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# Geology

## Influence of bedrock mineral composition on microbial diversity in a subglacial environment --Manuscript Draft--

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Cover letter

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1 Influence of bedrock mineral composition on microbial  
2 diversity in a subglacial environment

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14 **ABSTRACT**

15 Microorganisms in subglacial environments drive the chemical weathering of  
16 bedrock, however, the influence of bedrock mineralogy on the composition and activity  
17 of microbial assemblages in such environments is poorly understood. Here, using a  
18 combination of in situ mineral incubation and DNA fingerprinting techniques, we  
19 demonstrate that pyrite is the dominant mineralogical control on subglacial bacterial  
20 community structure and composition. In addition, we show that the abundance of Fe in  
21 the incubated minerals influences the development of mineral associated biomass. The  
22 ubiquitous nature of pyrite in many common bedrock types and high SO<sub>4</sub><sup>2-</sup> concentrations

23 in most glacial meltwaters suggests that pyrite may be a dominant lithogenic control on  
24 microbial communities in many subglacial systems. Mineral-based energy may therefore  
25 serve a fundamental role in sustaining subglacial microbial populations and enabling their  
26 persistence over glacial-interglacial timescales.

## 27 **INTRODUCTION**

28 Terrestrial ice masses currently cover  $\sim 15 \times 10^6$  km<sup>2</sup>, roughly 10% of Earth's land  
29 surface. The presence of viable microbes in terrestrial ice, subglacial sediments, and  
30 proglacial meltwaters has been demonstrated (Christner, 2002; Sharp et al., 1999;  
31 Skidmore et al., 2005; Skidmore et al., 2000) and *ex-situ* (Boyd et al., 2011; Christner,  
32 2002) and *in situ* (Boyd et al., 2010) evidence of microbial activities has been presented.  
33 The lack of light energy capable of driving photosynthesis in subglacial environments  
34 suggests that energy for cellular synthesis and the maintenance of microorganisms is  
35 supplied from chemical energy (chemosynthesis), likely derived from weathering of the  
36 local bedrock (e.g., minerals) (Luttge et al., 2005; Shock, 2009). Indeed, numerous lines  
37 of evidence suggest that many geochemical processes, including mineral weathering and  
38 redox transformations in subglacial environments, are driven by microorganisms (Boyd  
39 et al., 2011; Hamilton et al., 2013; Montross et al., 2013; Skidmore et al., 2005; Wadham  
40 et al., 2004; Wynn et al., 2007). Hydrological regimes (Tranter et al., 2005), nutrient  
41 availability and redox conditions (Wadham et al., 2004; Wynn et al., 2007) have been  
42 shown to impact the function of microorganisms in subglacial environments. However,  
43 the influence of bedrock mineralogy on the structure, composition and activity of  
44 microbial assemblages in subglacial systems remains poorly understood despite its

45 potential importance over a significant portion of Earth's surface both today and on  
46 glacial-interglacial time scales.

47 The importance of pyrite ( $\text{FeS}_2$ ) oxidation as a dominant geochemical process in  
48 subglacial environments has been documented (Bottrell and Tranter, 2002; Brown, 2002;  
49 Tranter et al., 2002), and more recent evidence suggests that this process is microbially  
50 mediated (Montross et al., 2013; Skidmore et al., 2005; Wadham et al., 2004). Sulfate is  
51 the second most dominant anion (after bicarbonate) in most proglacial and subglacial  
52 meltwaters from alpine, arctic and antarctic environments (Brown, 2002), and  
53 concentrations of  $\text{SO}_4^{2-}$  are always significantly enriched relative to possible input waters  
54 (e.g., ice- or snow-melt), indicating a lithogenic source. In addition to  $\text{SO}_4^{2-}$ , other  
55 products of pyrite weathering are often ubiquitous in glacial meltwaters and sediments  
56 (Mitchell et al., 2001), including nano particles of Fe-oxyhydroxides (Raiswell et al.,  
57 2009). Equally, surveys of subglacial microbial community 16S rRNA transcripts  
58 (Hamilton et al., 2013) and genes (Lanoil et al., 2009; Skidmore et al., 2005) often yield  
59 sequences that are closely affiliated with organisms that actively metabolize Fe, S, and/or  
60 FeS minerals. Therefore, while geochemical and microbiological evidence suggests Fe  
61 and S cycling are important in subglacial systems, the extent to which lithology and  
62 mineralogy shape community structure, composition, and activity is unknown. Here we  
63 employ a method to isolate the influence of mineralogy on the composition and structure  
64 of microbial communities in the subglacial environment at Robertson Glacier (RG),  
65 Alberta, Canada (115°20'W, 50°44'N; Item DR1 and Fig. DR1a in the GSA Data  
66 Repository<sup>1</sup>). This approach employs coupon samplers composed of capped stainless-  
67 steel mesh cylinders (Item DR1 and Fig. DR1b) that compartmentalize different minerals

68 during in situ incubation (Boyd et al., 2007) in the glacial outflow channel of RG ~10 m  
69 downstream of the glacier terminus, in order to assess the influence of mineralogy on the  
70 structure, composition, and abundance of the microbial community.

## 71 MATERIALS AND METHODS

72 Combustion-sterilized rocks and minerals (1.4–1.7 mm diameter) were incubated  
73 in the glacial meltwater stream at RG (RW [western drainage]) for 7 mo. over winter in  
74 order to promote colonization of mineral substrata contained in pre-sterilized coupon  
75 samplers by microorganisms that originated from the subglacial system (Item DR1 and  
76 Fig. DR1b). The close proximity of the coupons to the glacier and the subglacial source  
77 for the waters passing through the coupons provide confidence that the microbial  
78 colonization that occurred reflects subglacial microbial diversity. The selection of  
79 minerals, derived from mineral collections and chemically characterized by energy-  
80 dispersive X-ray spectroscopy (EDS) and X-ray diffraction (XRD) (Item DR1), included  
81 those that could serve as electron donors or acceptors (pyrite,  $[\text{Fe}^{2+}, \text{S}^-]$ , hematite and  
82 magnetite  $[\text{Fe}^{3+}]$ ) and olivine  $[\text{Fe}^{2+}]$  (90% forsterite + 10% fayalite;  $\text{Fo}_{90}\text{Fa}_{10} =$   
83  $\text{Mg}_{1.8}\text{Fe}_{0.2}\text{SiO}_4$ ;  $\text{Fe}^{2+} = 75,000 \pm 1425$  ppm [Item DR1 and Table DR1]). Minerals that  
84 offered no obvious metabolic substrate [quartz ( $\text{SiO}_2$ ), calcite ( $\text{CaCO}_3$ )] but were  
85 abundant in catchment bedrock (Table DR1; Sharp et al., 2002) were also included. In  
86 addition, we included representative rock from the RG catchment (dark shale containing  
87 quartz, microcline, calcite, and pyrite) which was prepared in the same way as the pure  
88 mineral phases. RG rock contained  $9800 \pm 764$  ppm  $\text{Fe}^{2+}$  and  $3000 \pm 360$  ppm S, and  
89 assuming all S came from pyrite, an estimated pyrite content of 0.56 weight % (Table  
90 DR1). Subglacial (RG water) and supraglacial meltwaters were collected aseptically and

91 filtered through sterilized filter apparatus with 0.22  $\mu\text{m}$  pore size polyvinylidene  
92 difluoride membrane at the time of coupon deployment to compare the native community  
93 with that which developed on the coupon minerals. Fine-grained subglacial sediment was  
94 also collected aseptically (RG sediment) from the site of coupon deployment at the time  
95 the coupon was retrieved. All mineral and sediment samples were immediately flash  
96 frozen on site using dry ice (Item DR1).

97 Coupon minerals, subglacial sediments (RG sediment), and filters with subglacial  
98 suspended sediments and planktonic (e.g., suspended) cells (RG water) were subjected to  
99 community DNA extraction, polymerase chain reaction (PCR) amplification, and  
100 bacterial 16S rRNA gene terminal-restriction length polymorphism (T-RFLP) (Item  
101 DR1). Distinct T-RFs were considered to be unique operational taxonomic units and were  
102 the unit by which individual phylotypes were demarcated. Comparison of T-RFLP  
103 profiles was performed using the Bray-Curtis index, which represents an abundance  
104 weighted metric describing the similarity of communities (Item DR1). Clone library  
105 construction was also performed on pyrite- and RG-sediment associated communities, as  
106 previously described (Boyd et al., 2007), to determine similarity with known  
107 microorganisms (Item DR1). Filtered waters (0.22  $\mu\text{m}$ ) were immediately measured for  
108 pH and electrical conductivity in the field, and were analyzed for major anions, cations,  
109  $\delta^{34}\text{S-SO}_4^{2-}$ ,  $\delta^{18}\text{O-SO}_4^{2-}$ , and  $\delta^{18}\text{O-H}_2\text{O}$  (Item DR1).

