distribution of thermophilic endospores for several reasons. First, the  
140 Arctic Ocean north of Iceland is considered a distinct biogeographic province (Longhurst, 1998;  
141 Costello et al., 2017), inclusive of Arctic surface water (Costello et al., 2017), deep waters (German et  
142 al., 2011; Costello et al., 2017) and its mid-ocean ridge system containing hydrothermal vents (Tyler  
143 and Young, 2003; German et al., 2011). A biogeographic province is an area particularly reflective of  
144 historical factors due to geological or physical forces and/or dispersal barriers that separate it from  
145 other regions (Martiny et al., 2006; Takacs-Vesbach et al., 2008), and for macro-organisms, is often  
146 distinguished by endemic taxa (Costello et al., 2017). Indeed, the Arctic is relatively more isolated  
147 from global ocean circulation relative to other oceans because of its many bordering large land masses  
148 and resulting shallow sills that restrict movement of deep waters between the Arctic and its  
149 neighbouring oceans (German et al., 2011). Secondly, the west coast of Svalbard is in close proximity  
150 to potential warm deep biosphere ecosystems from which thermophilic endospores are likely derived  
151 including mid-ocean ridge spreading centres and buried petroleum deposits (Gautier et al., 2009;  
152 Pedersen et al., 2010; Jaeschke et al., 2014). The Arctic Mid Ocean Ridge system (AMOR) lies  
153 approximately 200 km due west of Spitsbergen and the Gakkel Ridge lies approximately 650 km due  
154 north of Spitsbergen, both of which are hydrothermally active with plumes and vent fields (German et  
155 al., 2011; Pedersen and Bjerkgård, 2016). In addition, the region is a well-known repository of both  
156 explored and unexplored petroleum and gas reservoirs, with the western portion of the Svalbard shelf  
157 estimated to contain up to 1 billion barrels of undiscovered oil and 6 trillion cubic feet of undiscovered  
158 gas (Gautier et al., 2009). Thus, within this biogeographic province at the small scale of western  
159 Svalbard, observed biogeographic patterns for thermophilic endospores should highlight the effect of  
160 historical factors associated with one or more nearby warm source environments.

161  
162 Therefore, the aim of this study was to test whether historical factors associated with a former habitat  
163 shape the biogeography of themophilic endospores in sediments near Svalbard. To do this, we

164 characterised their diversity and distribution in ten sampling stations along the west coast of Svalbard  
165 using a heated enrichment approach. Diversity was assessed by analysis of organic acid resource use at  
166 the whole community level in sediment enrichment incubations and by 16S rRNA gene sequence  
167 analysis of the enriched communities. We hypothesised that enriched thermophilic endospore taxa  
168 would differ in their presence-absence distribution across our study area (16 - 240 km). If so, then a  
169 comparison of distribution patterns may reveal differences in the historical factors influencing those  
170 taxa. Our aim was not to identify the exact location or type of source environment(s), but rather to  
171 speculate on the nature of past ecological and evolutionary pressures and the environmental scenarios  
172 under which they could have occurred. Further, as past ecological and evolutionary pressures would  
173 have direct effects at the population-level, we hypothesised that the influence of historical factors  
174 would be relatively more apparent at higher levels of taxonomic resolution (i.e. population-level)  
175 (Berry et al., 2012; Hanson et al., 2012; Buttigieg and Ramette, 2014; Eren et al., 2014; Jones et al.,  
176 2016; Chase and Martiny, 2018). In particular, we expected biogeographic patterns to be weakest when  
177 diversity was defined using resource-use at the community level and greatest when defined using  
178 genetic “microdiversity” within 16 rRNA genes at the >97% sequence identity level.  
179

180

## 181 **Materials and Methods**

182

### 183 ***Sediment Samples and Locations***

184 Marine surface sediments (3 - 9 cm depth) were collected with a Haps corer (a single coring cylinder  
185 with top valve supported by a frame (Kannevorff and Nicolaisen, 1983)), aboard R/V *Farm* during the  
186 summer of 2006 or 2007 from 10 stations within 7 distinct fjords along the west coast of Spitsbergen,  
187 Svalbard (Fig. 1, Table 1). Surface sediments were chosen here because they represent recent  
188 deposition via sedimentation within the past 100 years (Hubert et al., 2009) and thus loss of  
189 thermophilic endospore viability over time (de Rezende et al., 2013; Volpi et al., 2017) is expected to  
190 have minimal influence on their abundance and diversity in surface sediments. Sediments were sealed  
191 in gas-tight plastic bags and stored at 4 °C. For all stations, the *in situ* surface sediment temperature  
192 range was -2 to +7 °C. Geodesic distance between stations ranged between 16 and 240 km. A map of  
193 stations (Fig. 1) was produced using Simple Mapper (<http://www.simplemapper.net/>).  
194

195

### 196 ***Sediment slurry preparation and incubations at 50 °C***

196 Sediment slurries were prepared for each of the 10 stations separately under a constant flow of N<sub>2</sub> by  
197 diluting sediment in a 1:2 (w/w) ratio with artificial seawater medium (Widdel and Bak, 1992) and  
198 sulphate concentration adjusted to 20 mM. To evenly enrich, detect, and assess the diversity of  
199 sulphate-reducing bacteria (SRB) across the different sediments, a combined pool of typical SRB  
200 substrates acetate, butyrate, ethanol, formate, lactate, propionate and succinate were also added to all  
201 incubations such that each substrate was provided to a final concentration of 1 mM (Hubert et al.,  
202 2010; de Rezende et al., 2013). Slurries were homogenized and dispensed into autoclaved and  
203 stoppered serum bottles under a flow of N<sub>2</sub>. These master slurries were divided into two identical  
204 portions: one was spiked with radioactive sulphate tracer (<sup>35</sup>S-sulphate) for determination of sulphate  
205 reduction, and the other was not spiked but used for organic acid analysis and DNA extraction  
206 (described below). To enrich for thermophilic SRB present in the samples as endospores, slurries were  
207 pasteurised at 80 °C for one hour, sub-sampled for an initial time-zero sample, then incubated at 50 °C  
208 for 253-277 hours. Subsamples (1-2 ml aliquots) were removed from the slurries every 12-24 hours  
209 using N<sub>2</sub>-flushed syringes, and analysed as described below. 50 °C was chosen because it is in the  
210 range of thermophilic growth previously observed in Svalbard sediments (45-64 °C) (Hubert et al.,  
211 2009; Hubert et al., 2010), yields maximal sulphate reduction rates and SRB diversity (Hubert et al.,  
212 2009; de Rezende et al., 2013), and to maintain consistency with previous studies (e.g. Müller et al.,

