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## **A genome compendium reveals diverse metabolic adaptations of Antarctic soil microorganisms**

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## 1 **Abstract**

2 A surprising diversity and abundance of microorganisms resides in the cold desert  
3 soils of Antarctica. The metabolic processes that sustain them, however, are poorly  
4 understood. In this study, we used metagenomic and biogeochemical approaches to  
5 study the microbial communities in 16 physicochemically diverse mountainous and  
6 glacial soils from remote sites in South Victoria Land, north of the Mackay Glacier.  
7 We assembled 451 metagenome-assembled genomes from 18 bacterial and  
8 archaeal phyla, constituting the largest resource of Antarctic soil microbial genomes  
9 to date. The most abundant and prevalent microorganisms are metabolically  
10 versatile aerobes that use atmospheric hydrogen and carbon monoxide to meet  
11 energy, carbon, and, through metabolic water production, hydration needs.  
12 Phylogenetic analysis and structural modelling infer that bacteria from nine phyla can  
13 scavenge atmospheric hydrogen using a previously unreported enzyme family, the  
14 group 1I [NiFe]-hydrogenases. Consistently, gas chromatography measurements  
15 confirmed most soils rapidly consume atmospheric hydrogen and carbon monoxide,  
16 and provide the first experimental evidence of methane oxidation in non-maritime  
17 Antarctica. We also recovered genomes of microorganisms capable of oxidizing  
18 other inorganic compounds, including nitrogen, sulfur, and iron compounds, as well  
19 as harvesting solar energy via photosystems and novel microbial rhodopsins.  
20 Bacterial lineages defined by symbiotic lifestyles, including Patescibacteria,  
21 Chlamydiae, and predatory Bdellovibrionota, were also surprisingly abundant. We  
22 conclude that the dominant microorganisms in Antarctic soils adopt mixotrophic  
23 strategies for energy and sometimes carbon acquisition, though they co-exist with  
24 diverse bacteria and archaea that adopt more specialist lifestyles. These  
25 unprecedented insights and associated genome compendium will inform efforts to  
26 protect biodiversity in this continent.

## 27 Introduction

28 Continental Antarctica is a relatively pristine but oligotrophic wilderness<sup>1</sup>. Terrestrial  
29 life on the continent is adapted to extremely low temperatures, low water  
30 bioavailability, highly limited organic carbon and nitrogen, salt accumulation and  
31 seasonal light/dark periodicity<sup>2-4</sup>. These cumulative pressures exclude most  
32 macroscopic fauna and flora, and instead microorganisms constitute most of the  
33 continent's biodiversity and biomass<sup>5</sup>. While historical observational surveys  
34 indicated that few microorganisms existed in terrestrial Antarctica, subsequent  
35 molecular studies have uncovered rich and abundant microbial communities,  
36 especially in the continent's ice-free regions<sup>6-10</sup>. Antarctic soil communities are  
37 comparable to mesophilic soils at the phylum level, with Actinobacteriota,  
38 Acidobacteriota, Chloroflexota and Proteobacteria often predominant<sup>2,8,9,11,12</sup>. These  
39 communities are highly specialised at lower taxonomic levels<sup>7,8</sup>, however, and have  
40 unique functional traits<sup>11,13</sup>. Complementary culture-based studies have also  
41 isolated a growing number of taxa from the continent, although from relatively few  
42 phyla<sup>14-17</sup>. Most community members are assumed to be extremely slow-growing or  
43 adopt dormant states to adapt to the physicochemical conditions of the continent<sup>18</sup>.  
44 In turn, the formation of a microbial 'seed bank' may provide a means to maintain  
45 biodiversity<sup>19,20</sup>.

46 An enduring question is what metabolic strategies enable soil microorganisms to  
47 meet energy and carbon needs on this continent<sup>2</sup>. Even in dormant states, cells still  
48 require a net energy input to maintain cellular integrity, repair damaged  
49 macromolecules, and generate a basal membrane potential<sup>21,22</sup>. Conventionally it  
50 was thought that Cyanobacteria and microalgae are the major primary producers in  
51 Antarctic soils and that they produce the organic carbon to sustain  
52 organoheterotrophic bacteria<sup>2,11</sup>. However, oxygenic photoautotrophs are typically in  
53 low abundance (<1% of total bacterial community) outside lithic niches<sup>11,23</sup> and  
54 hence are unlikely to produce sufficient organic carbon to sustain the energy and  
55 carbon needs of the dominant community members. More recently, some Antarctic  
56 soil bacteria were shown to conserve energy and acquire carbon independently of  
57 photoautotrophs<sup>12</sup>. Genome-centric metagenomic studies have revealed that  
58 bacteria from several phyla, including Actinobacteriota, consume molecular

59 hydrogen (H<sub>2</sub>) and carbon monoxide (CO) from the atmosphere. By liberating  
60 electrons from these ubiquitous and diffusible trace gases, these bacteria sustain  
61 aerobic respiration and fix carbon even when preferred organic substrates are  
62 limiting<sup>12,24</sup>. However, given the relatively few metagenome-assembled genomes  
63 (MAGs) recovered (21) and limited geographical scope of this previous study<sup>12</sup>, it is  
64 unknown whether trace gas oxidation is a widespread strategy among Antarctic  
65 bacteria. Several molecular and biogeochemical studies have detected signatures of  
66 carbon fixation through the Calvin-Benson-Bassham (CBB) cycle within the  
67 continent, though it is unclear whether this originates through activities of  
68 photoautotrophs or lithoautotrophs<sup>12,13,25–27</sup>. Molecular evidence also suggests that  
69 some Antarctic soil bacteria can also conserve energy through other means,  
70 including methanotrophy, nitrification, and rhodopsin-based light harvesting<sup>12,13,16,28–</sup>  
71 <sup>30</sup>.

72 Here we build on these initial findings to develop a holistic genome-resolved  
73 understanding of the metabolic capabilities of Antarctic soil microorganisms. We  
74 profiled 16 soils with distinct physicochemical properties from the Mackay Glacier  
75 region, a cold hyper-arid ice-free region to the north of the McMurdo Dry Valleys that  
76 comprises approximately 15% (~4,800 km<sup>2</sup>) of the ice-free regions on the continent.  
77 Soil microbial communities in this region are adapted to average annual  
78 temperatures of -20°C and annual precipitation below 50 mm<sup>31,32</sup>, as well as  
79 profound limitation for organic carbon (~0.1%) and nitrogen (~0.02%)<sup>33</sup>. Through  
80 deep metagenomic sequencing, we generated a resource of 451 metagenome-  
81 assembled genomes, covering all major microbial lineages in the region. We  
82 confirmed that the most abundant bacteria in the region are mixotrophs that  
83 scavenge atmospheric trace gases, and substantiated these findings with  
84 biogeochemical assays confirming rapid gas consumption and phylogenetic  
85 analyses revealing a novel hydrogenase family. These findings lend strong support  
86 to the recent hypothesis that survival in desert soils depends on continual harvesting  
87 of alternative energy sources<sup>18</sup>. Nevertheless, these metabolically versatile bacteria  
88 co-exist with microorganisms that adopt a wide range of other nutritional and  
89 ecological strategies, including apparent obligate parasites and predators.  
90 Altogether, Antarctic soils appear to harbour much more compositionally rich and  
91 functionally complex microbial life than previously assumed.



## 92 Results and Discussion

### 93 Genome-resolved metagenomics reveals phylogenetically diverse bacteria co- 94 exist across the Mackay Glacier region

95 We analyzed surface soils from sixteen glacial and mountainous sites sampled  
96 across the Mackay Glacier region of South Victoria Land. Physicochemical analysis  
97 confirmed that the soils varied in key properties (e.g. pH, salinity, micronutrients,  
98 texture), but in common with previously characterized soils from continental Antarctic  
99 regions<sup>8,34,35</sup>, all had exceptionally low organic carbon content (0.02 – 0.25%) (**Table**  
100 **S1**). These soils nevertheless supported moderately abundant bacterial and  
101 archaeal communities ( $1.7 \times 10^6$  to  $2.7 \times 10^7$  16S rRNA gene copies per gram soil  
102 wet weight) (**Figure 1a**). Based on high-resolution 16S rRNA amplicon sequencing<sup>36</sup>  
103 (**Figure S1a & S1b**), observed richness ( $832 \pm 258$ ) and Shannon index ( $5.27 \pm$   
104  $0.31$ ) were high in most samples, implying diverse community members co-exist in  
105 these soils (**Figure 1c; Figure S1d**). Beta diversity analysis confirmed microbial  
106 communities diverge between sampled regions and with geographic distance  
107 (**Figure 1d; Figure S1e**).

108 To determine the community composition of the samples, we retrieved and classified  
109 shotgun metagenomic reads of the universal single-copy ribosomal protein gene *rpIP*  
110 (**Table S2**). The dominant community members were from bacterial phyla known to  
111 predominate in soil ecosystems<sup>37,38</sup>. Actinobacteriota, Proteobacteria,  
112 Acidobacteriota, Chloroflexota, Gemmatimonadota, Verrucomicrobiota and  
113 Bacteroidota were particularly abundant (**Figure 1b**), in agreement with other  
114 Antarctic surveys<sup>2,18</sup>. Cyanobacteria were scarce in most soils except for Pegtop  
115 Mountain and Cliff Nunatak, accounting for an average of 0.50% in the soil  
116 communities. Likewise, Archaea were minor members of this ecosystem (av. 0.88%)  
117 and mainly comprised the ammonia-oxidizing order Nitrososphaerales (**Figure 1b**).  
118 More surprisingly, bacterial phyla that predominantly adopt a predatory  
119 (Bdellovibrionota)<sup>39</sup>, intracellular parasitic (Dependentiae and Verrucomicrobiota\_A /  
120 Chlamydiae)<sup>40,41</sup> or obligately symbiotic (Patescibacteria)<sup>42,43</sup> lifestyle were  
121 prevalent and sometimes highly abundant, for example together comprising 17% of  
122 the community at Mount Murray. This suggests that a range of symbiotic interactions

123 occur in these communities. These dominant and rare phyla were also detected by  
124 16S rRNA gene sequencing (**Figure S1c**; **Table S3**).

125 These inferences on the composition and metabolic capabilities of the microbial  
126 communities were supported by genome-resolved analysis. From the 99.5  
127 gigabases of sequencing data (**Table S4**), we reconstructed a non-redundant set of  
128 101 high-quality and 350 medium-quality<sup>44</sup> metagenome-assembled genomes  
129 (MAGs). The recovered genomes span 18 different phyla, the relative composition of  
130 which reflects the community structure patterns observed in the *rpIP* and 16S rRNA  
131 analysis (**Figure 1b**). In turn, they capture all major microbial lineages (present at  
132 >1% relative abundance across all samples) and map to an average of 26% of reads  
133 in each metagenome (**Table S5**). To the best of our knowledge, this represents the  
134 largest sequencing effort and most extensive genomic resource reported from  
135 terrestrial Antarctica to date.

136

### 137 **Most abundant lineages encode enzymes supporting trace gas oxidation,** 138 **including a novel family of [NiFe]-hydrogenases**

139 We sought to understand which metabolic strategies support the numerous bacteria  
140 in these hyper-oligotrophic soils. We profiled the distribution and affiliation of 52  
141 conserved marker genes representing different energy conservation and carbon  
142 acquisition pathways in both the metagenomic short reads (**Table S6**) and MAGs  
143 (**Table S5**). In line with expectations, almost all community members encoded genes  
144 for aerobic organotrophic respiration (CoxA, NuoF, SdhA, AtpA) (**Figure 2**), whereas  
145 capacity for anaerobic respiration and fermentation was low (**Figure S2**). In addition  
146 to formate dehydrogenase, the other most abundant markers were the catalytic  
147 subunits of [NiFe]-hydrogenases (present in average of 90% community members),  
148 form I carbon monoxide dehydrogenases (32%), and RuBisCO (27%) (**Figure 2**).  
149 Phylogenetic analysis revealed that most binned sequences of these enzymes were  
150 most closely related to clades that support atmospheric H<sub>2</sub> oxidation<sup>45–49</sup> (**Figure**  
151 **3a**), atmospheric CO oxidation<sup>12,50–53</sup> (**Figure S3**), and chemosynthetic CO<sub>2</sub> fixation  
152<sup>12,54–56</sup> (**Figure S4**). Recent pure culture studies have shown that energy liberated by  
153 atmospheric H<sub>2</sub> and CO oxidation supports bacterial persistence during carbon  
154 starvation and, in some cases, mixotrophic growth<sup>52,57–62</sup>. Thus, the ability of

155 bacteria to harvest these trace gases may confer a major selective advantage in the  
156 carbon-depleted soils of Antarctica. Moreover, in extension of findings made in the  
157 Windmill Islands region <sup>12</sup>, over a quarter of the community may fix carbon via the  
158 CBB cycle, providing a mean to generate biomass independently of photoautotrophy.