## 110 **RESULTS AND DISCUSSION**

### 111 **Geochemical Evidence of Mineral Weathering**

112 Enrichment of sulfate ( $\text{SO}_4^{2-}$ ) in subglacial waters, relative to supraglacial waters,  
113 indicates pyrite weathering in the subglacial environment of RG (Table DR2 and Item



114 DR2). The  $\delta^{34}\text{S-SO}_4^{2-}$  and  $\delta^{18}\text{O-SO}_4^{2-}$  values in meltwaters also indicate pyrite oxidation  
115 (Table DR2 and Item DR2). Our results are consistent with previous geochemical (Sharp  
116 et al., 1999; Tranter et al., 2002) and isotopic studies (Bottrell and Tranter, 2002;  
117 Wadham et al., 2004) of other glacial catchments, which indicate a role for pyrite  
118 weathering in solute liberation, which has been argued to be the result of microbial  
119 activity. If microorganisms are driving pyrite weathering in RG subglacial sediments,  
120 then the microbial community would be expected to harbor 16S rRNA genes affiliated  
121 with organisms capable of this physiological activity. Indeed, examination of microbial  
122 communities from RG (Hamilton et al., 2013) and from glacial catchments with similar  
123 geology to that of RG (Skidmore et al., 2005) reveal the presence of sequences affiliated  
124 with several organisms capable of oxidizing Fe and/or S.

### 125 **RG Subglacial Sediment Microbial Community Composition**

126 The majority of 16S rRNA genes recovered from sediments sampled adjacent to  
127 the mineral coupon incubation site (Fig. 1; Table DR3, RG sediment), exhibited close  
128 affiliation with  $\beta$ - and  $\gamma$ -protobacteria (e.g., *Thiobacillus* spp. [ $>94\%$  identity],  
129 *Acidithiobacillus ferrooxidans* [90% identity], and *Siderooxidans lithoautotrophicus*  
130 [96% identity]). These organisms have been demonstrated to catalyze the oxidation of  
131 soluble and/or solid phase ferrous iron and sulfur in pure cultures (Karavaiko et al., 2003;  
132 Okereke and Stevens, 1991; Suzuki et al., 1990; Item DR2). Indeed,  $\beta$ - and  $\gamma$ -  
133 Protobacteria are the dominant phylogenetic groups in nearly all subglacial systems  
134 investigated from alpine and polar environments (e.g., Foght et al., 2004; Skidmore et al.,  
135 2005) and all of these glaciers are underlain by bedrock that contains pyrite, similar to the  
136 bedrock at RG. Collectively, these observations suggest that pyrite weathering is an

137 important process and is likely an important determinant in structuring the composition of  
138 microbial communities in this and other subglacial systems.

### 139 **Mineralogical Controls on Community Structure and Composition**

140 In order to further examine the role of pyrite and other minerals in structuring the  
141 composition of bacterial communities in the subglacial system, we compared 16S rRNA  
142 gene T-RFLP profiles (each T-RF considered a unique taxonomic unit) of bacterial  
143 communities associated with the coupon incubated mineral surfaces to those associated  
144 with native sediments (RG sediment) using the Bray Curtis (BC) similarity index.

145 Importantly, such in situ experiments represent a discrete colonization interval (7 mo.)  
146 and the observations from such minerals may be confounded by differences in microbial  
147 colonization and successional dynamics. Notwithstanding, the bacterial community that  
148 associates with the RG subglacial sediment is most similar to that associated with pyrite  
149 (BC index = 0.40) (Fig. 2, Tables DR4 and DR5), adding further evidence that supports  
150 the influence of pyrite on the composition and structure of bacterial communities in the  
151 subglacial environment at RG. The RG sediment bacterial community was also similar to  
152 the communities that associated with the native rock from the RG catchment that was  
153 also incubated in the subglacial coupons, RG Rock (BC index = 0.37). For example, the  
154 RG sediment-associated bacterial community shared seven dominant phlotypes with the  
155 RG rock- and pyrite-associated communities. Together these phlotypes accounted for  
156 37.6% of the RG sediment-associated community, 70.9% of RG rock-associated  
157 community, and 53.4% of the pyrite-associated community (Table DR4). Interestingly,  
158 the communities that associated with pyrite, RG rock, and RG sediment formed a cluster  
159 along with the community from RG water (subglacial meltwater containing suspended

160 sediment and planktonic cells) associated community (Fig. 2), further evincing the  
161 compositional similarity of these communities to each other. These results, which  
162 demonstrate the similarity of communities on pyrite with those associated with native  
163 sediments, rocks and water, are supported by sequence-based comparisons of community  
164 16S rRNA genes which also reveal significant overlap in the phylogenetic composition of  
165 pyrite- and RG sediment-associated communities (Table DR3). Because pyrite is a  
166 constituent of RG rock (0.56 wt%), RG sediment (1.2 wt% [Table DR1]), and thus the  
167 suspended sediment in the RG water, these collective observations strongly suggest that  
168 pyrite, even in low concentrations, is a key control on the subglacial sediment-associated  
169 bacterial communities, perhaps due to a selective advantage to organisms that actively  
170 metabolize Fe and S in this mineral.

171       The other Fe-bearing minerals, hematite and magnetite, harbored microbial  
172 communities similar in structure and composition to each other (BC index = 0.42), which  
173 would be expected considering that they are both Fe-oxides. Importantly, the bacterial  
174 communities associated with hematite and magnetite were also similar to the  
175 communities associated with the RG sediment (BC index = 0.26 and 0.34, respectively),  
176 though less than when compared with the pyrite associated community (BC index = 0.40)  
177 (Fig. 2; Table DR5). While XRD was unable to detect Fe-oxides in the RG bedrock  
178 (Table DR2), Fe-oxyhydroxides, which are derived from the oxidation of pyrite (e.g.,  
179 Tranter et al., 2002), are likely to be present in the subglacial RG sediments. Moreover,  
180 direct measurements in other glacial environments indicate Fe-oxyhydroxides exist as  
181 single grain or aggregate nano-particles and/or as labile surface coatings of other mainly  
182 silicate sediments (Mitchell et al., 2001; Raiswell et al., 2009). Thus Fe-oxyhydroxides in

183 RG sediments may also influence microbial communities in subglacial environments, and  
184 account for the high similarity of hematite and magnetite communities with RG sediment  
185 communities. This hypothesis is further supported by the recovery of 16S rRNA gene  
186 clones from the RG sediment-associated library that are affiliated with *Rhodoferrax*  
187 *ferrireducens* (Table DR3), an iron reducing chemotroph (Finneran et al., 2003). Olivine,  
188 while containing Fe<sup>2+</sup> at 7.5 wt% (Table DR2) harbored communities that shared little  
189 resemblance to RG rock and RG sediment communities, presumably because it was not  
190 detected in the catchment bedrock.

191 Communities associated with quartz and calcite exhibited low similarity with, and  
192 that were distinct from those which associated with RG sediment, RG water and RG rock  
193 (Fig. 2) despite their abundance in the catchment bedrock (Table DR1). This is  
194 presumably due to a lack of any potential metabolic substrate associated with the calcite  
195 or quartz mineral phases, aside from the potential use of calcite derived carbonate as a  
196 source of carbon. Therefore T-RFLP add further evidence suggesting that Fe and S  
197 containing minerals, particularly pyrite, strongly influence the structure and composition  
198 of subglacial bacterial communities presumably due the selective advantage afforded to  
199 those that metabolize these available substrates.

## 200 **Mineralogical Controls on Biomass**

201 We compared the quantity of genomic DNA extracted from the mineral phases as  
202 a proxy for assessing the amount of biomass that accumulated on the mineral surfaces,  
203 with the premise that higher biomass loadings might indicate that the populations are  
204 using the minerals as a substrate to support their metabolism. We hypothesized that  
205 minerals containing Fe and S, which can support the metabolism of a wide variety of

206 microbial populations (Luttge et al., 2005; Shock, 2009), would harbor a higher biomass.  
207 Surface associated biomass was particularly pronounced on Fe bearing oxides and  
208 sulfides (hematite, magnetite, and pyrite; 204, 190, and 83 ng DNA/g mineral,  
209 respectively), but much lower on pyrite containing RG rock and Fe-bearing olivine  
210 [Fo<sub>90</sub>Fa<sub>10</sub>] (25 and 17 ng DNA/g mineral, respectively) (Fig. 3). These differences are  
211 correlated with the abundance of Fe in the minerals and rocks, which was greatest in  
212 hematite (70 wt%), magnetite (84 wt%) and pyrite (47 wt%), compared to only 7.5 wt%  
213 in olivine and 1.6 wt% in RG rock. The lowest surface-associated biomass was recovered  
214 from calcite and quartz (17 and 16 ng DNA/g mineral, respectively), which may reflect  
215 the lack of any Fe or other abundant metabolic substrate in these carbonate and silicate  
216 minerals (Fig. 3).