213 2014). This heated incubation approach has several advantages. First, the approach has previously been  
214 shown to enrich for a wide diversity of thermophilic spore-forming *Firmicutes* (Müller et al., 2014;  
215 Chakraborty et al., 2018), including those that are phylogenetically related to known spore-forming  
216 *Desulfotomaculum* spp (Tardy-Jacquenod et al., 1998; Vandieken et al., 2006; de Rezende et al.,  
217 2013). The enrichment is also necessary in order to effectively target only those cells which are present  
218 as endospores because it a) kills vegetative cells, allowing endospores to be distinguished (Müller et  
219 al., 2014) and b) induces germination of endospores, thus circumventing the problem of endospores  
220 being missed in direct sequencing libraries due to their resistance to commonly used DNA extraction  
221 methods (Wunderlin et al., 2013; de Rezende et al., 2017). The incubations do not necessarily elicit  
222 germination and growth of all thermophilic endospore taxa present in the samples, but are effective at  
223 enriching communities that are comparable across samples by employing equivalent incubation  
224 conditions and substrate amendments (Müller et al., 2014).

### 226 ***Organic acid concentrations and sulphate reduction***

227 Concentrations of low-molecular-weight organic acids in filtered subsamples were determined with a  
228 Sykam HPLC system equipped with an anion neutral pre-column (4 × 20 mm; Sykam GmbH) and an  
229 Aminex HPX-87H separation column (300 × 7.8 mm; Bio-Rad) at 60°C. The eluent was 5 mM H<sub>2</sub>SO<sub>4</sub>  
230 prepared using HPLC grade water, and the flow rate was 0.6 ml min<sup>-1</sup>. Succinate, lactate, formate,  
231 acetate, propionate and butyrate were quantified using a UV detector (210 nm) and a refractive index  
232 detector using single injections (one measurement per sample per timepoint).

234 The extent of sulphate reduction at each sampling time point was determined in parallel sediment  
235 slurries that contained ca. 100 kBq ml<sup>-1</sup> of <sup>35</sup>S-sulphate. Subsamples were fixed in 20% w/v zinc  
236 acetate and frozen until determination of sulphate reduction by cold chromium distillation of reduced  
237 sulphur compounds as described elsewhere (Kallmeyer et al., 2004). The fraction of added <sup>35</sup>S in the  
238 reduced sulphur pool was used to determine the proportion of sulphate reduced to sulphide, and is  
239 reported here as the extent of sulphate reduction. These proportions were calculated using sulphate  
240 concentrations in the supernatant that were measured following 100× dilution via non-suppressed  
241 anion exchange chromatography (Ferdelman et al., 1997).

### 243 ***Sequencing and OTU creation***

244 We focused on the genus *Desulfotomaculum*, as previous work indicated that sulphate reduction within  
245 similar sediment incubations was due to the growth of thermophilic members of this specific genus  
246 (Hubert et al., 2009; Hubert et al., 2010; Müller et al., 2014). Sequence analysis is based on a subset of  
247 previously published 16S rRNA gene sequences from one of these studies (NCBI Sequence Read  
248 Archive accession SRP028774; Müller et al. 2014). Briefly, pyrosequencing of PCR-amplified 16S  
249 rRNA genes (V6-V9 region) was performed on DNA extracted from sediment slurry subsamples  
250 before and after 120 hours of incubation, for one slurry per station. The subset of sequences that were  
251 significantly enriched after 120 hours were clustered into OTUs to a ≥97% similarity threshold (Müller  
252 et al., 2014). A subset of 10 OTUs related to *Desulfotomaculum* that were enriched in our 10 stations  
253 was retained for further analysis (Table S2). Here, we use the term “OTU”, equivalent to “phylotype”  
254 as used by Müller et al (2014). We retain here the OTU naming scheme as defined in that study, with  
255 the prefix “TSP.” Prior to statistical analyses, the OTU by sample table was randomly subsampled to  
256 the smallest sample size (226 reads; Table S2) one hundred times, and median Jaccard (presence-  
257 absence) and Bray-Curtis (read count) dissimilarity matrices were calculated using MacQIIME ver  
258 1.9.1 (Caporaso et al., 2010). Analyses were repeated on normalised raw data (standardised and  
259 square-root transformed OTU-by-sample read-count data) with little effect on the results (Table S3).  
260 Cluster analysis was performed using Primer-E version 6.1.5 (Plymouth, UK).

261

262 **Oligotyping**  
263 Oligotyping was employed to identify subtle 16S rRNA gene nucleotide variation (termed  
264 “oligotypes”) within individual *Desulfotomaculum* OTUs from the above analysis. Oligotyping uses  
265 Shannon entropy to identify individual nucleotide positions representative of biologically relevant  
266 genetic variation among closely related sequences (Eren et al., 2013; Eren et al., 2016) while  
267 minimising the potential influence of random sequencing error (Kleindienst et al., 2016). OTUs having  
268 greater than 100 total reads from at least one of the 10 stations were oligotyped individually using  
269 oligotyping version 1.7, following the recommendations by Eren et al. (2013) and described in SI. To  
270 ensure even comparisons, stations having less than 10 reads for a given OTU were removed from the  
271 oligotyping analysis, hence the number of stations in the oligotyping analysis was often less than the  
272 number of stations in which that OTU was originally detected (Table 2). Inverse Simpson’s Index  
273 (Table 2) and rarefaction curves (Fig. S1) were calculated using MOTHUR version 1.33.0 (Schloss et  
274 al., 2009). Prior to statistical analyses (below), we followed a similar normalisation approach as  
275 recently employed by Buttigieg and Ramette (2014). Specifically, for each OTU individually, any  
276 stations with fewer than 10 reads after oligotyping analysis were removed, and then oligotype-by-  
277 sample abundance tables were standardised by total number of reads per sample, square-root  
278 transformed, and converted into Bray-Curtis dissimilarity matrices.