159 Genes for trace gas oxidation were present in the most abundant and widespread  
160 community members. Uptake hydrogenases were encoded by MAGs affiliating with  
161 nine bacterial phyla (**Figure 2 & 3a**), including the seven dominant soil phyla (**Figure**  
162 **1**), whereas CO dehydrogenases were confined to Actinobacteriota and  
163 Chloroflexota (**Figure S3**). Indeed, 17 of the 20 most abundant Actinobacteriota and  
164 Chloroflexota MAGs encoded one or both enzymes (**Table S5**). Remarkably, the  
165 CBB pathway (**Figure S4; Table S7**) frequently co-occurs with hydrogenases (64%)  
166 and CO dehydrogenase (25%) in MAGs (**Figure 2; Table S5**), potentially enabling  
167 hydrogenotrophic, carboxydrotrophic or mixotrophic growth. This association was  
168 especially pronounced in the uncultivated classes Ellin6529 (Chloroflexota) and  
169 UBA4738 (Actinobacteriota) (**Table S6**), which respectively comprise an average of  
170 5.1% and 0.9% (maximum of 12.3% and 2.4%) of the communities across the region  
171 (**Table S2**). These classes are predicted to couple atmospheric H<sub>2</sub> and CO oxidation  
172 to fix carbon via their respective type IC and IE RuBisCO enzymes (**Figure S4;**  
173 **Table S7**). These traits in turn may contribute to their unexpectedly high relative  
174 abundance in Antarctica as well as other oligotrophic soils <sup>15,63–66</sup>. Indeed, given their  
175 abundance in the community and genetic potential for atmospheric chemosynthesis  
176 <sup>12,24</sup>, we hypothesize that both classes are major Antarctic primary producers. We  
177 propose replacing the placeholder names UBA4738 with *Candidatus* Aridivitia (arid  
178 Actinobacteriota class; based on high-quality type MAG MGR\_bin238, '*Candidatus*  
179 Aridivita willemsiae') and Ellin6529 with *Candidatus* Edaphomicrobia (edaphic  
180 Chloroflexota class; based on high-quality type MAG MGR\_130 '*Candidatus*  
181 Edaphomicrobium janssenii') (**Etymological Information**), as per recent taxonomic  
182 recommendations <sup>67,68</sup>.

183 Most microorganisms in the Mackay Glacier region encoded a novel hydrogenase  
184 family (**Figure 2**). We generated a maximum-likelihood tree of the conserved  
185 catalytic subunits of group 1 [NiFe] hydrogenases using amino acid sequences  
186 retrieved from 176 MAGs. All hydrogenase sequences form two major and  
187 tremendously diverse lineages that share less than 40% sequence identity with each

188 other and were supported by robust bootstrapping (**Figure 3a**). One branch is  
189 associated with characterized group 1h [NiFe] hydrogenases from multiple bacterial  
190 isolates <sup>45–47,51,61</sup>. The other forms a novel cluster, herein the group 1l [NiFe]-  
191 hydrogenase, which includes the previously unreported hydrogenases of McMurdo  
192 Dry Valleys isolate *Hymenobacter roseosalivarius* <sup>69</sup> and several other recently  
193 sequenced isolates. Group 1l is the prevailing hydrogenase family within the Mackay  
194 Glacier region, with an estimated abundance 2.3 times higher than group 1h (**Table**  
195 **S5**), and is encoded by all nine hydrogenase-bearing phyla and the two candidate  
196 classes. As elaborated in **Supplementary Note 1**, structural modelling shows that  
197 this enzyme shares common structural features with previously characterized group  
198 1h [NiFe]-hydrogenase <sup>70,71</sup>, but contains large sequence insertions and a key  
199 substitution in a residue ligating the proximal iron-sulfur cluster. Even more strikingly,  
200 the genes encoding this hydrogenase often have an unusual arrangement (**Figure**  
201 **S5; Table S7**), with five open reading frames predicted to encode small  
202 transmembrane proteins separating the small and large core structural subunits. On  
203 this basis, we predict that this enzyme is a *bona fide* high-affinity membrane-  
204 associated hydrogenase that relays electrons derived from atmospheric H<sub>2</sub> through  
205 the respiratory chain. The broad distribution and predominance of this hydrogenase  
206 suggests it is the primary mediator of H<sub>2</sub> oxidation in these soils. Moreover, given the  
207 strong positive correlation between this hydrogenase and RuBisCO based on the  
208 MAGs and metagenomic short reads ( $R^2 = 0.68$ ,  $p = 0.002$ ) (**Figure S6; Table S9**), it  
209 is likely that electrons yielded by this enzyme support carbon fixation either through  
210 direct transfer or reverse electron flow.

211

## 212 **Trace gas consumption occurs at sufficient rates to meet energy needs and** 213 **support hydration of Mackay Glacier region bacteria**

214 Our metagenomic analyses suggest that the most abundant soil bacteria across the  
215 Mackay Glacier region conserve energy and fix carbon by oxidizing atmospheric H<sub>2</sub>  
216 and CO. To test whether soil communities mediate these activities, we set up soil  
217 microcosms in which ambient air headspaces were amended with 10 parts per  
218 million (ppmv) of these gases and used high-sensitivity gas chromatography to  
219 measure their consumption over time. In line with predictions, H<sub>2</sub> was oxidized by

220 soils from all sixteen sites and all but three soils consumed CO (**Figure 4a**). Of  
221 these, all soils except Pegtop Mountain consumed H<sub>2</sub> to below atmospheric  
222 concentrations (0.53 ppmv)<sup>72</sup> and ten soils consumed atmospheric CO (0.09 ppmv)  
223 <sup>73</sup> during the timecourse of our experiments (**Figure S7**). These sub-atmospheric  
224 thresholds confirm that these microbial communities can harvest energy from the  
225 atmosphere, a virtually unlimited source of diffusive and energy-rich reduced gases  
226 <sup>74,75</sup>. The average rate of atmospheric H<sub>2</sub> oxidation (135 pmol hr<sup>-1</sup> g<sub>soil ww</sub><sup>-1</sup>) was much  
227 faster than for atmospheric CO oxidation (0.60 pmol hr<sup>-1</sup> g<sub>soil ww</sub><sup>-1</sup>) (**Table S8**). This  
228 finding, together with the higher abundance of putative H<sub>2</sub> oxidizers in the soil  
229 communities (**Figure 2**), suggests that atmospheric H<sub>2</sub> is likely to be the predominant  
230 energy source sustaining these communities. As elaborated in **Supplementary Note**  
231 **2**, considerable variations in bulk and normalized oxidation rates were measured for  
232 both gases, which was significantly correlated with several measured  
233 physicochemical variables (**Figure S6; Table S9**).

234 Cell-specific rates were calculated by normalizing bulk rates against soil microbial  
235 abundance and the proportion of trace gas oxidizers. Cell-specific atmospheric H<sub>2</sub>  
236 oxidation rates were high (av. 1.1 × 10<sup>-7</sup> nmol hr<sup>-1</sup> cell<sup>-1</sup>) and approximately two  
237 orders of magnitude higher than those of CO (av. 1.3 × 10<sup>-9</sup> nmol hr<sup>-1</sup> cell<sup>-1</sup>) (**Figure**  
238 **4b**). In line with our findings in the Windmill Islands region<sup>12</sup>, this rate of atmospheric  
239 H<sub>2</sub> consumption exceeds the theoretical maintenance requirements of trace gas  
240 oxidizers at the temperature tested (10°C) and is sufficient to support some growth  
241 <sup>76-78</sup>. It should also be noted that metabolic water is the major end-product of the  
242 aerobic respiration of atmospheric H<sub>2</sub> (2 H<sub>2</sub> + O<sub>2</sub> → 2 H<sub>2</sub>O). Given the reported  
243 cytosolic orientation of high-affinity hydrogenases and terminal oxidases<sup>62</sup>, the water  
244 produced would be retained in the cytosol, including as a solvent for  
245 macromolecules. Thus, trace gas oxidation may be a simple, but hitherto overlooked,  
246 mechanism for microorganisms to stay hydrated in the hyper-arid deserts of  
247 Antarctica. Based on cell-specific rates of atmospheric H<sub>2</sub> oxidation, a theoretical  
248 average of 1.1 million water molecules would be produced per cell each minute. For  
249 a cell with an expected 1 μm<sup>3</sup> volume and 70% water content<sup>79,80</sup>, such production  
250 rates would be sufficient to replace all cellular water over a 15-day period (**Table**  
251 **S8**). We therefore propose that the metabolic water continuously generated by trace

252 gas oxidation is a quantitatively significant source of hydration in this environment  
253 with minimal precipitation<sup>32</sup>.

254

### 255 **Metabolically constrained phototrophs, lithotrophs, and organotrophs co-exist** 256 **with versatile mixotrophs in Antarctic soils**

257 While the most abundant taxa in the Mackay Glacier ecotone appear to be versatile  
258 mixotrophs, the genome compendium revealed that these ecosystems also harbor  
259 diverse bacteria and archaea with specialist strategies for energy and carbon  
260 acquisition. Multiple chemolithoautotrophs were present, including those capable of  
261 oxidizing the trace amounts of ammonium, sulfur and iron detected in the soils  
262 (**Table S1**). Ammonium and nitrite oxidizers comprised an average of 2.9% and  
263 1.0% of the communities, but together comprised 23% and 15% of the community in  
264 Mount Seuss 6 and Benson Glacier samples, respectively (**Figure 2; Table S6**).  
265 Phylogenetic analysis confirmed that Nitrososphaerales (archaea) and  
266 Burkholderiales (bacteria) were the dominant ammonium oxidizers (**Figure S8**), in  
267 line with previous reports for McMurdo Dry Valley soils<sup>28</sup>, whereas Nitrospirota were  
268 the main nitrite oxidizers (**Figure S9**). These nitrifiers also respectively encoded the  
269 signature enzymes to fix carbon through the archaeal 4-hydroxybutyrate cycle  
270 (**Figure S10**), proteobacterial CBB cycle (**Figure S4**), and nitrospiral reverse  
271 tricarboxylic acid cycle (**Figure S11**), suggesting that multiple chemosynthetic  
272 primary production strategies sustain biodiversity in these oligotrophic soils. The  
273 marker genes for sulfide and thiosulfate oxidation (Sqr, FCC, SoxB) were each  
274 encoded by 1 - 4% of community members in most soils (**Figure 2; Table S5**),  
275 including multiple Burkholderiales MAGs and several other lineages (**Figure S12,**  
276 **S13, S14**). The genes to oxidize ferrous iron via the c-type cytochrome Cyc2 were  
277 widespread in Mount Seuss 6 (4.7%) and Cliff Nunatak samples (7.3%), and present  
278 in select MAGs from five major phyla (**Figure S15**). Thus, atmospheric and edaphic  
279 inorganic compounds alike are major energy sources for Antarctic soil communities,  
280 although their relative importance varies across the physicochemically diverse soils  
281 from the region.

282 Our metagenomic analysis suggests that light energy supports few photoautotrophs,  
283 but numerous photoheterotrophs, in the region. Reflecting cyanobacterial



284 distributions across the region (**Figure 1b**), photosystems associated with oxygenic  
285 photosynthesis were encoded by few community members except in the Pegtop  
286 Mountain and Cliff Nunatak samples (**Figure 2**). Some photosystem II sequences  
287 affiliated with proteobacterial anoxygenic phototrophs were also detected (**Figure**  
288 **S16**). In contrast, energy-converting microbial rhodopsins were prevalent and  
289 abundant across the region (**Figure 2**). These light-powered proton pumps are well-  
290 characterized for their role in energy conservation in marine and freshwater  
291 ecosystems<sup>81–85</sup>, though have been scarcely studied in desert environments<sup>86</sup>. As  
292 outlined by our ‘continual energy harvesting hypothesis’, sunlight (in common with  
293 atmospheric trace gases) is a relatively dependable energy source and hence  
294 lineages that harvest it may have a selective advantage in energy-poor desert soils  
295<sup>18</sup>. In line with this theory, putative energy-converting rhodopsins were present in  
296 several of the most dominant orders of Actinobacteriota and Chloroflexota in these  
297 soils (**Table S5**). They were also present in both cyanobacterial MAGs, thereby  
298 providing a means for photoautotrophs to conserve energy when water for oxygenic  
299 photosynthesis is limiting (**Figure S17**). Phylogenetic analysis confirmed the binned  
300 and unbinned sequences fell into diverse clades (**Figure S17**), including two novel  
301 clades that were most closely related (<50% sequence identity) to the biochemically  
302 characterized energy-converting rhodopsins of halophilic archaea  
303 (bacteriorhodopsins)<sup>87</sup> and *Pantoea* species (pantorhodopsins)<sup>88</sup>.