217         These data demonstrate that the abundance of Fe in subglacial minerals influences  
218 the respective surface-associated biomass, suggesting that these populations are using Fe  
219 in the minerals to support their metabolism. Importantly, the valence state of Fe in these  
220 minerals is different (pyrite 2<sup>-</sup>, hematite 3<sup>+</sup>, magnetite 2<sup>+</sup>/3<sup>+</sup>), consistent with community  
221 16S rRNA gene compositions, which indicate the presence of populations putatively  
222 involved in Fe oxidation or reduction in the RG sediments (Table DR3). Similarly, the  
223 metabolism of S in pyrite is also likely to have a strong influence on surface associated  
224 biomass, as evinced by pyrite associated 16S rRNA gene sequences with a high similarity  
225 to microorganisms known to metabolize sulfur (Table DR3), but this cannot be  
226 comparatively quantified in the minerals used. Together with molecular-based data that  
227 indicate that pyrite and Fe-oxides harbor communities that are most similar to the RG  
228 sediments, this strongly suggests that (1) pyrite and its weathering products (i.e., Fe

229 oxyhydroxides, thiosulfate) strongly influence subglacial microbial communities due to  
230 their ability to transform and utilize the mineral phase through redox reactions, and (2)  
231 the Fe concentration of Fe bearing minerals and rocks has a direct influence on surface-  
232 associated biomass in subglacial environments. Microbial interaction with mineral  
233 surfaces and the metabolism of key species such as Fe and S may therefore be a critical  
234 mechanism for sustaining life in subglacial systems at present, and over glacial-  
235 interglacial time scales. This is of global significance given that ice sheets covered 30%  
236 of Earth's continental land surface during Quaternary glaciations, and up to 100% during  
237 pervasive low latitude glaciations in the Neoproterozoic (Kirschvink, 1992).

## 238 **CONCLUSION**

239 Previous studies have demonstrated the importance of particulate-associated  
240 microbes in subglacial environments and indicate that this biomass represents a larger  
241 fraction of the community when compared with planktonic populations (e.g. Sharp et al.,  
242 1999; Skidmore et al., 2005). Our results indicate that mineralogy, due to the influence on  
243 community composition, structure, and abundance may help to explain these previous  
244 observations. Specifically, pyrite is the dominant mineralogical control on subglacial  
245 community structure at RG, and mineral associated biomass was proportional to the  
246 abundance of Fe in the incubated minerals. This suggests the importance of Fe and S  
247 metabolism at the mineral surface, as supported by the recovery of 16S rRNA gene  
248 sequences which were closely affiliated with organisms that are known to metabolize  
249 these species. Solid phase mineral utilization by microbial populations is likely a critical,  
250 life-sustaining strategy that enables subglacial ecosystems to persist during extended

251 glacial-interglacial time scales when ice masses have covered between 30% and 100% of  
252 Earth's continental land surface.

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## 352 **FIGURE CAPTIONS**

353 Figure 1. Phylogenetic affiliation of bacterial 16S rRNA genes in clone libraries  
354 generated from DNA extracted from pyrite (A) and Robertson Glacier (RG) sediment  
355 (B).

356

357 Figure 2. Hierarchical agglomerative clustering dendrogram depicting Bray–Curtis  
358 similarity of bacterial 16S rRNA gene assemblages (Table DR4 [see footnote 1])  
359 associated with mineral substrata, Robertson Glacier (RG) sediments, or RG meltwater.  
360 Data transformed to dendrogram using the Ward distance method. P-values supporting  
361 clustering at each node are depicted.

362

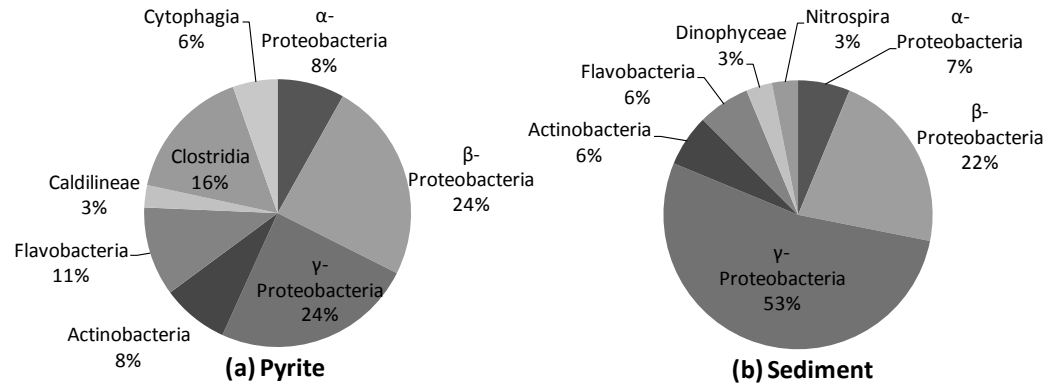
363 Figure 3. A: Biomass (extractable DNA as a proxy) associated with mineral substrata  
364 following 7 mo. colonization in subglacial stream. Error bars reflect standard deviation of

365 three replicate determinations of DNA concentration from a pool of three replicate  
366 mineral extractions. B: Correlation between biomass and Fe content, as determined by  
367 EDS (olivine, RG Rock, calcite and quartz; error bars smaller than symbols) or from pure  
368 phase stoichiometry (pyrite, hematite, and magnetite).

369

370 <sup>1</sup>GSA Data Repository item 2013xxx, xxxxxxxx, is available online at  
371 [www.geosociety.org/pubs/ft2013.htm](http://www.geosociety.org/pubs/ft2013.htm), or on request from [editing@geosociety.org](mailto:editing@geosociety.org) or  
372 Documents Secretary, GSA, P.O. Box 9140, Boulder, CO 80301, USA.

Figure 1.



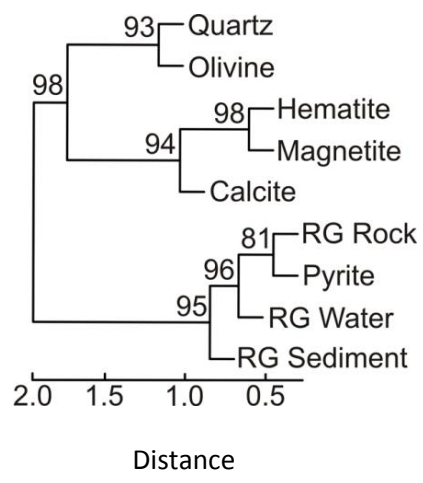
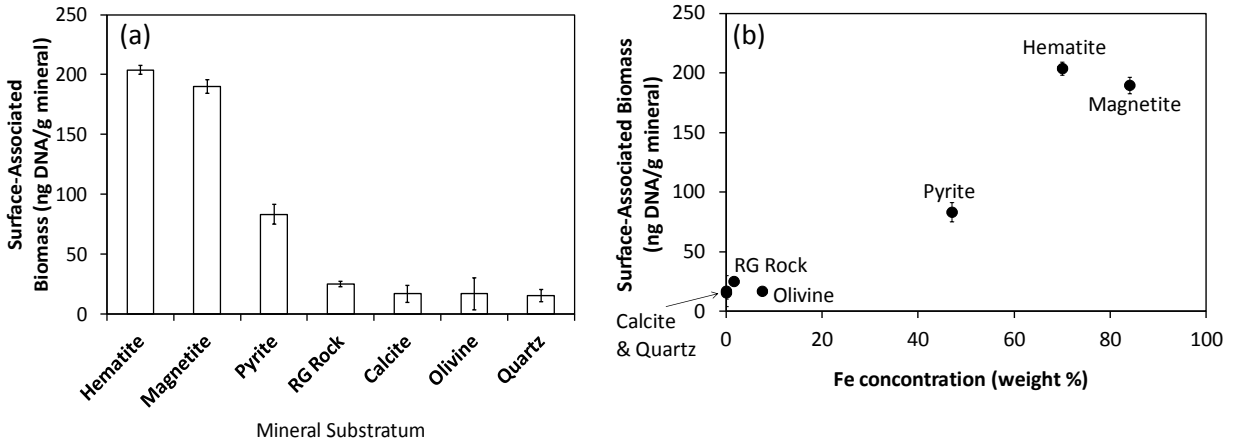


Figure 2.



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Figure 3.

**Supplemental Information. Data Repository.**

**Influence of bedrock mineral  
composition on microbial diversity  
in a subglacial environment**

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## **DR 1. DETAILED METHODS**

**DR 1.1. Field site description, sample collection and geology.** A detailed description of the hydrology and geology for Robertson Glacier (RG) has been previously reported (Sharp et al., 2002). Briefly, RG (115°20'W, 50°44'N) is a north facing valley glacier in Peter Lougheed Provincial Park, Kananaskis Country, Alberta, Canada. RG is approximately 2 km long, spans an elevation range from 2370 to 2900 m, and currently terminates on a flat till plain, although glacially smoothed bedrock surfaces are exposed along the glacier margins. Two principal subglacial meltwater streams (referred to here as RE [eastern drainage] and RW [western drainage]) drain from beneath the ice front (Fig. DR1a). The local bedrock is Upper Devonian in age (Mount Hawk, Palliser and Sassenach Formations) and consists of impure limestones, dolostones, and dolomitic limestones, with interbeds of shale, siltstone, and sandstone (McMechan, 1988).