279  
280 **Statistical analyses**  
281 To test for relationships between biotic similarity (community-wide traits, OTU composition, or  
282 oligotype composition) and geophysical variables (geographic distance or water depth), we performed  
283 simple Mantel-type tests with the RELATE function in Primer-E version 6.1.5 (Plymouth, UK) using  
284 weighted Spearman’s rank correlation coefficient ( $\rho$ ) with significance at  $p \leq 0.05$ . A geographic  
285 distance matrix was created using the open-source website “Geographic distance matrix generator”  
286 Version 1.2.3 ([http://biodiversityinformatics.amnh.org/open\\_source/gdmg/index.php](http://biodiversityinformatics.amnh.org/open_source/gdmg/index.php)) with WGS84  
287 spheroid mode. For water depth, a Euclidean distance matrix was calculated on normalised data (also  
288 see SI). For organic acid trait data (Table S1), a binary phenotype matrix (1 indicating consumption, 0  
289 indicating no consumption) for acetate, butyrate, formate, lactate and propionate in each of the 10  
290 sampling stations was converted into a dissimilarity matrix using the Czekanowski metric (Legendre  
291 and Legendre, 1998). Succinate was not considered in this analysis as its dynamics were not well-  
292 coupled to sulphate reduction, but rather it appeared to be consumed via succinate decarboxylation to  
293 propionate. Consumption was defined as a clear and consistent decrease in concentration at any point  
294 over the course of incubation. In some cases, “consumption” is the net result of production followed by  
295 depletion (e.g., acetate in Fig 2 panels B, C, and E).

296  
297 To test for relationships among number of oligotypes observed and number of reads, alignment length,  
298 or stations, Pearson correlations were performed in StatPlus for Mac version 5.9.33. To test for  
299 differences in overall genetic variation among stations, we performed Analysis of Molecular Variance  
300 (AMOVA) for each OTU separately in MOTHUR version 1.33.0 using the same sequence alignments  
301 as for oligotyping analysis. For AMOVA, we tested the null hypothesis that sequence reads from  
302 stations have the same level of nucleotide divergence as the entire dataset combined (sequences from  
303 all stations pooled together). We used the same alignments as used for oligotyping, such that the  
304 number of samples in each AMOVA analysis is equivalent to the number of stations in the oligotyping  
305 analyses (Table 2). At the oligotype level, AMOVA and RELATE tests were only performed for OTUs  
306 having greater than 2 stations in the oligotyping analysis.

307  
308 **Availability of Data and Research Materials**

309 The raw 16S rRNA gene amplicon sequence datasets analysed for this study can be found in the NCBI  
310 Sequence Read Archive under accession number **SRP028774**,  
311 <https://trace.ncbi.nlm.nih.gov/Traces/sra/?study=SRP028774> (Müller et al., 2014). The raw  
312 biogeochemical data, any other data files supporting the conclusions of this manuscript, and other  
313 materials such as protocols and analytical steps will be made available by the authors, without undue  
314 reservation, to any qualified researcher. Requests to access the datasets and other materials should be  
315 directed to the corresponding author.

316

## 317 **Results**

318

### 319 ***Organic acid resource use by enriched endospore-forming thermophiles***

320 Sulphate reduction was detected in sediments from all ten sampling stations (Fig. 1, Table 1) within 30  
321 hours of incubation at 50 °C (Fig. 2, Table S1), indicating the ubiquitous presence of viable, spore-  
322 forming thermophilic SRB along the west coast of Svalbard. Sulphate-reducing activity was in good  
323 correspondence with the consumption of different organic acids (Fig. 2). Initial sulphate reduction was  
324 always associated with the consumption of formate and lactate and the production of acetate.  
325 Relatively small amounts of sulphate were consumed (3 mM or less) before this initial activity levelled  
326 off, consistent with sulphate reduction coupled to incomplete oxidation of 1 mM lactate and complete  
327 oxidation of 1 mM formate, as well as other substrates that may be derived from the sediment organic  
328 matter (Hubert et al., 2010). This resource use pattern was observed in all stations, suggesting  
329 thermophilic SRB with corresponding metabolic capabilities were ubiquitous across the study area.

330

331 Beyond this initial response, however, sulphate reduction and organic acid consumption patterns were  
332 not uniform among stations. For all sediments except two (stations AB and EA) a second distinct  
333 exponential increase in sulphate reduction, with concomitant changes in the concentration of the  
334 organic acids, was apparent near or after 60 hours of incubation (Table S1). In these instances, this  
335 second phase encompassed most of the overall sulphate reduction observed (Fig. 2, Table S1). For  
336 most stations this second phase also exhibited corresponding consumption of butyrate and propionate,  
337 though the timing of consumption was not always clearly coupled to the onset of the second phase of  
338 sulphate reduction. In contrast, stations AB and EA lacked a second phase of sulphate reduction with  
339 concomitant butyrate and propionate consumption, suggesting that thermophilic SRBs capable of  
340 utilising these two organic acids were possibly absent from these stations or if present, did not grow;  
341 for example, due to the absence of other bacteria (see SI for further discussion). While acetate was  
342 always generated as a by-product of incomplete lactate oxidation during the initial phase of sulphate  
343 reduction, its subsequent depletion during the second phase was observed in only 3 out of 8 stations,  
344 suggesting patchy occurrence of thermophilic organisms capable of acetate utilisation in the different  
345 fjord sediments sampled. Stations E and A were the only sediments for which all six measured organic  
346 acids were consumed. Despite overall spatial differences in organic acid turnover, there was no  
347 relationship between organic acid consumption traits and geographic distance ( $\rho=-0.269$ ,  $p=0.753$ ) or  
348 water depth ( $\rho=0.042$ ,  $p=0.313$ ).