304 Twenty metagenome-assembled genomes were also recovered for the phyla known  
305 to adopt obligately symbiotic lifestyles, namely Patescibacteria, Chlamydiae,  
306 Dependientiae, and Bdellovibrionota (**Table S5**). All four phyla appear to be obligate  
307 organoheterotrophs that lack alternative pathways for energy conservation or carbon  
308 acquisition (**Figure 2**). Based on previous reports, all characterized Bdellovibrionota  
309 predate bacterial species<sup>39</sup>, whereas Chlamydiae and Dependientiae are likely to be  
310 parasites of protist or arthropod species<sup>40,41,89</sup> such as populations of springtails  
311 (Collembola) identified within the same sampling area<sup>90</sup>. Signature genes  
312 associated with the symbiotic lifestyles of each MAG were detected, for example  
313 host-targeted peptidoglycan metalloendopeptidases and self-protection proteins that  
314 Bdellovibrionota uses to invade cells of bacterial prey<sup>91,92</sup>, as well as ankyrin repeat  
315 and WD40 repeat proteins implicated in modulation of eukaryotic hosts by  
316 Dependientiae<sup>41,89</sup> (**Table S5**). Also in line with an obligately symbiotic lifestyle,



317 several lineages have ultra-small genomes when adjusted for completeness, namely  
318 the eight Patescibacteria MAGs (av. 1.3 Mbp), three Dependitiae MAGs (av. 1.8  
319 Mbp), and a Rickettsiaceae MAG (1.3 Mbp) (**Table S5**), and are predicted to be  
320 auxotrophic for multiple amino acids. Building on the discovery of unexpected  
321 symbionts in Antarctic lakes<sup>93,94</sup>, to our knowledge this is the first report that  
322 microbial parasitism is a major ecological strategy in terrestrial Antarctica. We also  
323 reveal oxic niches for phyla such as Patescibacteria that have, until now, primarily  
324 been studied in anoxic ecosystems<sup>42,95,96</sup>.

325 Finally, we obtained genomic and biogeochemical evidence that atmospheric  
326 methane oxidation occurs on non-maritime Antarctic soils. Based on methane  
327 monooxygenase levels in short reads, aerobic methanotrophs are members of the  
328 rare biosphere in most of the sampled Antarctic soils, but are present in very high  
329 levels in three soils, including Mount Seuss 5 (9.4%) (**Figure 2; Table S5**).  
330 Concordantly, two of these soils oxidized methane at high cell-specific rates to sub-  
331 atmospheric levels during microcosm incubations (**Figure 4; Figure S7**). Genome-  
332 resolved analysis suggested that this activity is primarily mediated by a single  
333 bacterial species within the gammaproteobacterial order UBA7966, which encodes a  
334 particulate methane monooxygenase clustering with sequences from the  
335 atmospheric methane-oxidizing clade USCy (**Figure S18**). While this bacterium has  
336 a restricted distribution, based on read mapping, it is among the most abundant  
337 single taxon across the entire region (**Table S5**). Thus, by adopting a relatively  
338 specialist lifestyle dependent on assimilating a widely available but catalytically  
339 demanding atmospheric substrate, this bacterium fills a distinct ecological niche.  
340 Importantly, although methanotroph genomes have previously been reported in  
341 Antarctic soils<sup>12,30</sup>, this is the first experimental report that such bacteria are  
342 biogeochemically active.

## 343 **Conclusions**

344 Altogether, these results demonstrate a remarkable diversity of both microbial  
345 lineages and metabolic strategies in the resource-poor soils of Antarctica. The most  
346 abundant and prevalent bacterial lineages in Antarctic soils appear to be free-living  
347 mixotrophs capable of meeting carbon, energy, and even hydration needs from  
348 atmospheric trace gases, i.e. 'living on air'<sup>97</sup>. Several bacteria and archaea also  
349 achieve high abundances in specific soils through more specialist strategies,  
350 spanning atmospheric methanotrophy, oxygenic photosynthesis and lithoautotrophic  
351 growth on trace edaphic substrates. This environment in turn has selected for a  
352 range of as-yet-uncultivated bacterial lineages (e.g. *Ca.* Edaphomicrobia and *Ca.*  
353 Aridivitia) and previously unreported gene families (e.g. encoding group 1I [NiFe]-  
354 hydrogenases and potential microbial rhodopsins). Also, surprisingly, a significant  
355 minority of community members gain resources through parasitism or predation of  
356 microorganisms. Through this combination of strategies, both free-living and  
357 symbiotic microorganisms can achieve stable niches in a polyextreme environment.

358 Additionally, the wealth of metagenomic sequencing data and 451 draft genomes  
359 generated by this study provides a valuable resource for two major areas of  
360 endeavor. First, these datasets support fundamental research and potentially inform  
361 decisions to secure Antarctica's environmental future, given forecasts of changing  
362 temperature and water availability<sup>98-100</sup>. Thus, in line with one of the six priorities for  
363 Antarctic science<sup>101</sup>, this resource will provide insights into how life has evolved and  
364 adapted on this microbially-dominated continent, and in turn may respond to climate  
365 changes. Secondly, these findings also contribute to considerations of what  
366 processes may sustain life on other cold, dry planets such as Mars. Antarctica has  
367 long been considered a potential analogue for life elsewhere in the solar system<sup>102</sup>.  
368 Our work brings that picture into sharper resolution.

## 369 **Materials and Methods**

### 370 **Soil physicochemical analysis**

371 This study used mineral soils previously sampled from 16 glacier- or mountain-  
372 associated sites in the Mackay Glacier region, South Victoria Land, Antarctica during  
373 January 2015 as previously described<sup>33,35</sup>. In brief, 50 g of surface soil (depth: 0 - 5  
374 cm) at each location was collected from an approximately 1 m<sup>2</sup> area and stored in  
375 sterile 50 ml polypropylene Falcon tubes (Grenier, Bio-One) aseptically. During  
376 storage and transportation to University of Pretoria, samples were kept at -80°C.  
377 They were later shipped to Monash University's quarantine approved facilities for  
378 further experiments. Details of soil samples can be found in **Table S1**. Prior to  
379 physicochemical measurements, approximately 35 g of soil of individual sample was  
380 aliquoted. Soil aliquots were treated with gamma irradiation at 50 kGy (Steritech Pty  
381 Ltd Victoria, Australia) for compliance with Department of Agriculture, Water and the  
382 Environment's quarantine good regulations. They were subsequently shipped to the  
383 Environmental Analysis Laboratory (EAL), Southern Cross University, Australia for  
384 physicochemical analyses in accordance with ISO/IEC 17025 standard procedures.  
385 Physicochemical parameters analysed included: basic soil colour and texture; pH  
386 and electrical conductivity (1:5 water); moisture content; total carbon, nitrogen,  
387 organic carbon, and organic matter; available calcium, magnesium, potassium,  
388 ammonium, nitrate, phosphate, sulfur; exchangeable sodium, potassium, calcium,  
389 magnesium, hydrogen, and aluminium; cation exchange capacity; Bray I, Bray II, and  
390 Cowell phosphorus; and available micronutrients zinc, manganese, iron, copper,  
391 boron, and silicon. These data are summarised in **Table S1**.

392

### 393 **Shotgun metagenome sequencing, assembly and binning**

394 Community DNA for metagenomic sequencing was extracted from 0.5 g of soil using  
395 the FastDNA SPIN Kit for soil (MP Biomedicals) according to the manufacturer's  
396 instructions. An extraction blank control was included. Metagenomic shotgun  
397 libraries were prepared using the Nextera XT DNA Sample Preparation Kit (Illumina  
398 Inc., San Diego, CA, USA) and subject to paired-end sequencing (2 × 150 bp) on an  
399 Illumina NextSeq500 platform at the Australian Centre for Ecogenomics (ACE),  
400 University of Queensland. Sequencing yielded 356,941,066 read pairs across the  
401 sixteen soil metagenomes and 556 read pairs for the negative control (**Table S4**),

402 indicating a minimal level of contamination from DNA extraction and sequencing  
403 processes. Raw metagenomic sequences were subjected to quality filtering using  
404 the BBDuk function of the BBTools v38.80 (<https://sourceforge.net/projects/bbmap/>);  
405 contaminating adapters (k-mer size of 23 and hamming distance of 1), PhiX  
406 sequences (k-mer size of 31 and hamming distance of 1), and bases from 3' ends  
407 with a Phred score below 20 were trimmed. After removing resultant reads with  
408 lengths shorter than 50 bp, 93% high-quality read pairs were retained for  
409 downstream analysis. Metagenomic reads from each sample were assembled  
410 individually with metaSPAdes v3.14.0<sup>103</sup> and collectively with MEGAHIT v1.2.9<sup>104</sup>  
411 (min k: 27, max k: 127, k step: 10). To generate corresponding coverage profiles for  
412 assembled contigs, short reads were mapped back using Bowtie2 v2.3.5<sup>105</sup> with  
413 default parameters. Subsequently, genome binning was performed using CONCOCT  
414 v1.1.0<sup>106</sup>, MaxBin2 v2.2.7<sup>107</sup>, and MetaBAT2 v2.15<sup>108</sup> on contigs with length over  
415 2000 bp. Resulting bins from the same assembly were then dereplicated using  
416 DAS\_Tool v1.1.2<sup>109</sup>. RefineM v0.0.25<sup>110</sup> was used to remove spurious contigs with  
417 incongruent genomic and taxonomic properties. Applying a threshold average  
418 nucleotide identity of 99%, bins from different assemblies were consolidated to a  
419 non-redundant set of metagenome-assembled genomes (MAGs) using dRep v2.5.4  
420<sup>111</sup>. Completeness and contamination of MAGs were assessed using CheckM v1.1.2  
421<sup>112</sup>. In total, 101 high quality (completeness > 90% and contamination < 5%) and 350  
422 medium quality (completeness > 50% and contamination < 10%)<sup>44</sup> MAGs from 18  
423 phyla were recovered. Their corresponding taxonomy was assigned by GTDB-TK  
424 v1.3.0<sup>113</sup> with reference to GTDB R05-RS95<sup>114</sup>. Open reading frames (ORFs) in  
425 MAGs were predicted using Prodigal v2.6.3<sup>115</sup>.

426

## 427 **Community analysis**

428 Soil microbial community structures were determined by using both metagenomic  
429 and 16S rRNA gene amplicon sequencing. Community profiles in sequenced  
430 metagenomes were generated by mapping quality-filtered reads to the universal  
431 single copy ribosomal marker genes and clustering at 97% identity using SingleM  
432 v.0.12.1 (<https://github.com/wwood/singlem>). To align with the latest GTDB  
433 taxonomy at the time of submission (R05-RS95; release 2020/07), we generated a  
434 SingleM package for the single-copy ribosomal protein-encoding gene *rplP*. In brief,  
435 all *rplP* sequences from Archaea and Bacteria genomes in GTDB R05-RS95