**DR 1.2. Rock / Mineral substrata and coupon preparation and incubation.** Pyrite ( $\text{FeS}_2$ ), hematite ( $\text{Fe}_2\text{O}_3$ ), magnetite ( $\text{Fe}_3\text{O}_4$ ), quartz ( $\text{SiO}_2$ ), calcite ( $\text{CaCO}_3$ ), and olivine (90% Forsterite + 10% Fayalite;  $\text{Fo}_{90}\text{Fa}_{10} = \text{Mg}_{1.8}\text{Fe}_{0.2}\text{SiO}_4$  [Table DR1]) were obtained from WARD's Natural Science or the mineralogical collection at the Department of Earth Sciences, Montana State University. Minerals were crushed and sieved to obtain a near uniform particle size of 1.4-1.7 mm diameter. Only particles in the 1.4-1.7 mm diameter size range were retained for use in loading biofilm coupons in an attempt to minimize differences in particle surface area.

In addition, representative rocks were collected from outcrops on the valley sides and moraines in the RG catchment. These consisted of various impure limestones (argillaceous and silty limestone) and shales, which are the predominant lithologies (McMechan, 1988). Five of these (R1 to R5) representing the range of rocks present, were analyzed by powder X-

Ray Diffraction (XRD) to determine the mineralogical composition (Table DR1). These rocks, as well as the coupon minerals were analyzed by Energy-dispersive X-ray spectroscopy (EDS) to determine elemental composition by weight. Samples were powdered and analyzed in variable pressure (VP) mode and against measured standards. All of the catchment rocks analyzed were found to contain quartz, calcite, and one or more aluminosilicate minerals (microcline, montmorillonite, muscovite, nontronite, sanidine) as determined by XRD (Table DR1). Pyrite was present in three of the rocks (R1 [Silty Limestone], R3 [Shale] and R5 [Shale]). R5, a dark shale containing quartz, microcline, calcite and pyrite) was crushed and prepared in the same way as the pure mineral phases for use in the coupon sampler (RG Rock, Table DR1). The pyrite content of RG rock and RG Sediment was estimated at 0.56 and 1.2 weight % respectively, assuming all S came from pyrite, which is valid given the apparent lack of any other S sources from XRD of the catchment rocks. The mineralogy of the rocks suggests that the major elements and their ions capable of serving as a substrate to support microbial metabolism in the catchment rocks were  $\text{Fe}^{2+}$  and  $\text{S}^-$  from pyrite ( $\text{FeS}_2$ ), since this was present in 3 of the 5 rocks analysed. While Fe containing nontronite ( $\text{Na}_{0.3}\text{Fe}_2\text{Si}_4\text{O}_{10}(\text{OH})_24\text{H}_2\text{O}$ ), and ankerite ( $\text{Ca}(\text{Fe},\text{Mg})(\text{CO}_3)_2$ ) were detected, these were only present in one of the five rocks. Olivine stoichiometry was determined from EDS results (Table DR1).

Mineral coupons, composed of capped stainless steel mesh cylinders (25.4 x 1.27 cm; 1-mm mesh size) (Figure DR1b), were prepared with approximately 5 g each of pyrite, hematite, magnetite, quartz, calcite, olivine [ $\text{Fo}_{90}\text{Fa}_{10} = \text{Mg}_{1.8}\text{Fe}_{0.2}\text{SiO}_4$ ], and RG Rock. All substrata were separated within the coupon by plugs of glass wool. Mineral-loaded coupons were sterilized by combustion (550°C, 6 h) under an atmosphere of  $\text{N}_2$ . Coupons were suspended in the glacial outflow channel of RW approximately 10 meters downstream of the

glacier terminus where the stream bed was well developed. Coupons were incubated for seven months, spanning October 2007 to July 2008 in order to promote colonization of substrata by those microorganisms with a propensity for attachment. The large coupon mesh size allowed access of the particle surfaces to bacteria suspended in the surrounding groundwater. After incubation, coupons were retrieved, placed in sterile bags, frozen on site using a dry ice/ethanol slurry, and transported frozen to Montana State University where they were stored at -80°C until further processed.

**DR 1.3. Subglacial Meltwater and Sediment Sampling and Processing.** All sample collection and processing was undertaken using aseptic techniques, as described below. Subglacial meltwaters were collected from RW at the site of coupon deployment during the summer ablation season (July 2007) and at the time that the coupon was deployed (October, 2007). Supraglacial meltwaters, from the surface of the glacier, were also collected during July 2007. Samples of subglacial meltwaters (RW water) were collected in autoclaved sterile bottles and approximately 500 mL was immediately filtered in an autoclaved Nalgene filter unit containing pre-sterilized 0.22 µm PVDF membrane filters that were emplaced using ethanol soaked, flame sterilized metal tweezers. Following filtration, the filters with accumulated suspended sediment were placed in sterile DNA free 2.0 mL screw-cap vials using ethanol soaked, flame sterilized metal tweezers and were immediately frozen using a dry ice/ethanol slurry. Samples were transported back to MSU frozen where they were stored at -80°C until processed for genomic DNA extraction. In the laboratory, filters were cut in half using a sterile spatula and were placed in separate bead beating tubes for genomic DNA extraction (see below). 500 mg aliquots of fine-grained subglacial sediment (RG Sediment) were also collected from the site of coupon deployment at the time that the coupon was retrieved using ethanol soaked, flame sterilized metal scoops. Triplicate subsamples of

sediment were placed in sterile bead beating tubes in the field with sterile spatulas and tubes and contents were immediately flash frozen on site in a dry ice/ethanol slurry. Sediment samples were kept at -80°C until further processed for genomic DNA extraction.

**DR 1.4. Mineral Substrata Processing.** Mineral coupons were thawed and disassembled in a sterile laminar flow hood. Each individual mineral phase was removed from the coupon and granules were placed in a sterile petri dish containing molecular-grade sterile water (Sigma-Aldrich, St. Louis, MO). Unattached microbial cells and/or fine-grained glacial till were removed from the mineral phases by gentle agitation of the petri dishes. This process was repeated three times, until no observable till was being released from the mineral phases. Triplicate 500 mg subsamples of individual mineral granules from the coupons were transferred directly to bead-beating DNA extraction vials

**DR 1.5. Community DNA Extraction and Community Bacterial 16S rDNA T-RFLP Analysis.** DNA was extracted from the 500 mg samples of individual mineral substrate, subglacial sediments, and from filter papers in triplicate (duplicate in the case of filtered water) using the Bio101 FastDNA SPIN Kit for Soil (MP Bio Medicals, Solon, OH) using a slightly modified protocol as described previously (Boyd et al., 2007b). Equal volumes of each extraction were pooled and genomic DNA was quantified fluorometrically using previously described methods (Boyd et al., 2007b). Approximately 15 ng of genomic DNA from each substratum type, RG sediment, and RG water filter paper pooled extracts was subjected to thirty cycles of PCR in triplicate using bacterial-specific primers 8F and 907R, according to previously described protocols (Boyd et al., 2007a, however the forward primer was modified with phosphoramidite fluorochrome 5-carboxyfluorescein (FAM) at the

5' terminus (Invitrogen). Replicate PCR products were pooled, purified, and subjected to T-RFLP digestion, electrophoresis, and analysis as previously described {Boyd, 2007 #32}.

**DR 1.6. Statistical Analysis of T-RFLP Electropherograms.** For the purposes of this study, distinct T-RFs were considered to be unique operational taxonomic units (OTU) and were the unit by which individual phylotypes were demarcated. The Bray-Curtis index (e.g., Sørensen index), which represents an abundance weighted metric describing the similarity of communities, ranges from 0 to 1, with higher values indicative of greater similarity. Bray-Curtis similarity for community comparisons were calculated using PAST (ver. 1.7.2) (Hammer et al., 2001). Hierarchical cluster analysis with multiple (1000) bootstrap was performed with the program pvclust (<http://www.is.titech.ac.jp/~shimo/prog/pvclust/>) and the base package within R (version 2.10.1) (<http://www.r-project.org/contributors.html>). Hierarchical clustering was based on Ward's agglomerative correlation method with the Bray-Curtis similarity indices serving as input for the analysis.