349

### 350 ***Diversity and biogeography of enriched *Desulfotomaculum****

351 Spatial differences between fjords in organic acid turnover coupled to different phases of sulphate  
352 reduction coincided with the sequential appearance of different *Desulfotomaculum* spp. (a time-  
353 resolved example is shown in Fig. S3 and discussed in SI). Therefore, we focused on sulphate-reducing  
354 *Desulfotomaculum* for subsequent 16S rRNA gene sequence analysis. We detected a total of ten  
355 enriched *Desulfotomaculum* OTUs (defined at  $\geq 97\%$  similarity; Table S2) across all stations, with one  
356 OTU, TSP004, present in all stations. The most OTU-rich *Desulfotomaculum* communities were found  
357 at Stations BE and A, each having 7 OTUs, with 4 in common. Stations AB and EA were the least



358 OTU-rich, sharing the same two *Desulfotomaculum* OTUs: TSP004 and TSP015. While not  
359 ubiquitous, TSP006 and TSP015 were still very common, occurring in 7 and 9 out of the 10 stations,  
360 respectively. In contrast, two other OTUs were detected in just one station each, both occurring in  
361 station BE (TSP036 and TSP072). While TSP004 was detected in all stations, the presence of the other  
362 9 OTUs was much more variable (Table S2). Despite these observations, there was no significant  
363 relationship between enriched *Desulfotomaculum* OTU composition and geographic distance or water  
364 depth (Fig. 3; Table S3). For example, stations AH and I are among the geographically most distant  
365 pairs (approx. 220 km), yet are identical with respect to the occurrence pattern of *Desulfotomaculum*  
366 OTUs (Fig. 3). In contrast, stations A and D were very different in terms of OTU occurrence (>60%  
367 dissimilar; Fig. 3) despite being one of the geographically closest pairs (approx. 23 km).

368

### 369 ***Microdiversity within Desulfotomaculum OTUs***

370 We further investigated the diversity of enriched *Desulfotomaculum* spp. in our samples by using  
371 oligotyping to resolve fine-scale variation within OTUs (<3% nucleotide differences). Oligotype  
372 diversity varied substantially among OTUs (Table 2, Fig. 4, Fig. S2). TSP006 was more diverse than  
373 TSP004 (Fig. S1; Table 2), even though TSP004 was the most abundant OTU overall and present in all  
374 stations. TSP004 mainly consisted of one highly predominant oligotype, TSP004 oligotype 1, which  
375 represented >87% of the reads for this OTU (Fig. 4A and C). By contrast, TSP006 consisted of 10  
376 oligotypes that were more even in overall relative abundance (Simpson's Diversity Index = 3.34; Table  
377 2), with the most abundant oligotype among these 10 representing less than 50% of the total diversity  
378 for this OTU (Fig. 4B and D). Accordingly, the number of oligotypes for a given OTU was not  
379 significantly correlated with the number of reads used in oligotyping analysis ( $R=0.66$ ;  $p=0.10$ ),  
380 number of stations the OTU was detected in ( $R=0.69$ ,  $p=0.13$ ), or the alignment length ( $R=0.41$ ,  
381  $p=0.36$ ). Therefore, the number of oligotypes per OTU does not seem to be affected by these  
382 methodological factors.

383

384 For all *Desulfotomaculum* OTUs, oligotype composition varied among stations (Fig. 4 and Fig. S2)  
385 and within-OTU genetic variation differed significantly among stations (AMOVAs:  $F_s > 40$  and  
386  $p < 0.001$  for all OTUs; Table 3). However, individual OTUs exhibited different patterns in the way  
387 oligotypes were distributed spatially. TSP004 was highly uniform among stations, with a single  
388 oligotype constituting nearly all of the reads in all but two of the 7 stations included in its analysis (Fig.  
389 4A, 4C); and even in these two stations (BE and D) the predominant oligotype made up >60% of the  
390 reads. These two stations explain the overall weaker AMOVA results with fewer significant pairwise  
391 station comparisons for TSP004. Consequently, there was no relationship between oligotype  
392 composition and geographic distance or water depth for TSP004 within Svalbard (Table 3). The most  
393 abundant TSP004 oligotype (TSP004 oligotype 1) was 99.4% identical to oligotype 2 (i.e. 2 bp  
394 difference over 351 total bp) and all oligotypes had at least 98.9% identity (no more than 4 bp  
395 difference) (Fig. 4A, 4C).

396

397 In contrast, TSP006 exhibited relatively more spatial variation in oligotype composition (Fig. 4B). In  
398 fact, TSP006 oligotype composition within Svalbard was significantly correlated with spatial distance  
399 (RELATE test:  $\rho=0.49$ ,  $p=0.045$ ; Table 3). Two TSP006 oligotypes were detected in all four stations  
400 in the oligotyping analysis, but the other eight TSP006 oligotypes were highly variable in occurrence  
401 and relative abundance among stations, displaying a north to south trend (Fig. 4B). For example,  
402 TSP006 oligotypes 3 and 6 were only detected in the northernmost stations. Further, unlike TSP004,  
403 all AMOVA post-hoc pairwise station comparisons for TSP006 were significant. TSP006 oligotypes  
404 are 98.2 – 99.7% identical to each other (at most 6 and at minimum 1 bp difference over 377 total bp).