436 (<https://data.ace.uq.edu.au/public/gtdb/data/releases/release95/95.0/>) were  
437 downloaded. GraftM v0.12.2 <sup>116</sup> was used to generate a phylogenetic package for  
438 the sequences which was then used to make a community classification package by  
439 SingleM v.0.12.1. For 16S rRNA gene amplicon sequencing, the DNeasy PowerSoil  
440 kit (Qiagen) was used to extract DNA from 0.4 g of soil sample as per manufacturer's  
441 instructions. The quality and concentration of DNA extracted were determined using  
442 a Nanodrop spectrophotometer (ND-1000) and a Qubit Fluorometer. Quantitative  
443 PCR (qPCR) using a 96-well plate in a pre-heated LightCycler 480 Instrument II  
444 (Roche, Basel, Switzerland) was used to quantify the copy number of the 16S rRNA  
445 genes in the samples as previously described <sup>117</sup>. For each sample, the V4  
446 hypervariable region for 16S rRNA gene was amplified using the universal Earth  
447 Microbiome Project primer pairs F515 (Parada) <sup>118</sup> and R806 (Apprill) <sup>119</sup>. Amplicons  
448 were sent to paired-end sequencing (2 × 300 bp) on an Illumina MiSeq platform at  
449 the Australian Centre for Ecogenomics (ACE), University of Queensland. BBDuk  
450 function of the BBTools v38.80 was used to trim adapter sequences and filter PhiX  
451 contaminants as described above. The sequences were further processed on the  
452 QIIME2 platform (release 2019/07) <sup>120</sup> to resolve amplicon sequence variants (ASVs)  
453 through the following steps: (i) stripping amplicons primers using cutadapt plugin <sup>121</sup>;  
454 (ii) merging paired-end reads using q2-vsearch plugin <sup>122</sup>; (iii) quality filtering using a  
455 sliding window of four bases with an average Phred score 20; and (iv) de-noising  
456 and truncating sequences at 250 base pairs using deblur <sup>123</sup>. A total of 657,975  
457 reads remained in the dataset (min: 13248, max: 102382) (**Table S3**). For taxonomic  
458 assignment, ASVs were independently annotated with trained naïve Bayes  
459 classifiers of 16S rRNA reference databases Silva release 138 <sup>124</sup> and Greengenes  
460 13.8 <sup>125</sup> (**Table S3**). Multiple sequence alignment of the sequences and subsequent  
461 phylogenetic tree building were performed using MAFFT <sup>126</sup> and FastTree <sup>127</sup>,  
462 respectively, implemented in QIIME2. We then used R packages phyloseq <sup>128</sup>,  
463 picante <sup>129</sup>, vegan <sup>130</sup>, betapart <sup>131</sup> and ggplot2 <sup>132</sup> for downstream statistical analysis  
464 and visualizations. Alpha diversity including observed richness, Chao1, Shannon  
465 index, and Faith's phylogenetic diversity were computed using estimate\_richness  
466 function in phyloseq and pd function in picante. For beta diversity analysis, all  
467 samples were rarefied at the lowest sample sequencing depth, i.e. 13248 sequences  
468 per sample and rarefaction plots before and after rarefaction were shown in **Figure**  
469 **S1a-b**. Bray-Curtis dissimilarity was calculated and visualized using a non-metric

470 multidimensional scaling ordination (NMDS) plot. To examine community turnover in  
471 relations to increasing geographic separation, a distance decay relationship of beta  
472 diversity (Bray-Curtis dissimilarity) against pairwise geographic distance was  
473 computed using the `decay.model` function fitted with a negative exponential law  
474 function in `betapart`. A  $p$  value was calculated using the same function with 999  
475 permutations (**Table S3**).

476

### 477 **Functional analysis**

478 To estimate the metabolic capability of the soil communities, metagenomes and  
479 derived genomes were searched against custom protein databases of representative  
480 metabolic marker genes using DIAMOND v.0.9.31 (query cover > 80%)<sup>133</sup>.  
481 Searches were carried out using all quality-filtered unassembled reads with lengths  
482 over 140 bp and the ORFs of the 451 MAGs. These genes are involved in sulfur  
483 cycling (AsrA, FCC, Sqr, DsrA, Sor, SoxB), nitrogen cycling (AmoA, HzsA, NifH,  
484 NarG, NapA, NirS, NirK, NrfA, NosZ, NxrA, NorB), iron cycling (Cyc2, MtrB, OmcB),  
485 reductive dehalogenation (RdhA), phototrophy (PsaA, PsbA, energy-converting  
486 microbial rhodopsin), methane cycling (McrA, MmoA, PmoA), hydrogen cycling  
487 (catalytic subunit of [NiFe]-hydrogenases, catalytic domain of [FeFe]-hydrogenases,  
488 and Fe-hydrogenases), isoprene oxidation (IsoA), carbon monoxide oxidation (CoxL,  
489 CooS), succinate oxidation (SdhA), fumarate reduction (FrdA), and carbon fixation  
490 (RbcL, AcsB, AclB, Mcr, HbsT, HbsC)<sup>48,52,134</sup>. Results were filtered based on an  
491 identity threshold of 50%, except for group 4 [NiFe]-hydrogenases, [FeFe]-  
492 hydrogenases, CoxL, AmoA, and NxrA (all 60%), PsaA (80%), PsbA and IsoA  
493 (70%), and HbsT (75%). Subgroup classification of reads was based on the closest  
494 match to the sequences in databases. To search for the presence of an additional  
495 set of genes involved in oxidative phosphorylation (AtpA), NADH oxidation (NuoF),  
496 aerobic respiration (CoxA, CcoN, CyoA, CydA), formate oxidation (FdhA), arsenic  
497 cycling (ARO, ArsC), and selenium cycling (YgfK), corresponding in-house  
498 databases were generated for this study. All archaeal and bacterial non-redundant  
499 proteins were retrieved from NCBI Refseq protein database release 99<sup>135</sup>, which  
500 were then screened by hidden Markov models (HMM)<sup>136</sup>, with search cutoff scores  
501 as described previously<sup>137</sup>. Resulting hits were manually inspected to remove false  
502 positives and genes with lengths that deviated more than 20% from the average  
503 were discarded. The search of these genes in unassembled reads and ORFs of



504 MAGs was carried out using the DIAMOND blastp algorithm with a minimum  
505 percentage identity of 60% (NuoF), 70% (AtpA, ARO, YgfK) or 50% (all other  
506 databases). Read counts for each gene were normalized to reads per kilobase per  
507 million (RPKM) by dividing the actual read count by the total number of reads (in  
508 millions) and then dividing by the gene length (in kilobases). In order to estimate the  
509 gene abundance in the microbial community, high-quality unassembled reads were  
510 also screened for the 14 universal single copy ribosomal marker genes used in  
511 SingleM v.0.12.1 and PhyloSift<sup>138</sup> by DIAMOND (query cover > 80%, bitscore > 40)  
512 and normalized as above. Subsequently, the average gene copy number of a gene  
513 in the community was calculated by dividing the read count for the gene (in RPKM)  
514 by the mean of the read counts of the 14 universal single copy ribosomal marker  
515 genes (in RPKM).

516

### 517 **Phylogenetic analysis**

518 Maximum-likelihood phylogenetic trees were constructed to verify the presence and  
519 visualise the evolutionary history of key metabolic genes in the metagenome-  
520 assembled genomes and assembled unbinned reads. Trees were constructed using  
521 the amino acid sequences for subunits of ten enzymes involved in energy  
522 acquisition: group 1 [NiFe]-hydrogenase (HhyL, HylL); form I carbon monoxide  
523 dehydrogenase (CoxL), particulate methane monooxygenase (PmoA), ammonia  
524 monooxygenase (AmoA), nitrite oxidoreductase (NxrA), sulfide-quinone  
525 oxidoreductase (Sqr), flavocytochrome *c* sulfide dehydrogenase (FCC),  
526 thiosulfohydrolase (SoxB), iron-oxidizing *c*-type cytochrome (Cyc2), photosystem II  
527 (PsbA), and energy-converting rhodopsins. Trees were also constructed of the  
528 amino acid sequences for subunits of three enzymes involved in carbon fixation:  
529 ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO; RbcL),  
530 thaumarchaeotal 4-hydroxybutyrate synthase (HbsT), and ATP-citrate lyase (AclB).  
531 In all cases, protein sequences retrieved from the MAGs or assembled metagenome  
532 sequences by homology-based searches were aligned against a subset of reference  
533 sequences from the custom protein databases using ClustalW<sup>139</sup> in MEGA X<sup>140</sup>.  
534 Evolutionary relationships were visualized by constructing maximum-likelihood  
535 phylogenetic trees; specifically, initial trees for the heuristic search were obtained  
536 automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of  
537 pairwise distances estimated using a JTT model, and then selecting the topology



538 with superior log likelihood value. All residues were used and trees were  
539 bootstrapped with 50 replicates. To characterise the genetic context of [NiFe]  
540 hydrogenases and ribulose-1,5-bisphosphate carboxylase / oxygenase (RuBisCO)  
541 from the MAGs, up to 10 genes upstream and downstream of the catalytic subunits  
542 were retrieved. These flanking genes were annotated against Pfam protein family  
543 database v33.1<sup>141</sup> using PfamScan v1.6<sup>142</sup> and NCBI Refseq protein database  
544 release 99<sup>135</sup> using DIAMOND<sup>133</sup> blastp algorithm (default parameters). Alignments  
545 with the highest score were retained and are summarised in **Table S7**. The R  
546 package gggenes (<https://github.com/wilkox/gggenes>) was used to construct gene  
547 arrangement diagrams.

548

### 549 **Hydrogenase sequence analysis and homology modelling**

550 The amino acid sequence for the large (HylL; GBID = SMB94678) and small  
551 subunits (HylS GBID = SMB94698) of the group 1I [NiFe]-hydrogenase from *H.*  
552 *roseosalivarius* were inputted into the Phyre2 webserver using default parameters  
553<sup>143</sup>. The highest confidence output model for both subunits was derived from the  
554 structure of the group 1h [NiFe]-hydrogenase (HhyLS) from *Cupriavidus necator* H16  
555 (PDB ID = 5AA5)<sup>71</sup>. The structure of the group 1I [NiFe]-hydrogenase tetramer was  
556 assembled using Pymol, based on the tetrameric structure of the *C. necator* group  
557 1h [NiFe]-hydrogenase for further analysis. To identify transmembrane helix  
558 presence, position and topology in the HylTM proteins associated with group 1I  
559 [NiFe]-hydrogenases, the amino acid sequences from *H. roseosalivarius* were  
560 inputted into the TMHMM 2.0 webserver<sup>144</sup>.

561

### 562 **Gas chromatography assays**

563 Soil microcosms were used to determine the capacity of soil microbial communities  
564 to oxidize H<sub>2</sub>, CO, and CH<sub>4</sub> by gas chromatography. For each of the 16 Mackay  
565 Glacier region samples in technical duplicate, 2 g of soil was placed in a 120 ml  
566 serum vial and incubated at 10°C. The ambient air headspace was amended with  
567 H<sub>2</sub>, CO, and CH<sub>4</sub> (via a mixed gas cylinder containing 0.1 % v/v H<sub>2</sub>, CO, and CH<sub>4</sub>  
568 each in N<sub>2</sub>, BOC Australia) to give starting mixing ratios of approximately 10 parts  
569 per million (ppmv) for each gas. At each time interval, 2 ml of headspace gas was  
570 sampled using a gas-tight syringe and stored in sealed a 3 ml glass exetainer that  
571 had been flushed with ultra-high purity N<sub>2</sub> (99.999% pure, BOC Australia) prior to

572 measurement. A VICI gas chromatographic machine with a pulsed discharge helium  
573 ionization detector (model TGA-6791-W-4U-2, Valco Instruments Company Inc.) and  
574 an autosampler was used to measure gas concentrations as previously described<sup>51</sup>.  
575 The machine was calibrated against ultra-pure H<sub>2</sub>, CO and CH<sub>4</sub> standards down to  
576 the limit of quantification (H<sub>2</sub>: 20 ppbv; CO: 9 ppbv; CH<sub>4</sub>: 500 ppbv). Calibration  
577 mixed gas (10.20 ppmv of H<sub>2</sub>, 10.10 ppmv of CH<sub>4</sub>, 9.95 ppmv of CO in N<sub>2</sub>, Air  
578 Liquide Australia) and pressurized air (Air Liquide Australia) with known trace gas  
579 concentrations were used as internal reference standards. Four pooled heat-killed  
580 soils (2 g of pooled soil; treated at 121°C, 15 p.s.i. for 60 mins) were prepared as  
581 negative controls. For kinetic analysis, measurement time points with individual gas  
582 concentration over 0.4 ppmv were used. First order reaction rate constants were  
583 calculated by fitting an exponential model as determined by the lowest overall Akaike  
584 information criterion value when compared to a linear model. Actual reaction rate  
585 constants of the sample were obtained by correcting against means of negative  
586 controls and only resultant values higher than the magnitude of measurement errors  
587 of negative controls were retained. Bulk atmospheric gas oxidation rate for each  
588 sample was calculated with respect to mean atmospheric mixing ratio of  
589 corresponding trace gases (H<sub>2</sub>: 0.53 ppmv; CO: 0.09 ppmv; CH<sub>4</sub>: 1.9 ppmv)<sup>73,145,146</sup>.  
590 Soil cell abundance was estimated using 16S rRNA gene copy number from qPCR  
591 corrected with a reported average number of 16S rRNA gene copy per genome (i.e.  
592 4.2)<sup>147</sup>. Cell specific gas oxidation rates were then inferred by dividing estimated soil  
593 cell abundance and the proportion of corresponding gas oxidizers from metagenomic  
594 data. To identify factors potentially influencing gas oxidation rates, a two-tailed all-vs-  
595 all Spearman correlation matrix was generated that encompassed gas oxidation  
596 rates, gas oxidation gene abundances, and soil physicochemical variables for each  
597 of the 16 samples.