**DR 1.7. PCR Amplification of 16S rRNA Genes for Sequence Analysis.**

Approximately 15 ng of genomic DNA from the subglacial sediment and pyrite substratum DNA extracts was subjected to thirty cycles of PCR in triplicate using bacterial-specific primers 8F and 907R according to previously described protocols (Boyd et al., 2007a). Replicate PCR products were pooled, purified using the Promega Wizard purification kit (Madison, WI), quantified using the Low Mass DNA Ladder (Invitrogen), cloned using the pGEM Easy Vector System (Promega), and sequenced using the M13F and R primer pair as previously described (Boyd et al., 2009). Sequences were assembled using BioEdit (ver.

7.0.9.0) (Hall, 1999) and were checked for chimeric artifacts using Mallard (ver. 1.0.2) (Ashelford et al., 2005).

**DR 1.8. Phylogenetic analysis.** Bacterial 16S rRNA genes from both the subglacial sediment- and pyrite-associated communities were compiled and aligned using ClustalX (ver. 2.0.9) (Thompson et al., 1994) specifying the IUB substitution matrix and default gap extension and opening penalties. The phylogenetic position of bacterial 16S rRNA genes was assessed using PhyML (ver. 3.0) (Guindon and Gascuel, 2003) using the GTR substitution model with gamma-shaped rate variation and a proportion of invariable sites as recommended by ModelTest (ver. 3.8) (Posada, 2006). The 16S rRNA genes from *Acidilobus sulfurireducens* str. 18D70 and *Caldisphaera draconis* str. 18U65, both of which are members of the Crenarchaeota, served as outgroups. The 16S rRNA gene phylogram was rate-smoothed with a molecular clock approach using the multidimensional version of Rambaut's parameterization as implemented in PAUP (ver 4.0) (Swofford, 2001). *P*-values indicating the probability that each environment has more unique branch length (e.g., are not overlapping phylogenetically) than expected by chance were determined using 100 permutations and the weighted Unifrac significance test of each pair of environments as implemented in the program Unifrac (Lozupone and Knight, 2005). If the assemblages did not harbor significant unique branch length at a *P* value of < 0.05, they were considered to overlap phylogenetically (Lozupone and Knight, 2005).

**DR 1.9. Anions, cations and stable isotopes.** Anion and cation analyses were performed on aliquots of filtered water (0.22 µm) that were stored on ice in plastic scintillation vials without headspace. Major anions and cations were measured using a Dionex 3000 ICS ion chromatography system (Dionex, Sunnyvale, CA) according to

previously published methods (Lewis et al., 2012). The precision (based on repeats of standards and samples) was better than 2% for all analytes. pH and electrical conductivity were measured in the field by using an Orion 4 Star pH/Conductivity meter and probes designed for low ionic strength solutions. Alkalinity was determined using a Hach Digital Titrator.

The sulfur ( $^{34}\text{S}/^{32}\text{S}$ ) and oxygen ( $^{18}\text{O}/^{16}\text{O}$ ) isotope ratios of  $\text{SO}_4^{2-}$  ( $\delta^{34}\text{S}-\text{SO}_4^{2-}$  and  $\delta^{18}\text{O}-\text{SO}_4^{2-}$ ) were analyzed at the University of Calgary Isotope Lab following standard procedures (University of Calgary, 2010a, b, c). Briefly,  $\text{BaSO}_4$  was precipitated from 1-2L of filtered sample by acidification with HCl and addition of a saturated  $\text{BaCl}_2$  solution (University of Calgary, 2010b). The sulfur isotope ratio of the  $\text{BaSO}_4$  precipitate was measured using Continuous Flow-Isotope Ratio Mass Spectrometry (CF-EA-IRMS) on a Carlo Erba NA 1500 elemental analyzer interfaced to a VG PRISM II mass spectrometer. The sulfur isotope results are expressed in the usual per mil notation ( $\delta^{34}\text{S} \text{‰}$ ) relative to the international VCDT standard. The precision of  $\delta^{34}\text{S}_{\text{BaSO}_4}$  using this technique, is generally better than  $\pm 0.25$  (n=10) based on daily reproducibility tests (University of Calgary, 2010c). The  $\delta^{18}\text{O}$  of  $\text{BaSO}_4$  is determined using a high temperature, pyrolysis reactor (Finnigan MAT TC/EA) coupled with an isotope ratio mass spectrometer (Finnigan Mat Delta+XL) in continuous flow mode (using Conflow III open split/interface). Results are expressed in the usual per mil notation ( $\delta^{18}\text{O} \text{‰}$ ) relative to the international VSMOW standard. Accuracy and precision of  $\delta^{18}\text{O}$  of  $\text{BaSO}_4$  is generally better than  $\pm 0.3\text{‰}$  (one standard deviation based on n=50 lab standards) (University of Calgary, 2010a). Oxygen ( $^{18}\text{O}/^{16}\text{O}$ ) isotope ratios of  $\text{H}_2\text{O}$  ( $\delta^{18}\text{O}-\text{H}_2\text{O}$ ) were determined using the  $\text{CO}_2-\text{H}_2\text{O}$  equilibrium technique with subsequent analysis using continuous flow input to a ThermoFinnigan DELTAplusXP mass spectrometer (Richardson et al., 2009).  $\delta^{18}\text{O}-\text{H}_2\text{O}$  values are expressed in the usual per mil notation ( $\delta^{18}\text{O} \text{‰}$ ) relative to the international VSMOW standard.

## DR 2. EXTENDED RESULTS

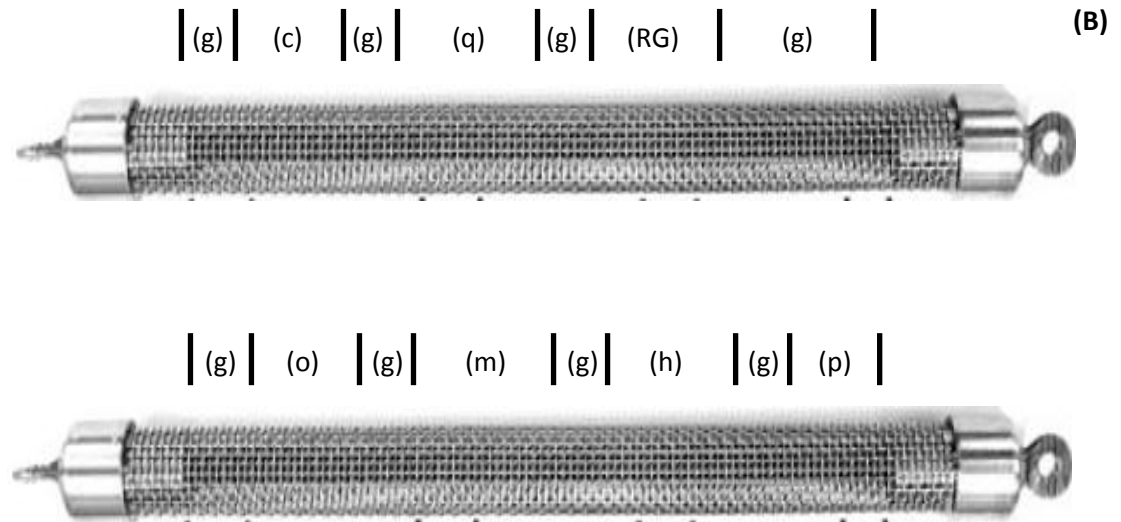
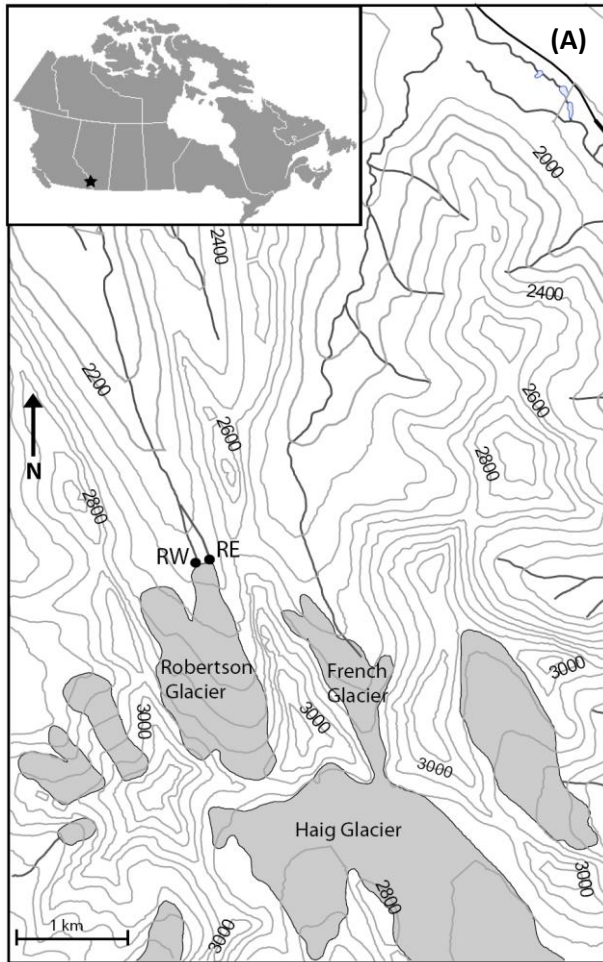


Fig DR1. (A) Topographic map of RG showing the two bulk meltwater streams RW and RE. The coupons were incubated ~ 10m from the snout of the glacier in RW. Gray shaded areas are glaciers. Contour interval is 100 m. The inset image (top left) indicates the location of RG (star) within Alberta, Canada. Map was prepared from NTS 1:50,000 maps 82J/14 and 82J/11 (© Department of Natural Resources Canada. All rights reserved). (B) Biofilm coupon (25.4 × 1.27 cm) used to retain mineral phases during incubation in subglacial environment. Two coupons were required to contain all the mineral phases. These were located in each, as shown above. Glass wool spacer (g), pyrite (p), hematite (h), magnetite (m), quartz (q), calcite (c), olivine (o), catchment rock (RG).