405

406 Oligotyping other OTUs also revealed spatial variation (Fig. S2). For example, for TSP015, no single  
407 oligotype was detected in all 4 analysed stations. TSP045 consisted of just 3 oligotypes across 2  
408 stations, with none being found in both stations. Finally, although TSP085 was dominated by a single  
409 oligotype, it also comprised several additional rare oligotypes appearing in just one station.  
410

## 411 **Discussion**

412  
413 In accordance with our hypothesis, we found that enriched thermophilic endospores vary strikingly in  
414 their spatial distributions on the western coast of Svalbard with respect to all three metrics of diversity  
415 – organic acid-use traits at the community-level, OTU composition, and sub-OTU level microdiversity  
416 within the 16S rRNA gene. Patterns at all definitions of diversity displayed a general trend that some  
417 taxa are widespread while others have more limited distributions. One *Desulfotomaculum* OTU  
418 (TSP004) and two community-wide traits (formate and lactate consumption) were ubiquitously present  
419 at all stations. In contrast, other OTUs were more patchily distributed, being detected in as few as a  
420 single station to as many as 9 stations. At the sub-OTU level, we also observed oligotypes that were  
421 more widely distributed across multiple stations and others that were geographically more restricted  
422 (Fig. 4 and Fig. S2). Importantly, however, a relationship with geographic distance only emerged at the  
423 highest level of taxonomic resolution achieved with oligotyping. For instance, TSP006 oligotype  
424 composition was significantly correlated with geographic distance, even though there was no evidence  
425 for a correlation between geographic distance and overall OTU composition or organic acid-use traits.  
426 Community-wide organic acid-use traits generally mirrored the spatial variation detected at the OTU  
427 level, with neither being sufficient for delineating clear biogeographic patterns in enriched  
428 thermophilic communities.  
429

430 The biogeographic patterns observed here for thermophilic endospores at the small scale of western  
431 Svalbard are best explained by historical factors associated with one or more allocthonous warm  
432 source environments where the thermophilic spore-forming populations were once active. As such,  
433 distribution patterns in Svalbard sediments can be used to speculate on the nature of these source  
434 environments. For example, since *Desulfotomaculum* OTUs vary in their presence-absence and in their  
435 diversity and distribution of oligotypes, there appear to be multiple sources that vary in their  
436 environmental conditions and/or spatial locations. In this way, historical factors other than (or in  
437 addition to) dispersal vary among taxa of thermophilic endospores and thus shape their contemporary  
438 distribution in cold sediments. This is one of the first studies to demonstrate that historical factors  
439 associated with a past environment drive the contemporary biogeographic distribution of a marine  
440 microbe in sediments.  
441

442 These findings support previous suggestions that in other parts of the world, thermophilic endospores  
443 could be derived from multiple sources, including different marine deep biosphere environments  
444 (Hubert et al., 2009; Hubert et al., 2010; de Rezende et al., 2013; Müller et al., 2014; Bell et al., 2018;  
445 Chakraborty et al., 2018). Potential marine deep biosphere sources include: 1) exposed crust and off-  
446 axis hydrothermal systems at the seafloor in areas flanking mid-ocean ridge spreading centres (Summit  
447 and Baross, 2001), where crustal-seawater fluid flow occurs at temperatures in the range of growth for  
448 diverse bacteria (Orcutt et al., 2011), including thermophilic *Desulfotomaculum* spp.; and 2) oil or gas  
449 reservoirs, which consist of deeply buried sediments at geothermally elevated temperatures. Reservoir  
450 environments are also capable of supporting thermophilic microbial communities (Head et al., 2003;  
451 Magot, 2005) and are connected to the overlying ocean by upward fluid flow at hydrocarbon seeps  
452 (Hubert and Judd, 2010). Indeed, *Desulfotomaculum* spp have previously been identified in off-axis  
453 hydrothermal samples in other parts of the world (Brazelton et al., 2006; Cha et al., 2013; Müller et al.,  
454 2014; Robador et al., 2016) and in oil reservoir samples in the nearby North Sea (Rosnes et al., 1991;

455 Nilsen et al., 1996) and elsewhere (Tardy-Jacquenod et al., 1998; Çetin et al., 2007; Chakraborty et al.,  
456 2018). These environments have unique geology that could hypothetically constrain the ecological and  
457 evolutionary history of their *in situ* microbial populations in different ways (Medlin, 2007). Here, we  
458 use these environments as examples to consider how differences at potential source habitats could  
459 equate to differences in past ecological and evolutionary pressures, and thus explain the observed  
460 patterns of diversity in Svalbard surface sediments. Then, we discuss alternative explanations.

461  
462 For instance, TSP004 is ubiquitous along the west coast of Svalbard, consists of a single dominant  
463 oligotype, and lacks a relationship with geographic distance. Because a single oligotype of TSP004 is  
464 widely distributed across all stations, the source population is apparently not highly genetically  
465 differentiated with respect to the 16S rRNA gene at the regional scale investigated here. Therefore, we  
466 postulate that the majority of individuals of TSP004, representing the main dominant oligotype, have a  
467 common history (i.e. a common gene pool (Medlin, 2007)) that consists of origins in a spatially  
468 extensive, inter-connected, and well-mixed warm marine ecosystem that is far enough offshore to  
469 impact the entire north-south fjord system that we investigated. The nearby Arctic and northern Mid-  
470 Atlantic ridges are one set of possible sources that satisfy these criteria (Crane et al., 2001; Kelley et  
471 al., 2002; Edmonds et al., 2003), especially with recent discoveries of off-axis venting close to  
472 Svalbard (Pedersen et al., 2010; Jaeschke et al., 2014). At local to regional scales within warm ridge  
473 environments, relatively high rates of both dispersal and colonisation by microbial individuals could  
474 occur through a constant exchange of crustal fluids and seawater (Elderfield and Schultz, 1996; Kelley  
475 et al., 2002; Hutnak et al., 2008; Fisher and Wheat, 2010; Orcutt et al., 2011; Jungbluth et al., 2016).  
476 This could result in a widespread, well-mixed *Desulfotomaculum* population that is relatively  
477 genetically homogenous with respect to the 16S rRNA gene (Slatkin, 1987; Cohan and Perry, 2007;  
478 Hartl and Clark, 2007), like the dominant oligotype we observe for TSP004. This perspective contrasts  
479 with other studies reporting an island-like habitat structure for hydrothermal submarine crustal  
480 environments and inhibited bacterial dispersal between individual venting mounds (Huber et al., 2010;  
481 Mino et al., 2013). However, we speculate that because *Desulfotomaculum* is spore-forming, it may  
482 more easily overcome these potential dispersal barriers compared to other thermophilic and  
483 hyperthermophilic residents.