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- 984

## 985 Footnotes

986

### 987 Etymological information:

988 *Candidatus* Edaphomicrobium (E.da.pho.mi.cro'bi.um. Gr. neut. n. *edaphos*, soil;  
989 N.L. neut. n. *microbium*, a microbe; N. L. neut. n. *Edaphomicrobium*, a soil  
990 microbium)

991 *Candidatus* Edaphomicrobium janssenii (jans.sen'i.i. N.L. gen. n. *janssenii*, of  
992 Janssen, named after Peter H. Janssen, for his pioneering isolation-based studies  
993 that first described this lineage <sup>148</sup>)

994 *Candidatus* Edaphomicrobiaceae (former candidate Chloroflexota family CSP1-4)  
995 (E.da.pho.mi.cro.bi.a.ce'ae. N.L. neut. n. *Edaphomicrobium* a (Candidatus) bacterial  
996 genus; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Edaphomicrobiaceae*,  
997 family of the genus *Edaphomicrobium*)

998 *Candidatus* Edaphomicrobiales (former candidate Chloroflexota order CSP1-4)  
999 (E.da.pho.mi.cro.bi.a'les. N.L. neut. n. *Edaphomicrobium* a (Candidatus) bacterial  
1000 genus; suff. *-ales* ending to denote an order; N.L. fem. pl. n. *Edaphomicrobiales*,  
1001 order of the family *Edaphomicrobiaceae*)

1002 *Candidatus* Edaphomicrobia (former candidate Chloroflexota class Ellin6529)  
1003 (E.da.pho.mi.cro'bi.a. N.L. neut. n. *Edaphomicrobium* a (Candidatus) bacterial  
1004 genus; *-ia* ending to denote a class; N.L. neut. pl. n. *Edaphomicrobia*, class of the  
1005 order *Edaphomicrobiales*)

1006

1007 *Candidatus* Aridivita (A.ri.di.vi'ta. L. masc. adj. *aridus*, dry; L. fem. n. *vita*, life; N.L.  
1008 fem. n. *Aridivita*, a dry life)

1009 *Candidatus* Aridivita willemsiae (wil.lems'i.ae. N.L. gen. n. *willemsiae*, of Willems,  
1010 named after Anne Willems, for her contributions to Antarctic microbiology using  
1011 isolation-based approaches)

1012 *Candidatus* Aridivitaceae (A.ri.di.vi.ta.ce'ae. N.L. neut. n. *Aridivita* a (Candidatus)  
1013 bacterial genus; suff. *-aceae* ending to denote a family; N.L. fem. pl. n.  
1014 *Aridivitaceae*, family of the genus *Aridivita*)

1015 *Candidatus* Aridivitales (A.ri.di.vi.ta'les. N.L. neut. n. *Aridivita* a (Candidatus)  
1016 bacterial genus; *-ales* ending to denote an order; N.L. fem. pl. n. *Aridivitales*, order  
1017 of the family *Aridivitaceae*)



1018 *Candidatus* Aridivitia (former candidate Actinobacteriota class UBA4738)  
1019 (A.ri.di.vi'ti.a. N.L. neut. n. *Aridivita* a (*Candidatus*) bacterial genus; -ia ending to  
1020 denote a class; N.L. neut. pl. n. *Aridivitia*, class of the order *Aridivitales*)

1021

1022 **Data availability statement:**

1023 All amplicon sequencing data, raw metagenomes, and metagenome-assembled  
1024 genomes were deposited to the NCBI Sequence Read Archive under BioProject  
1025 accession PRJNA630822.

1026

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1044 **Author contributions:**

1045 D.A.C., C.G., M.O., S.L.C., and I.D.H. conceived this study. C.G. and D.A.C.  
1046 supervised this study. C.G., P.M.L., D.A.C., and M.O. designed experiments. P.M.L.  
1047 and G.S. performed experiments. P.M.L., C.G., R.G., G.S., M.O., and D.A.C.  
1048 analyzed data. P.M.L., C.G., R.G., D.A.C., M.O., and S.L.C. wrote the manuscript  
1049 with input from all authors. Different authors were specifically responsible for the  
1050 original sampling campaign (D.A.C., I.D.H.), metagenomic sequencing and assembly  
1051 (P.M.L., C.G.), community analysis (P.M.L., G.S., C.G.), metabolic annotation



1052 (P.M.L., C.G., M.O., D.A.C.), phylogenetic analysis (C.G., P.M.L., M.O., D.A.C.,  
1053 S.K.B.), genetic organization analysis (P.M.L., R.G., C.G., M.O., D.A.C.), molecular  
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1057

1058 The authors declare no conflict of interest.

## 1059 **Figures**

1060

1061 **Figure 1. Abundance, composition, and diversity of the microbial communities**  
1062 **from the Mackay Glacier region. (a)** Boxplot showing the estimated abundance of  
1063 bacterial and archaeal taxa, based on 16S rRNA copy number determined by  
1064 quantitative PCR. **(b)** Stacked bar chart showing phylum-level community  
1065 composition based on metagenomic reads of the single-copy marker gene *rplP* and  
1066 metagenome-assembled genomes. Bacterial and archaeal taxonomy is based on  
1067 Genome taxonomy database (GTDB) release 05-RS95. Phyla with less than 1%  
1068 abundance in the sample were grouped to “Other phyla”. **(c)** Boxplot showing alpha  
1069 diversity (Observed richness, Chao1, Shannon, Faith’s phylogenetic diversity) of  
1070 microbial communities based on 16S rRNA gene amplicon sequence variants. **(d)**  
1071 Beta diversity of rarefied 16S rRNA gene amplicon sequencing data based on Bray-  
1072 Curtis dissimilarity and visualised by a non-metric multidimensional scaling  
1073 ordination (NMDS) plot.

1074

1075 **Figure 2. Metabolic potential of the microbial communities to use inorganic**  
1076 **compounds, organic compounds, and light for energy and carbon acquisition.**  
1077 Homology-based searches were used to identify signature genes encoding enzymes  
1078 associated with (from top to bottom): oxidative phosphorylation, trace gas oxidation,  
1079 sulfur compound oxidation, nitrification, other oxidative processes, photosynthesis,  
1080 and carbon fixation. The left heatmap shows the percentage of total community  
1081 members predicted to encode each signature metabolic gene. To infer abundance,  
1082 read counts were normalized to gene length and the abundance of single-copy  
1083 marker genes. The right heatmap shows the presence of these genes across the  
1084 451 metagenome-assembled genomes spanning 18 phyla. Abundance was  
1085 normalized by predicted MAG completeness.