Table DR1. Mineralogical (X-Ray Diffraction [XRD]) and elemental composition (Energy-dispersive X-ray spectroscopy [EDS]) of a selection of catchment rocks, and proglacial sediment from the Robertson catchment. Elemental composition of minerals used in incubated coupons also shown. Main rocks types observed and collected were limestone (with impurities) and shale. \* Indicates rock which was crushed and used in mineral coupons (RG Rock). General rock types: Silty limestone (SL); Argillaceous limestone (AL); Dark Shale (S).

		Catchment Rocks					RG						
Mineral	Formula	R1	R2	R3	R4	R5*	Sediment						
		SL	AL	S	AL	S							
Calcite	CaCO <sub>3</sub>	✓	✓	✓	✓	✓	✓						
Quartz	SiO <sub>2</sub>	✓	✓	✓	✓	✓	✓						
Pyrite	FeS <sub>2</sub>	✓		✓		✓	✓						
Microcline	KAlSi <sub>3</sub> O <sub>8</sub>		✓				✓						
Montmorillonite-14A	Na <sub>0.3</sub> (Al,Mg) <sub>2</sub> Si <sub>4</sub> O <sub>10</sub> (OH) <sub>2</sub> H <sub>2</sub> O		✓										
Montmorillonite-18A	Na <sub>0.3</sub> (Al Mg) <sub>2</sub> Si <sub>4</sub> O <sub>10</sub> OH <sub>2</sub> 6H <sub>2</sub> O		✓										
Muscovite	KAl <sub>2</sub> Si <sub>3</sub> AlO <sub>10</sub> (OH) <sub>2</sub>			✓		✓							
Nontronite-15A	Na <sub>0.3</sub> Fe <sub>2</sub> Si <sub>4</sub> O <sub>10</sub> (OH) <sub>2</sub> 4H <sub>2</sub> O	✓											
Sanidine	K(Si <sub>3</sub> Al)O <sub>8</sub>				✓	✓							
Ankerite	Ca(Fe,Mg)(CO <sub>3</sub> ) <sub>2</sub>			✓									
Element	(weight %) by EDS							Coupon minerals					
								Oli-vine	Pyrite	Hem-atite	Mag-netite	Cal-cite	Qua-rtz
O		55	54	55	54	55	54	46		30	16	48	70
Na		nd	nd	0.34	nd	0.27	0.2						
Mg		0.71	1.5	1.5	1.4	1.9	2.1	28					
Al		1.1	4	3.4	6	2.3	4.2						
Si		5.8	28	17	26	29	15	18					30
S		0.79	0.35	1.3	0.3	0.3	0.6		53				
K		0.58	4.1	2.4	4.1	2.2	2.4						
Ca		36	6.3	17	7.2	8.8	20					40	
Fe		0.89	1.8	2.6	0.9	0.98	1.6	7.5	47	70	84		
Cr		nd	nd	nd	nd	nd	nd	0.36					
Ni		nd	nd	nd	nd	nd	nd	0.49					

Table DR2. Meltwater chemistry from the supraglacial and subglacial (bulk meltwater) environment at RG. Units, dissolved ions, in  $\mu$  moles  $L^{-1}$ . Enrichment factors shown are calculated as the ratio of the average concentration of species in the July supraglacial meltwater relative to concentrations in RW bulk meltwater in July (17/07/2007) and on coupon installation day in October (13/10/2007). See section DR2.1 and 2.2 for extended description.

	Supraglacial (17/7/2007)				Subglacial / bulk meltwaters (RW) (17/7/2007)				Subglacial / bulk meltwaters (RW) (13/10/2007)	Enrichment factor (17/7/2007)	Enrichment factor (13/10/2007)
	min	max	Average (n=5)	var%	min	max	Average (n=2)	var%			
<b>Time</b>									n = 1		
<b>EC (<math>\mu</math>S/cm)</b>	3.5	27	11	84	79	87	83	7.1	460	7.6	42
<b>pH</b>	6.8	8.6	7.7	8.2	7.2	7.4	7.3	1.3	6.8	0.95	0.89
<b>Cl<sup>-</sup></b>	0.17	0.99	0.54	66	0.48	2.3	1.4	92	8.5	2.6	16
<b>SO<sub>4</sub><sup>2-</sup></b>	0.36	0.91	0.58	37	110	160	140	21	2100	230	3600
<b>NO<sub>3</sub><sup>-</sup></b>	0	1.2	0.63	81	1.6	3.4	2.6	47	1.3	4.0	2.1
<b>Na<sup>+</sup></b>	0.20	1.4	0.78	76	1.9	3.4	2.6	41	44	3.3	55
<b>NH<sub>4</sub><sup>+</sup></b>	0.17	1.7	0.72	94	1.2	1.3	1.3	2.6	0.67	1.7	0.91
<b>K<sup>+</sup></b>	0.049	1.2	0.36	120	2.0	2.8	2.3	23	28	6.4	77
<b>Mg<sup>2+</sup></b>	1.5	4.0	2.3	43	44	60	52	21	990	22	41
<b>Ca<sup>2+</sup></b>	15	140	57	92	270	270	270	1.1	2200	4.8	40
<b>Measured HCO<sub>3</sub><sup>-</sup></b>	43	260	120	74	390	410	390	1.7	1500	3.3	13
<b><math>\delta^{34}\text{S-SO}_4^{2-}</math></b>	-	-	-	-	-	-	-	-	3.0	-	-
<b><math>\delta^{18}\text{O-SO}_4^{2-}</math></b>	-	-	-	-	-	-	-	-	-13.2	-	-
<b><math>\delta^{18}\text{O-H}_2\text{O}</math></b>	-	-	-	-	-19.1	-19.2	-19.2	0.50%	-18.7	-	-

Table DR3. Phylogenetic affiliation of phylotypes associated with 16S rRNA gene clone libraries from DNA extracted from (a) pyrite and (b) RG sediment. See section DR2.3 for extended description. Organisms that have been demonstrated to catalyze the oxidation of soluble and/or solid phase ferrous iron and sulfur in pure cultures (Karavaiko et al., 2003; Okereke and Stevens, 1991; Susuki et al., 1990) are shown in comments.