484  
485 In contrast, all other OTUs are much more patchily distributed with patchily distributed oligotypes.  
486 One explanation for this pattern is past circumstances that facilitated spatial isolation, allowing genetic  
487 divergence of different oligotypes. Such circumstances could have arisen in environments that are not  
488 as spatially connected as mid-ocean ridge systems. Subsurface petroleum reservoirs can be separated at  
489 depth within geologic formations, such that adjacent reservoirs even just a few kilometres apart can be  
490 physically isolated allowing divergent evolution in deep biosphere “islands” (Lewin et al., 2014).  
491 Reservoir fluids escaping to the surface at seabed hydrocarbon seeps could serve as patchily distributed  
492 localised point sources of closely-related but non-identical taxa, like the oligotypes we have mapped  
493 for TSP006. In other words, microbial populations residing in reservoirs are relatively more closed to  
494 exchange with outside populations as compared to exposed axial ocean crustal fluid systems. In fact,  
495 the finding by Lewin et al. (2014) that similar taxa derived from adjacent but separated reservoirs share  
496 ca. 98% similarity across metagenomes agrees with the microdiversity distinguished within TSP006  
497 (oligotypes are highly identical, 98.2 – 99.7% in the variable V6-V9 region). The pool of independent  
498 populations derived from multiple isolated reservoirs would be expected to have at least some genetic  
499 variation, resulting in a relatively patchy distribution once dispersed to cold sediments especially if  
500 fine scale genetic analysis is employed. TSP006 is consistent with this as it consists of multiple  
501 genetically differentiated populations (corresponding to different oligotypes), many of which are  
502 patchily distributed, i.e. found in no more than a few stations (Fig. 4B, 4D). Further, TSP006 is not  
503 present in all stations and exhibits a north-south decrease in compositional similarity (Fig. 4B) which

504 could be caused by dispersal-limitation from localised point source(s). The closest match to TSP006  
505 oligotypes 1 and 2 in Genbank at 98% sequence identity is derived from a subsurface hydrocarbon  
506 contaminated aquifer (JQ086982; Tischer et al., 2013).

507  
508 It is also possible that instead of multiple habitats or spatial isolation, different oligotypes and/or OTUs  
509 arose within the same source habitat where they coexist. In order for multiple, closely related  
510 populations to coexist, ecological theory suggests that those populations must be ecologically  
511 differentiated somehow. In such a scenario, TSP006, for example, could be derived from a single  
512 reservoir, or from a ridge flank environment, where different TSP006 oligotypes represent coexisting  
513 populations adapted to different ecological conditions or with different functional traits (as discussed  
514 in Larkin and Martiny, 2017). Indeed, this has been observed for other bacterial groups sampled  
515 directly from marine hydrothermal systems (Huber et al., 2007; Brazelton et al., 2010; Anderson et al.,  
516 2017). However, when TSP006 is detected, regardless of oligotype composition, it always emerges late  
517 in our enrichment incubation experiments and is associated with the removal of propionate and  
518 butyrate (SI and Fig. S3), suggesting that TSP006 oligotypes could share similar functions. Our study  
519 does not resolve whether and how coexisting oligotypes differ with respect to their physiology,  
520 ecology, or genomic content. Competing explanations of the historical factors determining TSP006  
521 distributions highlight the ongoing need for further research on the causes and consequences of  
522 microbial microdiversity at the sub-OTU level.

523  
524 Another explanation is that different *Desulfotomaculum* taxa and/or oligotypes have origins in other  
525 habitats, such as naturally geothermal or anthropogenic terrestrial sources. This has been suggested for  
526 *Desulfotomaculum* endospores enriched from an urban river estuary in the UK (Bell et al., 2018). We  
527 speculate that this may be the case for the distinct and minor TSP004 oligotypes that we detected  
528 uniquely within Isfjorden's stations BE and D (Fig.4). Isfjorden is not only the largest fjord on the west  
529 coast of Svalbard but also the location of Longyearbyen, the capital and main settlement of Svalbard's  
530 2500 inhabitants. Isfjorden sediments may receive input related to human activities, e.g. mining and/or  
531 wastewater, which can be non-marine habitats for thermophilic SRB (Tasaki et al., 1991; Kaksonen et  
532 al., 2006; Widdel, 2006). Diversity at the broader 97% OTU-level also suggests the possibility of a  
533 localised source specifically influencing Isfjorden, as we also detected 3 OTUs (TSP032, TSP036, and  
534 TSP072) that were restricted to one or more of the four stations in Isfjorden. While acknowledging  
535 these possibilities, we speculate that most of the *Desulfotomaculum* taxa detected in this study are  
536 more likely derived from marine geothermal sources because 1) enrichment medium selects for  
537 sulphate-reducers capable of growing in brackish to saline conditions, 2) Svalbard experiences little,  
538 and only very local, human influence due to its sparse population, and 3) we have detected similar  
539 *Desulfotomaculum* taxa in isolated coastal fjords in northern Svalbard (Magdalenefjorden, station I, in  
540 this study, and even further north in Smeerenburgfjorden, in previous work (Hubert et al., 2009;  
541 Hubert et al., 2010)), which have no human settlements.

542  
543 The explanations above offer plausible past ecological scenarios that could have generated the  
544 observed patterns. These hypothesized origins and scenarios should be tested further, for example, by  
545 including samples directly from the proposed source environments. Most endospores are resistant to  
546 commonly used DNA extraction methods; thus, alternative, specialised methods like that tested by  
547 Wunderlin et al. (2013) may be necessary in order to capture DNA directly from endospores.  
548 Therefore, currently published sequence libraries from mid-ocean ridge and petroleum reservoir  
549 environments may not always include taxa highly related to those observed in our study. Additionally,  
550 many bacteria, including *Desulfotomaculum* spp., carry multiple copies of the 16S rRNA gene in their  
551 genomes (Větrovský and Baldrian, 2013), with both copy number and 16S sequence varying both  
552 among and within species. Therefore, it is possible that different oligotypes are derived from the same

553 cell or strain. However, oligotypes were not detected in similar proportions across all samples, but are  
554 generally distributed very unequally (Fig. 4 and Fig. S2) suggesting they represent distinct yet related  
555 organisms.