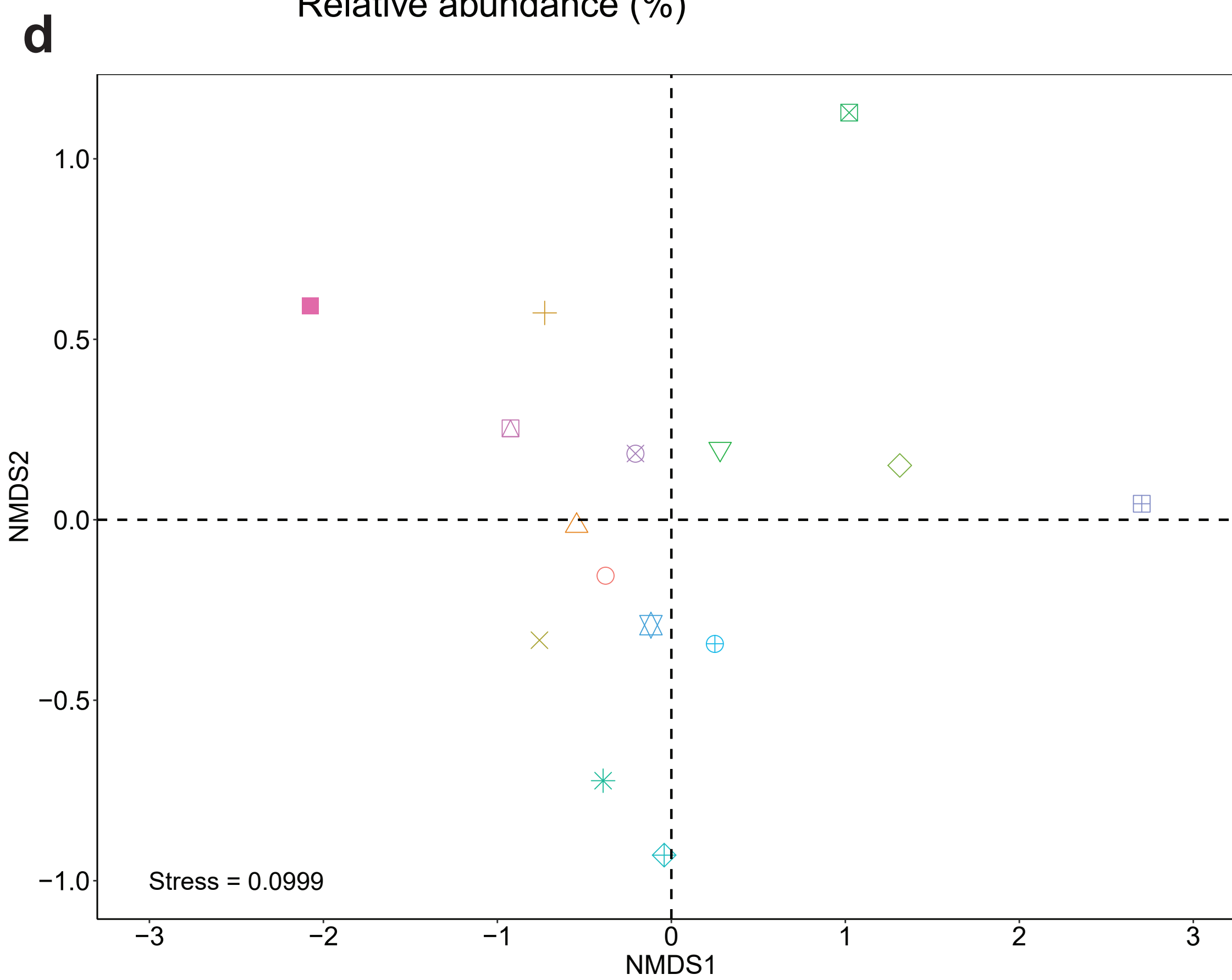
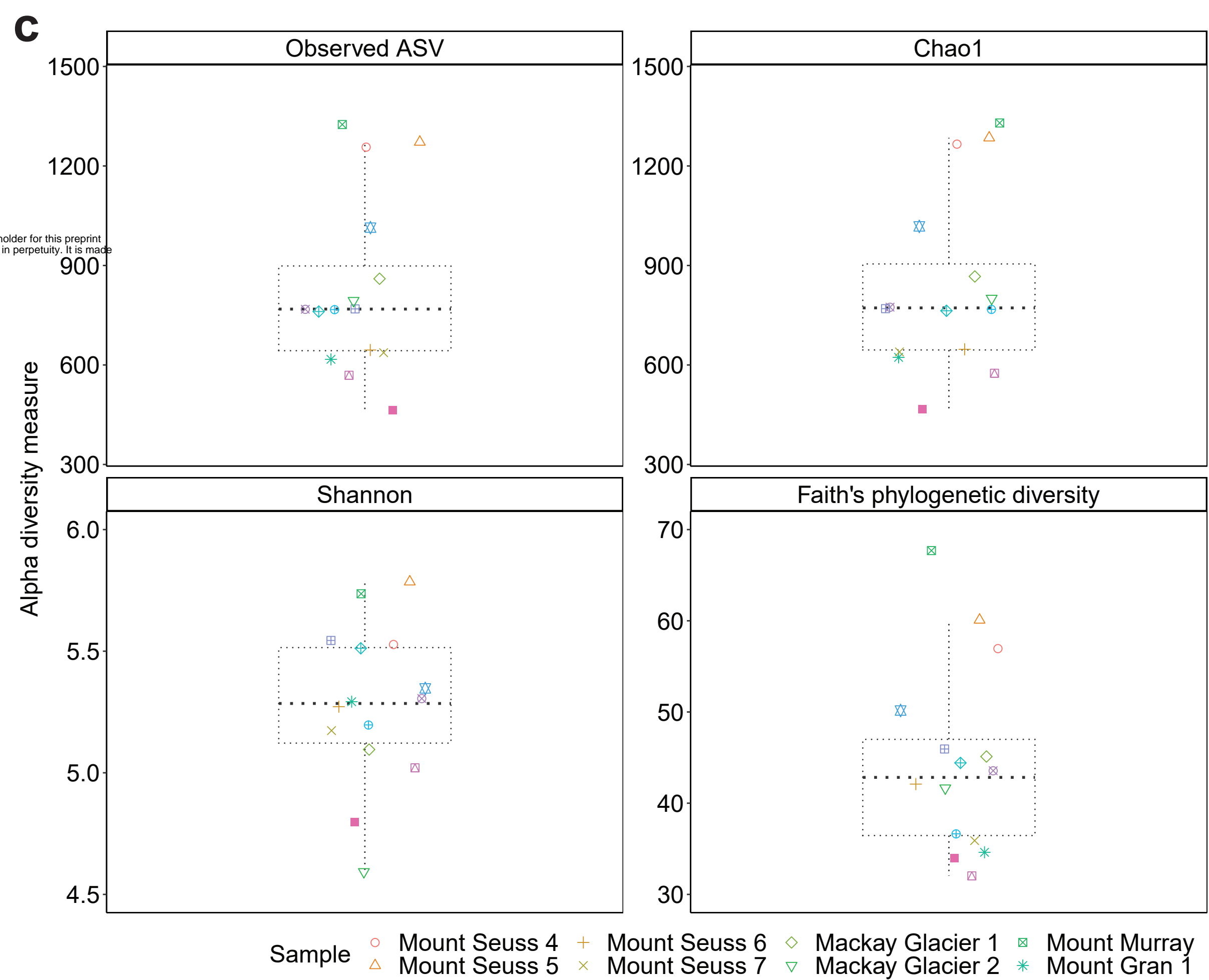
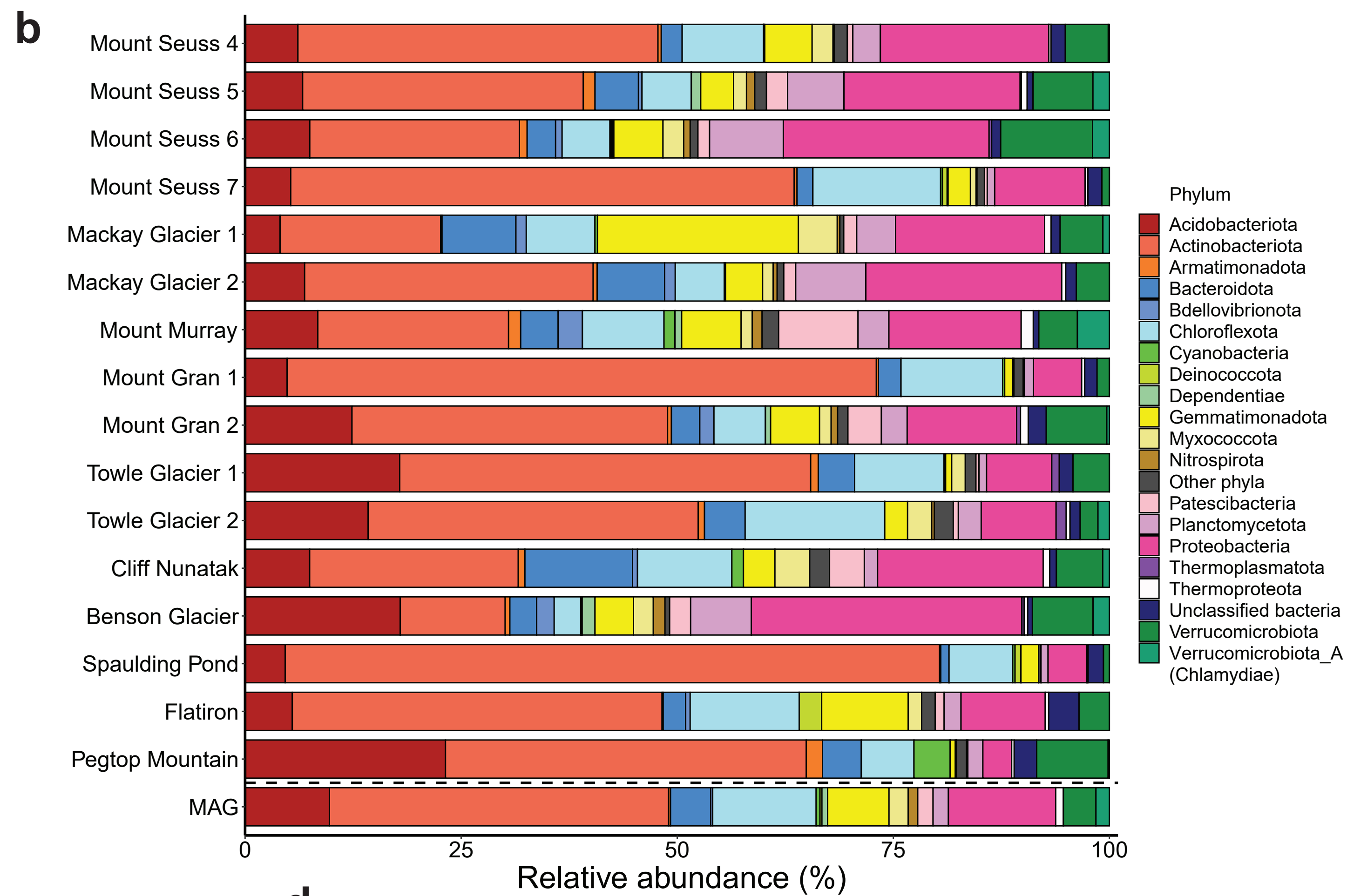
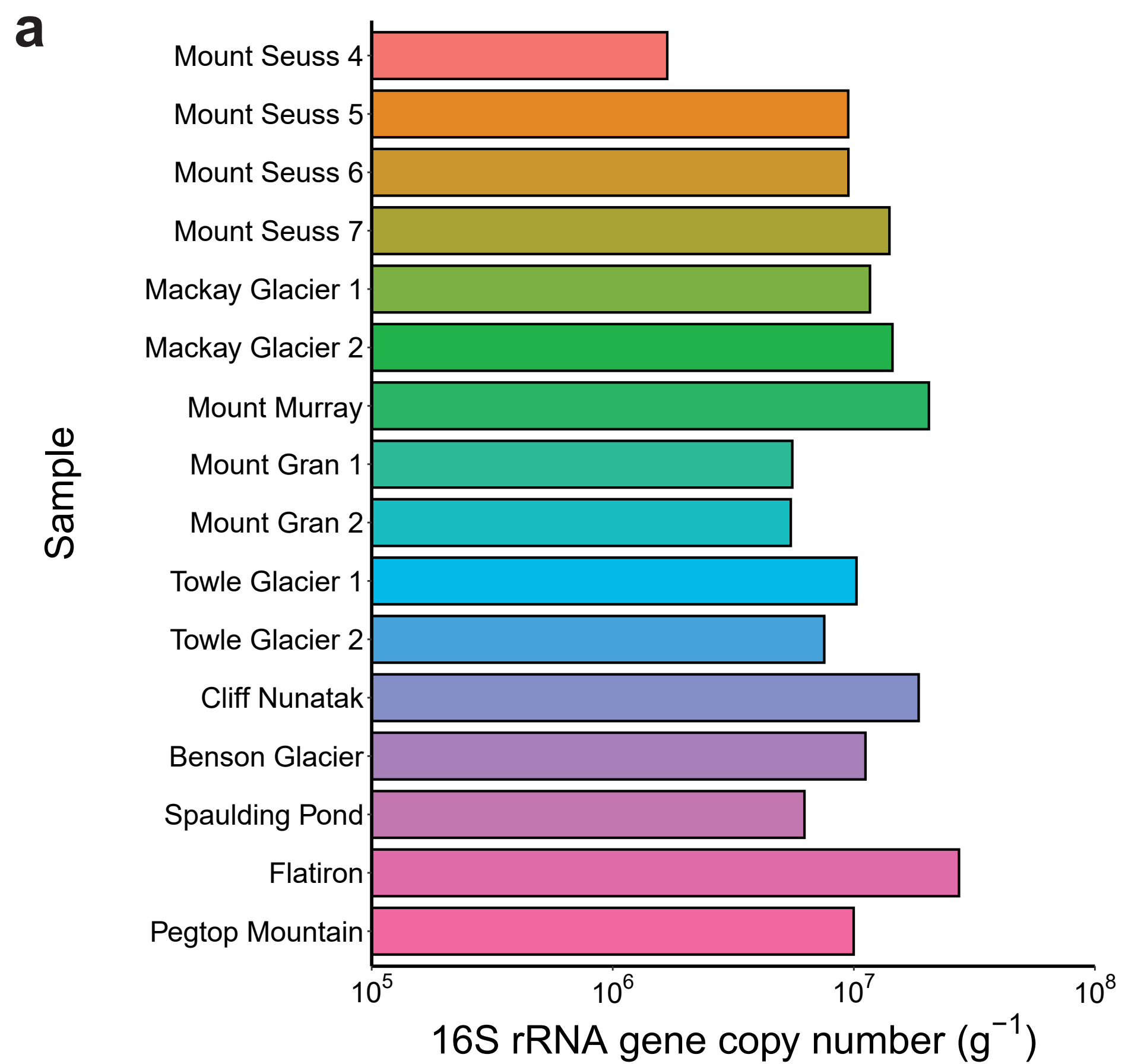
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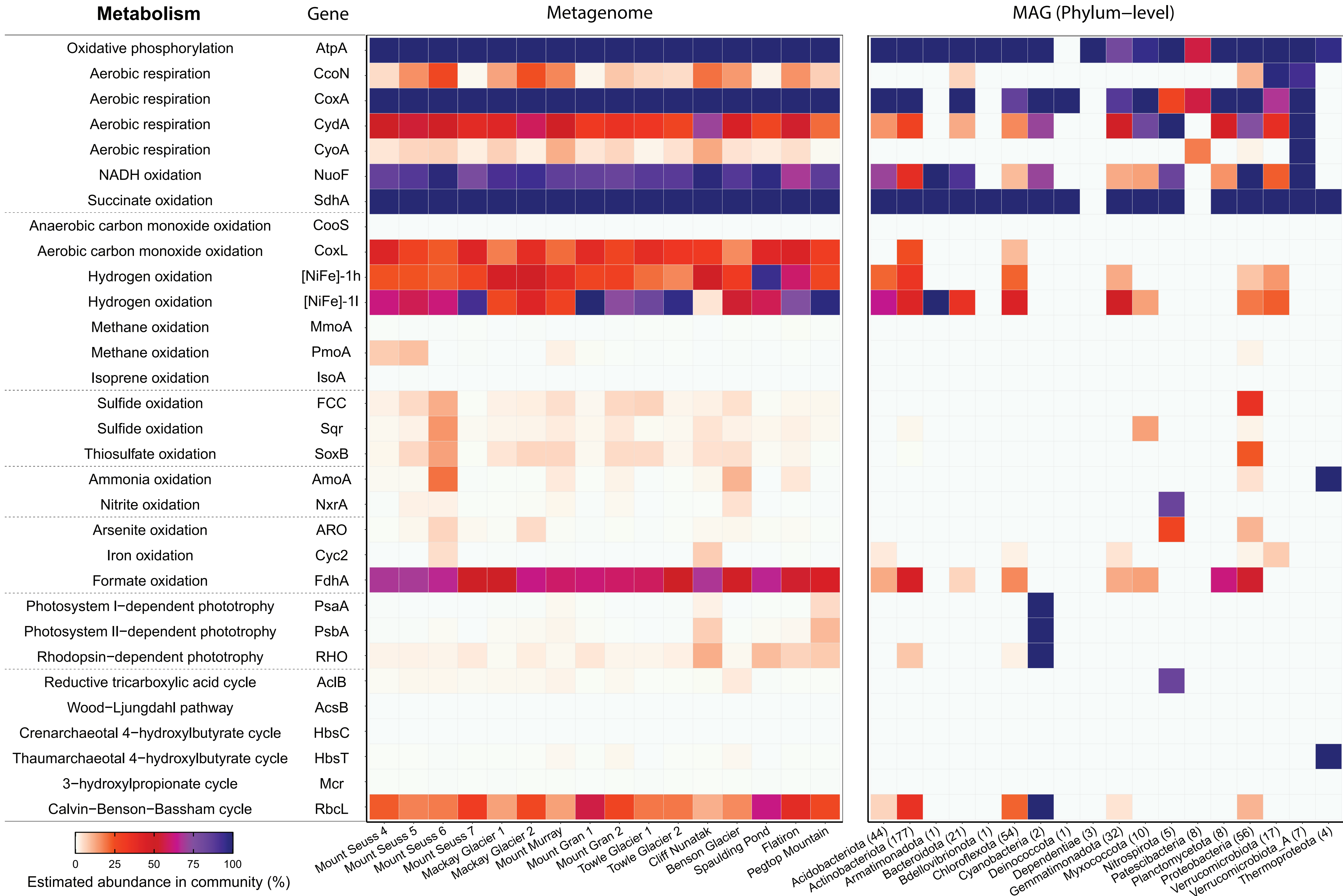
1087 **Figure 3. Identification of the novel group 1I family of [NiFe] hydrogenases**  
1088 **widespread in the Antarctic soil bacterial communities. (a)** Maximum-likelihood  
1089 phylogenetic tree showing the sequence divergence of group 1 [NiFe] hydrogenases  
1090 identified in MAGs from this study. Amino acid sequences retrieved from the  
1091 reconstructed genomes were aligned against reference sequences (bootstrapped