97% similarity threshold for OTU clustering

Clone (Accession#)	Freq	Closest BLAST Hit (Accession #) (% identity)	Closest Cultivated Organism (Accession #) (% identity)	Class	Comments
<b>(a) PYRITE</b>					
P1 (HM635783)	5	Uncultured glacial foreground soil bacterium clone (GQ397041) (98%)	<i>Steroidobacter denitrificans</i> strain FS (EF605262) (88%)	$\gamma$ -Proteobacteria	
P3 (HM635784)	1	Uncultured marine sediment bacterium clone (EF459825) (97%)	<i>Demequina lutea</i> strain SV47 (79%)	Actinobacteria	
P4 (HM635785)	1	Uncultured marine bacterium clone (EU803726) (97%)	<i>Ilumatobacter fluminis</i> (AB360343) (88%)	Actinobacteria	
P5 (HM635786)	3	Uncultured freshwater sediment bacterium clone (DQ463271) (98%)	<i>Flavobacterium granuli</i> (AB180738) (96%)	Flavobacteria	
P9 (HM635787)	1	Flavobacterium sp. FB7 (AM933500) (96%)	Flavobacterium columnare (AY747592) (96%)	Flavobacteria	
P16 (HM635788)	1	Uncultured glacial foreground soil bacterium clone (GQ396822) (99%)	<i>Thiobacillus denitrificans</i> strain ME16 (EU546130) (94%)	$\beta$ -Proteobacteria	iron/sulfur cycling
P18 (HM635789)	2	Uncultured high arctic desert bacterium clone (AM940601) (96%)	<i>Sporocytophaga</i> sp. 4v (FJ372724) (85%)	Cytophagia	
P19 (HM635790)	6	<i>Clostridium pasteurianum</i> (M23930) (99%)	<i>Clostridium pasteurianum</i> (M23930) (99%)	Clostridia	
P20 (HM635791)	1	Uncultured glacial sediment clone 39B (EU919774) (94%)	<i>Thiobacillus denitrificans</i> strain ME16 (EU546130) (94%)	$\beta$ -Proteobacteria	iron/sulfur cycling
P21 (HM635792)	1	Uncultured wastewater clone (AB305033) (96%)	<i>Caldilinea aerophila</i> (AB067647) (87%)	Caldilineae	
P22 (HM635793)	1	Uncultured glacial sediment clone 39B (EU919774) (95%)	<i>Thiobacillus thiophilus</i> strain D24TN (EU685841) (94%)	$\beta$ -Proteobacteria	iron/sulfur cycling
P23 (HM635794)	1	Uncultured gold mine bacterium (AF337875.2) (98%)	<i>Acidithiobacillus ferrooxidans</i> (AJ459800) (90%)	$\gamma$ -Proteobacteria	iron/sulfur cycling
P26 (HM635795)	4	Uncultured Svalbardsoil clone (EU919753) (99%)	<i>Thiobacillus denitrificans</i> strain ME16 (EU546130) (97%)	$\beta$ -Proteobacteria	iron/sulfur cycling
P29 (HM635796)	1	Uncultured Subglacial clone (DQ628916) (95%)	<i>Pseudorhodobacter incheonensis</i> strain KOPRI (DQ001322) (94%)	$\alpha$ -Proteobacteria	
P30 (HM635797)	1	Uncultured gold mine bacterium (AF337868.2) (94%)	<i>Nitrosospira multiformis</i> ATCC 25196 (CP000103) (93%)	$\beta$ -Proteobacteria	
P32 (HM635798)	1	Uncultured cave clone (DQ823151) (98%)	<i>Sphingomonas jaspisi</i> (AB264131) (97%)	$\alpha$ -Proteobacteria	
P36 (HM635799)	1	Uncultured gold mine bacterium (AF337875.2) (98%)	<i>Acidithiobacillus ferrooxidans</i> (AJ459800) (90%)	$\gamma$ -Proteobacteria	iron/sulfur cycling
P38 (HM635800)	1	Uncultured freshwater clone (EU117950) (99%)	<i>Terracoccus luteus</i> strain DSM 44267 (NR_026412) (89%)	Actinobacteria	
P42 (HM635801)	1	Uncultured Antarctic sub ice clone (DQ521467) (99%)	<i>Brevundimonas</i> sp. AKB-2008-JO103 (AM988997) (99%)	$\alpha$ -Proteobacteria	
P44 (HM635802)	1	Uncultured aquifer clone (EU735705) (97%)	<i>Thiobacillus denitrificans</i> strain ME16 (EU546130) (92%)	$\beta$ -Proteobacteria	iron/sulfur cycling
P47 (HM635803)	1	Uncultured subglacial clone (DQ228359) (98%)	<i>Siderooxidans lithoautotrophicus</i> strain ES-1 (DQ386264) (95%)	$\gamma$ -Proteobacteria	iron/sulfur cycling
P48 (HM635804)	1	Uncultured dust bacterium (FM874454) (96%)	<i>Arenimonas</i> sp. YC6267 (EU376961) (94%)	$\gamma$ -Proteobacteria	
<b>(b) RG SEDIMENT</b>					
S3 (HM635805)	10	Uncultured subglacial sediment bacterium clone (DQ228359) (91%)	<i>Siderooxidans lithoautotrophicus</i> str. ES-1 (DQ386264) (96%)	$\gamma$ -Proteobacteria	iron/sulfur cycling
S5 (HM635806)	1	Uncultured subglacial sediment bacterium clone (DQ228359) (99%)	<i>Rhodoferrax ferrereducens</i> T118 (CP000267) (98%)	$\beta$ -Proteobacteria	iron/sulfur cycling
S9 (HM635807)	3	Uncultured glacial sediment bacterium clone (EU919753) (99%)	<i>Thiobacillus denitrificans</i> str. ME16 (EU546130) (97%)	$\beta$ -Proteobacteria	iron/sulfur cycling
S11 (HM635808)	2	Uncultured freshwater bacterium clone (FJ694303) (98%)	<i>Flavobacterium</i> sp. SOC A4(12) (DQ628951) (97%)	Flavobacteria	
S15 (HM635809)	1	Uncultured aquatic bacterium clone (EU800906) (98%)	<i>Paucimonas lemoignei</i> (X92554) (91%)	$\beta$ -Proteobacteria	
S22 (HM635810)	2	Uncultured subglacial sediment bacterium clone (AF479323) (97%)	<i>Rhodoglobus</i> sp. 01WB01-49 (FM161352) (97%)	Actinobacteria	
S26 (HM635811)	1	Uncultured glacial ice bacterium clone (EU978700) (100%)	<i>Brevundimonas</i> sp. 39(2008) (FJ197848) (98%)	$\alpha$ -Proteobacteria	
S27 (HM635812)	3	Uncultured glacial foreground soil bacterium clone (GQ397041) (97%)	<i>Beggiatoa</i> sp. 402 (AY583996) (91%)	$\gamma$ -Proteobacteria	
S34 (HM635813)	1	Uncultured Antarctica freshwater bacterium clone (EU869545) (99%)	<i>Rhodobacter</i> sp. ZS5-10 (FJ196040) (99%)	$\alpha$ -Proteobacteria	
S35 (HM635814)	1	Uncultured microbial mat clone (GQ441301) (98%)	<i>Kryptoperidinium foliaceum</i> strain CCMP1326 (GU591328)	Dinophyceae	
S39 (HM635815)	1	Uncultured glacial foreground soil bacterium clone (GQ397021) (94%)	<i>Sideroxydans lithotrophicus</i> ES-1 (CP001965) (90%)	$\beta$ -Proteobacteria	iron/sulfur cycling
S40 (HM635816)	1	Uncultured glacial sediment bacterium clone (DQ228387) (97%)	<i>Thiobacillus denitrificans</i> str. ME16 (EU546130) (94%)	$\beta$ -Proteobacteria	iron/sulfur cycling
S46 (HM635817)	3	Uncultured subglacial sediment bacterium clone (DQ228367) (95%)	<i>Siderooxidans lithoautotrophicus</i> str. ES-1 (DQ386264) (93%)	$\gamma$ -Proteobacteria	iron/sulfur cycling
S47 (HM635818)	1	Uncultured glacial foreground soil bacterium clone (GQ397041) (98%)	<i>Steroidobacter denitrificans</i> str. FS (EF605262) (88%)	$\gamma$ -Proteobacteria	
S48 (HM635819)	1	Uncultured aquifer sediment bacterium clone (EU266776) (97%)	<i>Candidatus Magnetobacterium bavaricum</i> (FP929063) (87%)	<i>Nitrospira</i>	

Table DR4. Relative abundance<sup>a,b</sup> of T-RFs recovered from filtered water (RG water), RG sediment, or coupon rock- (RG rock) and mineral-associated bacterial communities.

T-RF (bp) <sup>c</sup>	RG Water	RG Sediment	Pyrite	Calcite	Magnetite	Hematite	RG Rock	Olivine	Quartz
67.9								3.3 (0.2)	
80.6		16.5 (5.6)	3.0 (1.1)	7.1 (3.6)	7.5 (0.5)	5.3 (1.6)			7.5 (0.6)
94.9								3.1 (0.4)	
117.5								13.2 (1.1)	
118.6	15.2 (1.0)	2.6 (0.5)	4.5 (1.4)				6.6 (0.7)	5.0 (0.3)	
120.5		11.4 (1.7)			3.2 (0.5)		2.5 (0.2)		
134.6	4.7 (0.2)	3.1 (0.7)					8.8 (0.7)		
136.6	4.6 (0.2)	3.2 (0.4)		2.8 (0.3)	3.3 (0.7)			11.8 (0.9)	
138.7	3.0 (0.2)			3.6 (0.3)	7.7 (0.4)		3.3 (0.3)	2.7 (0.3)	
141.1						4.7 (1.2)			
144.9		4.5 (0.7)					3.1 (0.4)		25.0 (0.8)
147.4	3.0 (0.1)	5.6 (1.7)			4.8 (1.2)			2.9 (0.1)	
150.3					3.6 (1.0)				
161.6								5.1 (0.4)	
176.9									25.2 (0.6)
206.7					4.5 (0.4)				
268.2								5.0 (0.2)	
282.3								2.8 (0.5)	
292.8								2.8 (0.1)	
294.6								2.9 (0.3)	3.5 (0.0)
297.9								4.6 (0.1)	4.5 (0.1)
401.4			2.9 (0.4)		4.8 (1.3)	7.0 (1.8)			5.8 (0.2)
402.7									4.4 (0.2)
427.6		5.7 (1.4)							
429.4	5.4 (0.2)	9.4 (1.2)	9.7 (0.8)				15.5 (0.6)		
430.9	11.5 (0.5)	3.0 (1.3)	11.1 (1.0)				14.4 (0.7)		4.3 (0.3)
433.0		3.8 (1.3)	7.2 (0.7)						