556  
557 Overall, this study highlights how the ability to detect the role of historical factors on microbial  
558 biogeography requires more sensitive taxonomic resolution than standard 97% OTU definitions  
559 (Hanson et al., 2012; Chase and Martiny, 2018). Oligotyping analysis was able to reveal biogeographic  
560 variation within *Desulfotomaculum* OTUs at levels representing no more than 1.2% divergence across  
561 351-377 bp within the V6-V9 region of the 16S rRNA gene. Recent studies have also uncovered  
562 distributional patterns in marine bacteria using oligotyping of 16S rRNA gene sequences. For example,  
563 oligotypes of sediment bacteria at an Arctic Long-Term Ecological Research site, roughly 100 km west  
564 of Svalbard, showed relationships with environmental variables that were not predictable by analyses  
565 based on 97% OTU community composition (Buttigieg and Ramette, 2014). In another study, the  
566 relative abundance of *Pelagibacter* oligotypes that were >99% identical to each other exhibited a  
567 strong, annually recurring seasonal pattern that was masked at the 97% OTU level (Eren et al., 2013).  
568 Similarly, oligotypes of aquatic *Vibrio* and estuarine *Synechococcus* demonstrated fine-scale  
569 differentiation according to environmental differences (Schmidt et al., 2014; Mackey et al., 2017,  
570 respectively). Building on these examples, our study has not only used oligotyping to unmask  
571 biogeographic structure, but to further reveal that such structure can be generated by differences in  
572 historical factors among and within OTUs. Thus, populations within OTU-level groupings do not  
573 necessarily have a common ecological and evolutionary history and higher resolution methods must be  
574 employed to tease apart the influence of history on contemporary microbial diversity. This probably at  
575 least partially explains the lack of agreement to-date on the relative importance of historical factors in  
576 shaping biogeographic patterns for microbes (Martiny et al., 2006; Hanson et al., 2012).

577  
578 This work substantiates that historical factors associated with former environments are capable of  
579 generating ecologically relevant biogeographic patterns in spore-forming marine microbes. Even for  
580 dormant spores, everything is not everywhere, and individual populations can exhibit different  
581 distributional patterns as a result of divergent histories. High-resolution analysis of microdiversity  
582 within the 16S rRNA gene was necessary to detect contrasting historical signatures that could have  
583 been shaped by different conditions of potential deep biosphere source habitats. From this, we have  
584 developed further testable hypotheses on the ecological nature of potential former source habitats and  
585 how this could have influenced the biogeography of microdiverse lineages of thermophilic  
586 *Desulfotomaculum* in Arctic sediments. Because subsurface environments are difficult to sample  
587 directly and to reproduce artificially, these taxa may serve as more accessible indicators for 1) the  
588 presence of undiscovered subsurface environments and resources (Hubert and Judd, 2010) and 2) the  
589 ecological and evolutionary history of microbial life within those environments.

#### 590 591 **Author Contributions**

592 CAH, CRJH, AM, AL, and BBJ designed the study. CRJH collected the samples. CRJH, AM, CD, and  
593 RA performed laboratory analysis. AM conducted bioinformatics. CAH performed bioinformatics  
594 (oligotyping) and analysed the data. CAH wrote the manuscript with contributions from all of the co-  
595 authors.

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601

602 **Conflict of Interest Statement**

603 The authors declare that the research was conducted in the absence of any commercial or financial  
604 relationships that could be construed as a potential conflict of interest

605

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818 **Figure Legends**

819

820 **Fig. 1** Map showing the ten sediment sampling stations along the west coast of Spitsbergen, Svalbard.

821

822 **Fig. 2** Progress in sulphate reduction (measured on the right axis) and organic acid concentrations  
823 (measured on the left axis) over time in sediment incubations for seven out of ten stations, ordered  
824 north to south. Organic acid concentrations were not determined for stations CN, BE, and AH (not  
825 shown; see Table S1).

826

827 **Fig. 3** Similarity in *Desulfotomaculum* OTU composition among sampling stations based on presence-  
828 absence of 10 OTUs.

829

830 **Fig. 4** Distribution and relative abundances of oligotypes within *Desulfotomaculum* OTUs TSP004 (A,  
831 C) and TSP006 (B, D). (A, B) Numbers to the right indicate the total number of reads detected in each  
832 station, followed in parentheses by the number of reads retained in the final oligotyping analysis.  
833 “ND”, grey bars = not determined, i.e. oligotyping not performed due to low numbers of reads.  
834 White/blank indicates stations in which the OTU was not detected in pyrosequencing libraries.  
835 Sampling stations are ordered in approximately northern-most to southern-most on the y-axis. (C, D)  
836 Rank-abundance showing number of sequence reads per observed oligotype. For all panels, different  
837 colours represent different oligotypes; oligotyping results based on the shorter alignment for TSP004  
838 are shown.

839 **Supplementary Material**

840

841 **SI. Supplementary Text**

842

843 **Table S1.** Summary of sulphate reduction, organic acid use traits, and presence of TSP004 and  
844 TSP006 in pyrosequencing libraries for each station.

845

846 **Table S2.** OTU table showing the number of pyrosequencing reads per *Desulfotomaculum* OTU per  
847 sampling station; and the total number of reads by OTU, by station, and combined.

848

849 **Table S3.** Results of RELATE tests for correlations between similarity in *Desulfotomaculum* OTU  
850 composition and geographic distance or water depth.

851

852 **Fig. S1.** Rarefaction curves for number of oligotypes observed for each of six *Desulfotomaculum*  
853 OTUs. \* = Oligotyping was repeated for TSP004 to allow for a longer alignment but inclusive of fewer  
854 total reads (see Methods and Table 2 in the main text). Error bars represent 95% confidence intervals.  
855 Note that the y-axis has been truncated to 900 for clarity, as number of oligotypes observed did not  
856 increase beyond this.

857

858 **Fig. S2.** Distribution and relative abundances of oligotypes within four other *Desulfotomaculum*  
859 OTUs. Numbers to the right indicate the total number of reads detected in each station, followed in  
860 parentheses by the number of reads retained in the final oligotyping analysis. “ND”, grey bars = not  
861 determined, i.e. oligotyping not performed due to low numbers of reads. White/blank indicates stations  
862 in which the OTU was not detected in pyrosequencing libraries. Sampling stations are ordered in  
863 approximately northern-most to southern-most on the y-axis. Within a panel, different colours  
864 represent different oligotypes.