1092 with 50 replicates). Branches of group 1 [NiFe] hydrogenases are shaded according  
1093 to the subgroup classification and tips are colored based on phylum-level affiliation of  
1094 the sequence. All sequences from MAGs of the Mackay Glacier region clustered with  
1095 either the well-characterized group 1h [NiFe]-hydrogenases or the previously  
1096 unreported group 1l [NiFe]-hydrogenases. **(b)** Representative genetic organization of  
1097 group 1l [NiFe] hydrogenase gene cluster derived from the Antarctic bacterium  
1098 *Hymenobacter roseosalivarius*. This shows the predicted open reading frames for  
1099 the large (HylL) and small (HylS) hydrogenase subunits, the five interposing short  
1100 predicted transmembrane proteins (HylTM1-5), a predicted electron-relaying Rieske-  
1101 type protein (HylE), and a maturation endopeptidase (HupD). Conserved open  
1102 reading frames with no predicted function are shown but not labelled. **(c)** Three-  
1103 dimensional model of the group 1l [NiFe] hydrogenase. This shows a structural  
1104 homology model of a heterotetramer of HylL and HylS subunits as a ribbon  
1105 representation and a cartoon of a speculative complex between the hydrogenase  
1106 and genetically associated HylTM proteins. **(d)** The location of conserved residues  
1107 coordinating the [NiFe]-centre of the HylL subunit and [FeS] clusters of the HylS  
1108 subunit of the group 1l [NiFe] hydrogenase. **(e)** Putative location of [FeS] clusters  
1109 and [NiFe] centre (spheres) in one half of the group 1l [NiFe] hydrogenase tetramer,  
1110 with conserved coordinating residues (sticks) color coded as in panel C.

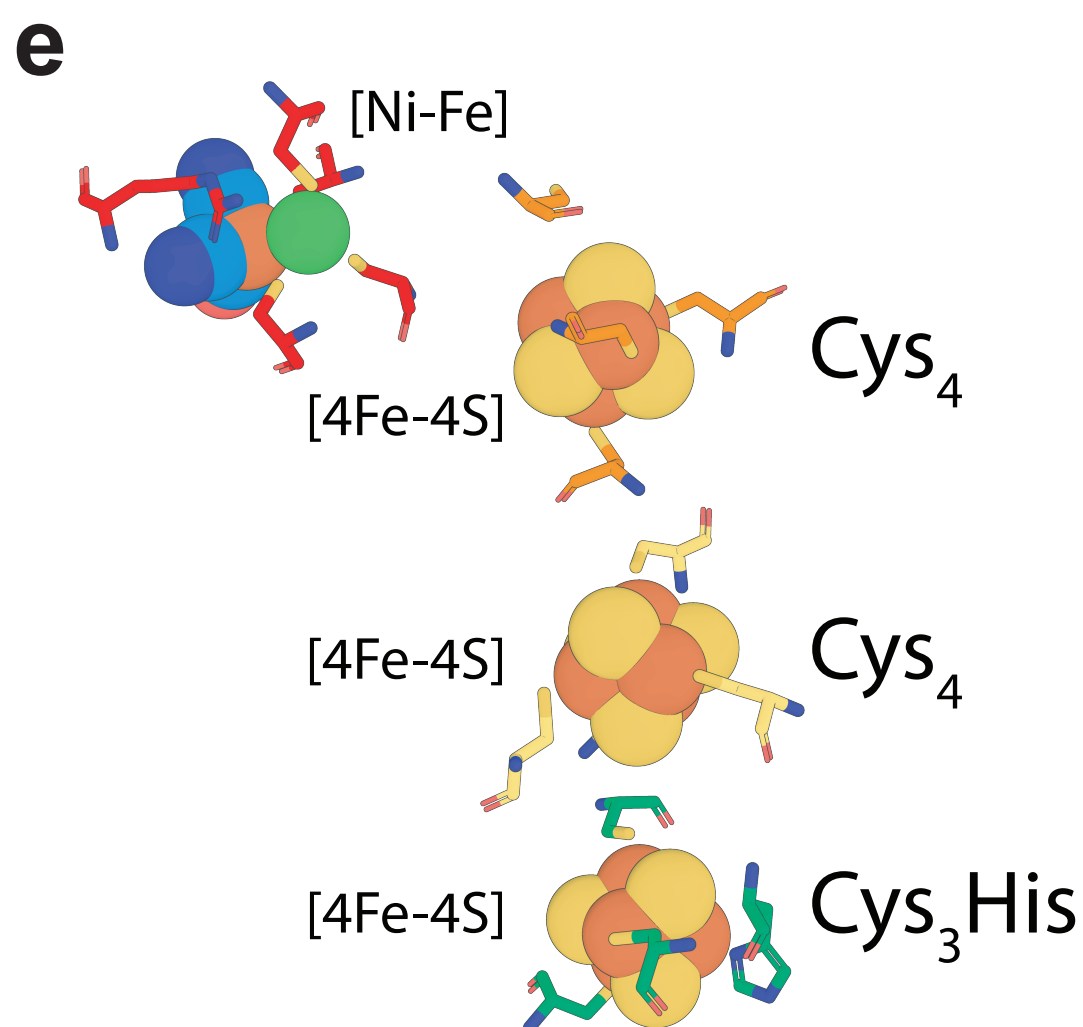
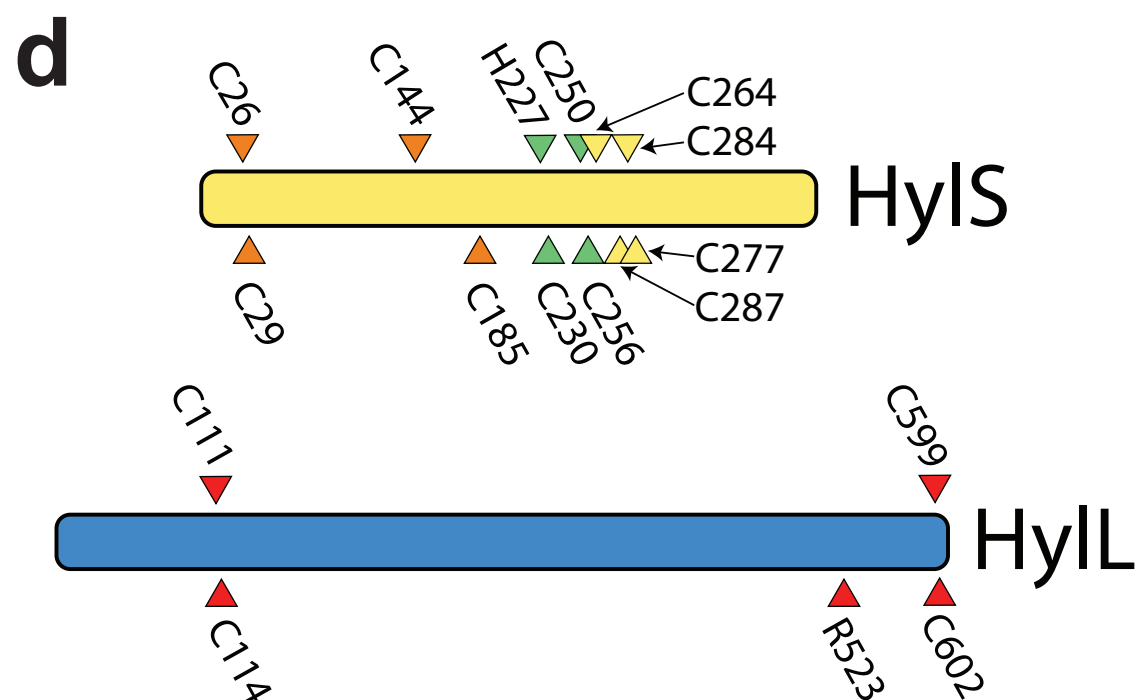
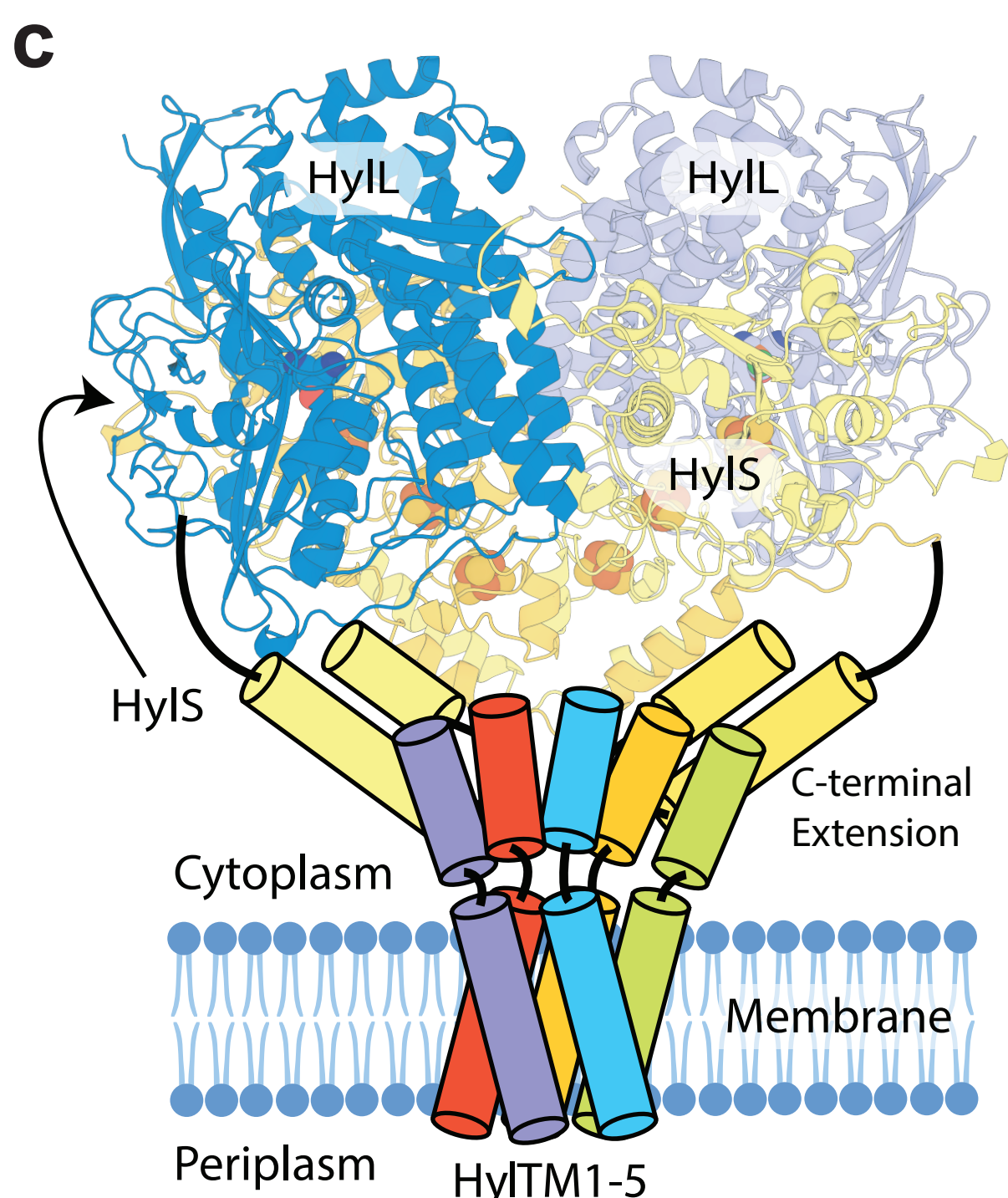
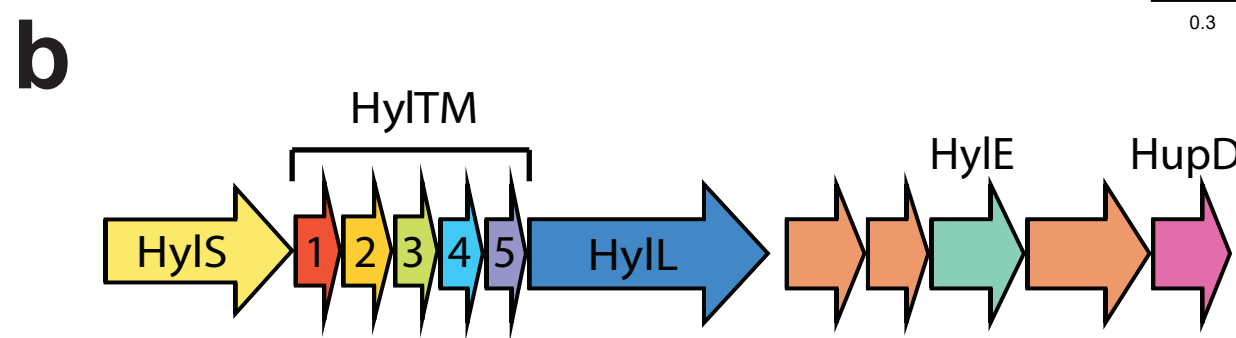
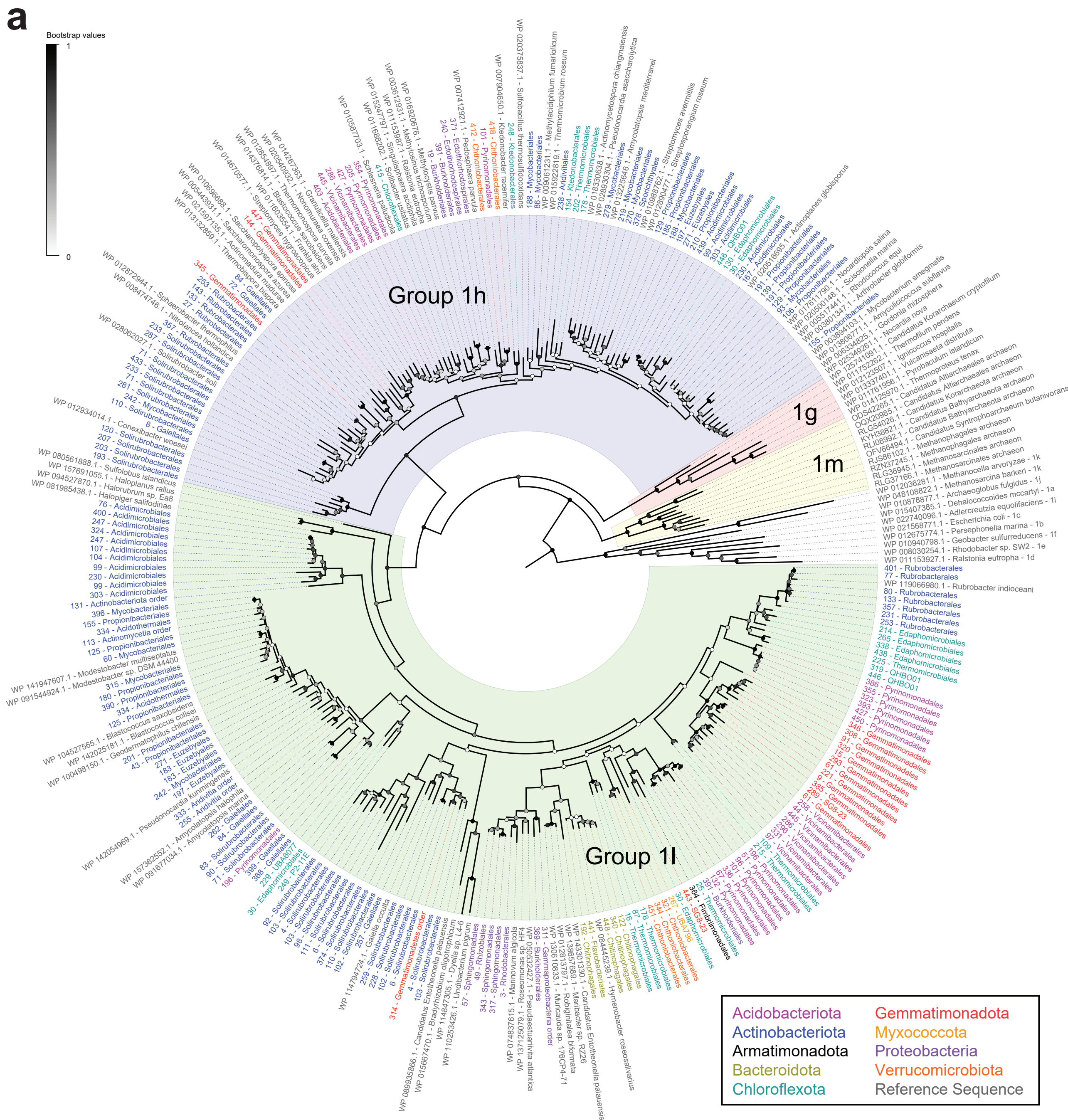
1111