436.9					3.3 (0.6)	3.7 (0.8)			
439.3		5.0 (1.6)							
456.8						3.2 (0.8)	3.8 (0.3)		7.2 (0.5)
460.6						3.4 (1.0)			
468.3					3.9 (0.7)				
477.6			2.5 (0.2)						
482.2				3.1 (0.9)				18.9 (1.4)	
485.7	9.6 (0.6)	7.4 (3.8)	3.0 (0.2)	5.3 (1.5)	13.8 (2.4)	15.3 (3.2)	2.6 (0.2)		
488.7						5.8 (5.1)			
489.8	40.2 (0.7)	3.9 (2.0)	8.6 (1.6)	12.8 (3.7)	15.9 (8.5)	7.1 (5.2)	13.4 (3.5)		
492.4	2.8 (0.2)	7.4 (1.9)	7.2 (1.3)			5.8 (5.1)	3.2 (0.3)	3.1 (0.4)	4.1 (0.2)
494.6		3.9 (1.4)	9.3 (0.4)				15.2 (1.1)		
498.6						6.6 (1.6)			
499.7						3.2 (0.8)			
502.1						3.1 (0.8)			
511.8			13.9 (0.7)	45.0 (9.4)					
531.5						8.9 (2.4)			
534.7			3.3 (0.3)	3.0 (0.9)	5.3 (1.4)	3.8 (0.9)			
539.6						8.6 (2.6)			
542.0					11.3 (1.5)		4.3 (0.4)		8.5 (0.8)
559.2								3.3 (0.4)	
570.6			6.5 (0.6)	8.0 (2.4)					
600.7			4.0 (0.3)		3.3 (0.5)		3.4 (0.2)		
605.5				2.7 (0.7)				5.8 (0.8)	
688.2		3.6 (1.4)			3.6 (0.4)	4.6 (1.6)			
872.0								3.7 (0.6)	
887.5			3.1 (0.2)	6.4 (1.9)					
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

<sup>a</sup>Percent of total fluorescence (TF) of phylotypes representing greater than 2.0% TF. <sup>b</sup>Standard deviations of three replicate profiles reported in parentheses. <sup>c</sup>Terminal restriction fragment (T-RF) as base-pair (bp) length.

Table DR5. Bray–Curtis similarity of bacterial 16S rRNA gene assemblages derived from T-RFs (Table DR4) associated with different mineral substrata, Robertson Glacier (RG) sediments, or RG meltwater (RG Water).

<b>Bray-Curtis</b>	<i>RG Water</i>	<i>Sediment</i>	<i>Pyrite</i>	<i>Calcite</i>	<i>Magnetite</i>	<i>Hematite</i>	<i>RG Rock</i>	<i>Olivine</i>	<i>Quartz</i>
<i>RG Water</i>	1.00	0.34	0.35	0.24	0.35	0.19	0.50	0.18	0.07
<i>Sediment</i>		1.00	0.40	0.19	0.34	0.26	0.37	0.12	0.19
<i>Pyrite</i>			1.00	0.41	0.24	0.25	0.52	0.08	0.14
<i>Calcite</i>				1.00	0.35	0.21	0.19	0.11	0.07
<i>Magnetite</i>					1.00	0.42	0.29	0.09	0.21
<i>Hematite</i>						1.00	0.16	0.03	0.18
<i>RG Rock</i>							1.00	0.11	0.19
<i>Olivine</i>								1.00	0.11
<i>Quartz</i>									1.00

1           **DR 2.1. Meltwater chemistry.** Meltwaters collected from the supraglacial environment  
2 were dilute, with an average EC = 11  $\mu\text{S cm}^{-1}$ . Conversely, subglacial meltwaters collected at the  
3 site of coupon deployment in RW stream, were more concentrated than supraglacial waters by 7  
4 times on average in July 2007 (EC = 83  $\mu\text{S cm}^{-1}$ ) and 42 times in October 2007 (EC = 460  $\mu\text{S}$   
5  $\text{cm}^{-1}$ ) when the coupons were deployed. The dominant cation in the subglacial meltwaters was  
6  $\text{Ca}^{2+}$  (average, July = 270  $\mu\text{M L}^{-1}$ ) and the dominant anion was typically bicarbonate (average,  
7 July = 390  $\mu\text{M L}^{-1}$ ), followed by  $\text{SO}_4^{2-}$  (average, July = 140  $\mu\text{M L}^{-1}$  (Table DR2). Although, at  
8 the time of deployment  $\text{SO}_4^{2-}$  concentration (2100  $\mu\text{M L}^{-1}$ ) exceeded bicarbonate (1500  $\mu\text{M L}^{-1}$ ).  
9 The enrichment of individual ions from the supraglacial to subglacial environment was greatest  
10 for  $\text{SO}_4^{2-}$  (230 to 3600 times enriched),  $\text{Na}^+$  (3 to 55 times enriched),  $\text{K}^+$  (6 to 77 times  
11 enriched),  $\text{Mg}^{2+}$  (22 to 41 times enriched) and  $\text{Ca}^{2+}$  (5 to 40 times enriched), with the greatest  
12 enrichment in October when subglacial meltwaters were most concentrated. Conversely,  $\text{NH}_4^+$   
13 and  $\text{NO}_3^-$  were less enriched in subglacial meltwaters in October, with greatest enrichment in  
14 July (Boyd et al., 2011). The pH of the subglacial meltwaters was neutral to mildly basic,  
15 ranging between 6.8 and 7.4.

16  
17           **DR 2.2. Isotope Geochemistry**  $\delta^{34}\text{S}$ - $\text{SO}_4^{2-}$  and  $\delta^{18}\text{O}$ - $\text{SO}_4^{2-}$  values from RW at the time of  
18 the coupon deployment suggest pyrite as the dominant  $\text{SO}_4^{2-}$  source (Table DR2). The  $\delta^{34}\text{S}$   
19 values from pyrite are usually slightly positive (e.g. 0 to 10‰), although reported values vary  
20 widely (-20 to 15‰, (Nielsen et al., 1991)). However, the  $\delta^{34}\text{S}$  values of sulfate in Upper  
21 Devonian evaporites in North America are very positive, with  $\delta^{34}\text{S}$  values ranging from  
22 approximately 22 to 34‰ (Nielsen et al., 1991). Thus, the  $\delta^{34}\text{S}$  value of sulfate in RW at 3 ‰ is  
23 consistent with a pyrite rather than evaporite source. Further, the  $\delta^{18}\text{O}$  value of sulfate in Upper

24 Devonian evaporites in North America varies between approximately 13 to 18‰ (Nielsen et al.,  
25 1991) while, the  $\delta^{18}\text{O-SO}_4^{2-}$  values in the RW sample is negative (-13‰). A plausible process  
26 that could account for the negative  $\delta^{18}\text{O-SO}_4^{2-}$  value is that a portion of the  $\text{SO}_4^{2-}$  was produced  
27 via anoxic sulfide oxidation, a process where most or all of the oxygen in  $\text{SO}_4^{2-}$  are derived from  
28 the water ( $\delta^{18}\text{O-H}_2\text{O}$ , -18.7 ‰) (Bottrell and Tranter, 2002; Lafrenière and Sharp, 2005).

29

30 **DR 2.3. Bacterial Community Composition.** To characterize the influence of pyrite on  
31 subglacial sediment-associated bacterial community composition, we characterized the diversity  
32 of 16S rRNA genes recovered from pyrite-associated minerals, as well as from native subglacial  
33 sediments sampled adjacent to the mineral coupons (main paper, Figure 1; Table DR3). A large  
34 proportion of the clones were most closely affiliated with lithotrophic organisms that metabolize  
35 Fe and /or S, (main paper, Figure 1; Table DR3). For example, 56% of the 16S rRNA gene  
36 clones recovered from the sediment-associated community were most closely affiliated with  
37 *Sideroxydans lithotrophicus* ES-1 and *Thiobacillus denitrificans* ME16, both of which have been  
38 shown to oxidize ferrous iron in pure culture (Emerson et al., 2007; Karavaiko et al., 2003;  
39 Okereke and Stevens, 1991; Suzuki et al., 1990). Additionally the closest BLAST hits for 46%  
40 of the pyrite-associated clones and 84% of the sediment-associated clones were from uncultured  
41 microorganisms recovered from other glacial or polar soils and subglacial sediments (Table  
42 DR3). This finding is consistent with the proposal that glacierised environments are selecting for  
43 similar communities, due to mineralogical controls imparted by similar bedrock geology  
44 (Skidmore et al., 2005), in concert with prevailing hydrological regimes (Tranter et al., 2005),  
45 nutrient availability and redox conditions (Wadham et al., 2004; Wynn et al., 2007).



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