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866 **Fig. S3.** Denaturing Gradient Gel Electrophoresis (DGGE) image showing the diversity of enriched  
867 *Desulfotomaculum* over time in sediment incubation experiments for selected stations. This image  
868 demonstrates 1) the existence of spatial differences in *Desulfotomaculum* composition and 2) a  
869 successional pattern of bands over time during the course of incubations (i.e., the band corresponding  
870 to TSP004 becomes weaker over time in some cases). We assume that different bands represent  
871 *Desulfotomaculum* diversity at the approximate species level, and therefore make no inference about  
872 different oligotypes here. Solid arrows indicate the approximate gel migration position for TSP004  
873 amplicons. Hatched arrows indicate the approximate gel migration position for TSP006 amplicons.

874

875 **Table 1.** Sediment sampling stations, geographic coordinates, and water depth

<b>Station ID</b>	<b>Name</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Water depth (m)</b>
A	Adventfjorden	78°15.51'N	15°30.53'E	69
AB	Van Keulenfjorden	77°35.19'N	15°05.24'E	100
AH	Van Mijenfjorden	77°45.75'N	15°03.23'E	116
BE	Nordfjorden	78°30.73'N	15°04.48'E	192
CN	Krossfjorden	79°08.40'N	11°44.18'E	334
D	Isfjorden	78°10.91'N	14°34.12'E	243
E	West of Stor Jonsfjorden	78°32.71'N	12°17.70'E	168
EA	Ymerbukta	78°16.80'N	14°00.31'E	0 (intertidal)
F	Kongsfjorden	78°54.97'N	12°16.04'E	109
I	Magdalenefjorden	79°35.05'N	11°03.59'E	126

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**Table 2.** Summary of oligotyping analyses for each of six *Desulfotomaculum* OTUs, including oligotype richness, diversity, and Genbank blast hits.

OTUs	No. stations detected	No. stations used in oligotyping analysis (No. reads)	Alignment length (bp)	Entropy positions	No. oligotypes observed (raw no. before noise filtering <sup>a</sup> )	No. reads for most abundant oligotype (% of total)	Simpson's Diversity Index (1/D)	Top Genbank blast hit for most abundant oligotype (% identity, % coverage)
TSP004	10	7 (2684)	351	58, 130, 138, 144	8 (14)	2343 (87.3)	1.30	JQ304695 <i>Desulfotomaculum</i> sp. Lac-2 (99.7, 94.3)
<i>TSP004<sup>b</sup></i>		<i>6 (2308)</i>	<i>393</i>	<i>1, 34, 179, 186</i>	<i>6 (7)</i>	<i>2020 (87.5)</i>	<i>1.29</i>	<i>same as above</i>
TSP006	7	4 (1819)	377	5, 7, 8, 146, 324, 334	10 (12)	829 (45.6)	3.34	JQ304697 <i>Desulfotomaculum</i> sp. For-1 (99.3, 94.3)
TSP015	9	4 (751)	277	41, 46, 52, 66	7 (9)	553 (73.6)	1.77	JQ741985 Uncultured <i>Clostridiales</i> bacterium clone 22 (98.5, 100)
TSP032	2	1 (458)	352	9, 42, 280	5 (6)	291 (63.5)	2.18	FN396785 Uncultured marine bacterium clone s5_8_I_31 (100, 93.3)
TSP045	2	2 (234)	284	70, 75	3 (4)	209 (89.3)	1.66	AY918123 <i>Desulfotomaculum salinum</i> strain 781 (95.4, 100)
TSP085	3	2 (890)	353	6, 38, 44	5 (5)	701 (78.8)	1.56	AY918123 <i>Desulfotomaculum salinum</i> strain 781 (95.4, 100)

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<sup>a</sup> To reduce noise, oligotypes consisting of 3 or fewer reads were eliminated from further analysis, as explained in the Methods. <sup>b</sup> Oligotyping was repeated for TSP004 to allow for a longer alignment but inclusive of fewer total reads.



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**Table 3.** Results for Analysis of Molecular Variance (AMOVA) and RELATE tests on oligotype composition for three *Desulfotomaculum* OTUs

	AMOVA <sup>a</sup>			Abundance (Bray-Curtis)				Presence-Absence (Jaccard)			
				Geographic Distance <sup>b</sup>		Water Depth <sup>b</sup>		Geographic Distance <sup>b</sup>		Water Depth <sup>b</sup>	
	Fs	<i>Global</i> <i>p</i> -value	pairwise station comparisons	<b><i>ρ</i></b>	<i>p</i> -value	<b><i>ρ</i></b>	<i>p</i> -value	<b><i>ρ</i></b>	<i>p</i> -value	<b><i>ρ</i></b>	<i>p</i> -value
TSP004	42.40	<0.001**	14 out of 21, p<0.05	-0.47	0.956	0.40	0.155	-0.17	0.703	0.45	0.070
<i>TSP004<sup>c</sup></i>	<i>40.55</i>	<i>&lt;0.001**</i>	<i>13 out of 15, p&lt;0.05</i>	<i>-0.48</i>	<i>0.901</i>	<i>-0.14</i>	<i>0.539</i>	<i>-0.23</i>	<i>0.761</i>	<i>0.10</i>	<i>0.270</i>
TSP006	84.41	<0.001**	all significant, p<0.001	0.49	0.045*	-0.62	0.954	0.91	0.088	-0.16	0.520
TSP015	172.11	<0.001**	all significant, p<0.001	0.68	0.170	0.32	0.172	0.96	0.058	0.71	0.045*

885 <sup>a</sup> AMOVAs were performed on the alignments used for oligotyping analysis. <sup>b</sup> Results of Spearman rank correlation tests between similarity  
886 in oligotype composition and geographic distance or water depth. Significant relationships (p≤0.05) are indicated by asterisk in bold. <sup>c</sup>  
887 Oligotyping was repeated on TSP004 to allow for a longer alignment but inclusive of fewer total reads (see Table 1).  
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