1112 **Figure 4. Rates of atmospheric trace gas oxidation by soils sampled from the**  
1113 **Mackay Glacier region.** Boxplots show rates of oxidation of atmospheric H<sub>2</sub>, CO,  
1114 and CH<sub>4</sub> for each soil in duplicate soil microcosms at 10°C, based on gas  
1115 chromatography measurements. Only rates for samples with detectable gas  
1116 oxidation are shown. **(a)** Atmospheric gas oxidation rate for each microcosm  
1117 normalized to wet weight of soil. **(b)** Cell-specific reaction rates for each microcosm.  
1118 These rates were calculated by dividing the estimated soil cell abundance and  
1119 proportion of gas oxidizers based on quantitative qPCR and metagenome short read  
1120 analysis (HhyL and HylL abundance for H<sub>2</sub>, CoxL abundance for CO, PmoA and  
1121 MmoX abundance for CH<sub>4</sub>).

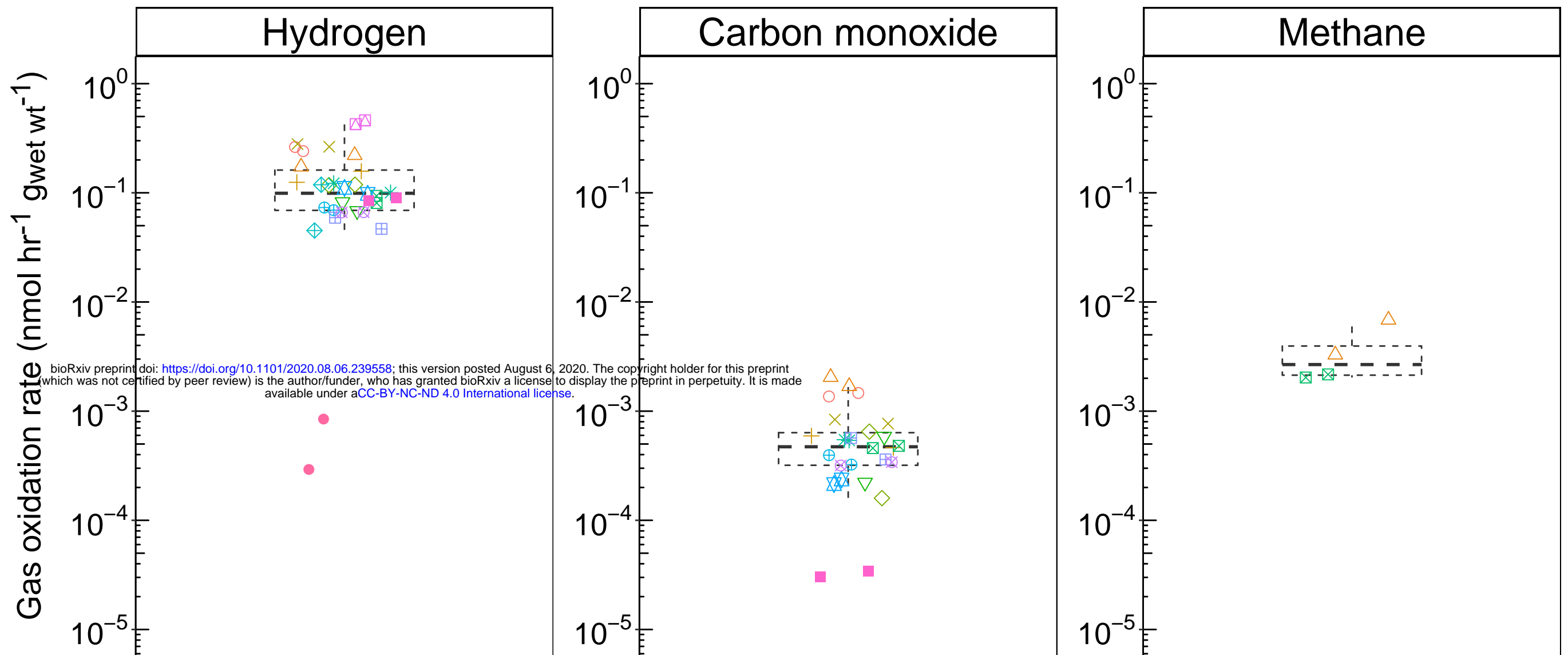
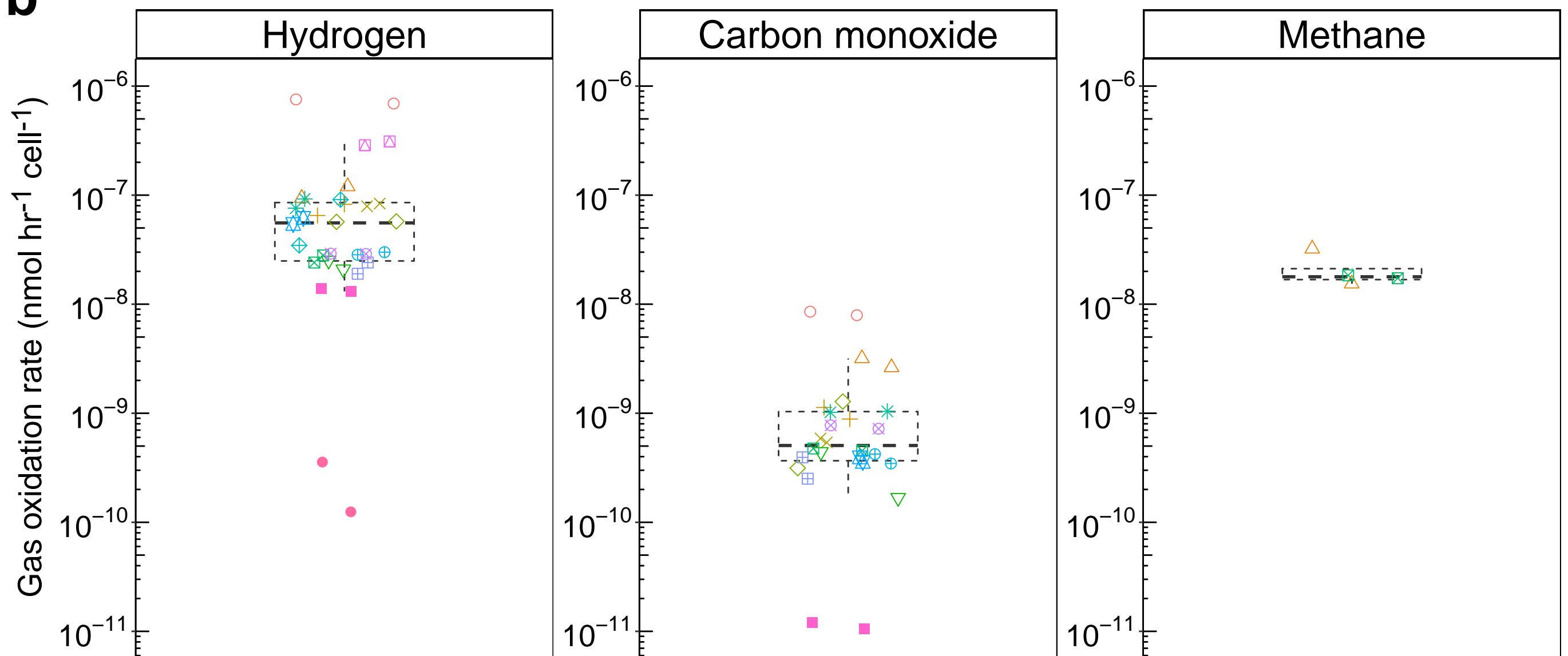










**a****b**

**Sample**

○ Mount Seuss 4	◇ Mackay Glacier 1	◊ Mount Gran 2	⊗ Benson Glacier
△ Mount Seuss 5	▽ Mackay Glacier 2	⊕ Towle Glacier 1	◊ Spaulding Pond
+ Mount Seuss 6	⊠ Mount Murray	⊗ Towle Glacier 2	■ Flatiron
× Mount Seuss 7	* Mount Gran 1	⊠ Cliff Nunatak	● Pegtop Mountain