

1 **Summer dynamics of microbial diversity on a mountain glacier**

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17 **Running title:** Temporal dynamics of glacier microbes

18

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20

21 **Abstract:**

22 Under climate change, glaciers are rapidly receding worldwide. A melting cryosphere will

23 dramatically alter global sea levels, carbon cycling, and water resource availability. Glaciers also

24 host rich biotic communities that are dominated by microbial diversity and this biodiversity can

25 impact surface albedo, thereby driving a feedback loop between biodiversity and cryosphere

26 melt. However, the microbial diversity of glacier ecosystems remains largely unknown outside of

27 major ice sheets, particularly from a temporal perspective. Here, we characterized temporal
28 dynamics of bacteria, eukaryotes, and algae on the Paradise Glacier, Mount Rainier, USA, over
29 the summer melt season. During our study, the glacier surface steadily darkened as seasonal
30 snow melted and darkening agents accumulated until new snow fell in late September. From a
31 community-wide perspective, the bacterial community remained generally constant and
32 eukaryotes exhibited a clear temporal progression of community change while fungal diversity
33 was intermediate. Individual taxonomic groups, however, exhibited considerable stochasticity.
34 We found little support for our *a priori* prediction that autotroph abundance would peak before
35 heterotrophs. Notably, two different trends in snow algae emerged—an abundant early- and
36 late-season OTU with a different mid-summer OTU that peaked in August. Overall, our results
37 highlight the need for temporal sampling to clarify microbial diversity on glaciers and that caution
38 should be exercised when interpreting results from single or few timepoints.

39

40 **Body:**

41 Glacier ecosystems are key components of global biodiversity and support diverse, mostly
42 microbial communities comprised of bacteria, photosynthetic algae, and fungi [8, 9, 15].
43 However, beyond point estimates of biodiversity, seasonal variation of these biota is poorly
44 understood. To date, the majority of biological research on glaciers has focused on establishing
45 baselines of biodiversity [4], understanding the ecophysiology of resident organisms [3],
46 resource availability and use [7, 13], and clarifying drivers of biological albedo reduction [where
47 pigmented organisms darken the cryosphere and promote melt, 9]. However, temporal
48 perspectives of biodiversity in glacier ecosystems remain rare [but see 5, 14, 16].

49

50 Glacier surfaces are highly dynamic and experience substantial environmental fluxes in space
51 and time. Early season “spring” conditions on temperate glaciers are typically marked by

52 increasing periods of daylight with intense temperature swings and relatively little biological
53 activity. By summer, temperature swings have moderated and biotic activity including
54 photosynthesis, respiration and nutrient cycling near annual peaks [1]. In fall, days shorten,
55 temperatures decrease, and snowfall events limit primary productivity [1].

56

57 Here, we present a temporal perspective of microbial community change on the Paradise
58 Glacier, Mount Rainier, WA, USA (Fig. 1a,b), a temperate alpine glacier that hosts a diverse,
59 representative community of glacier biota. From May to September, 2019, we collected triplicate
60 snow samples from ~2255 m on the eastern margin of the glacier and tracked changes in
61 microbial communities by sequencing 16S and 18S small subunit rRNA and fungal ITS
62 amplicons (detailed methods provided in Supporting Information). We expected to uncover a
63 rich biological community on the glacier and evidence of successional dynamics with primary
64 producer abundance peaking early in summer followed by an increase in heterotrophs later in
65 the season.

66

67 During our study, the Paradise Glacier surface darkened as seasonal snow receded, debris
68 accumulated, and biotic processes (e.g., snow algal blooms) transpired until late September
69 when new snow fell (Fig. 1a-b). Overall, we recovered 4724 bacterial OTUs (16S), 1246
70 eukaryotic OTUs (18S), and 3007 fungal OTUs (ITS). The bacterial community was distinct
71 month-to-month, particularly later in the season (Fig. 1d). The eukaryotic and fungal
72 communities were less clearly differentiated month-to-month but exhibited more seasonal
73 progression than bacteria (i.e., the amount of time between sampling events appeared to
74 generally scale with community turnover; Figs. 1e,f). Alpha diversity (Shannon's) was temporally
75 stable for bacteria (Fig. 1g), steadily increased for eukaryotes (Fig. 1h), and was variable for

76 fungi (Fig. 1i). The effects of September snowfall had little effect on the community composition,
77 alpha diversity, nor relative abundances (Figs. 1d-i, 2).

78

79 The most abundant bacterial OTUs were affiliated with Bacteroidetes and Proteobacteria (Fig.
80 2a-b). Within the Bacteroidetes, OTUs assigned to *Ferruginibacter* and *Solitalea* were most
81 abundant and OTUs assigned to *Pseudomonas* (Gammaproteobacteria) and *Exiguobacterium*
82 (Bacillota) were also common. For eukaryotes, OTUs assigned to green algae were abundant,
83 including four Chlorophyta OTUs; three were assigned to the snow algae genus *Chlainomonas*
84 while the fourth belonged to Cyanidiales. Basidiomycota OTUs were prevalent in the fungal
85 data, including six of the 10 most abundant OTUs. These six OTUs were affiliated with the
86 Microbotryomycetes including *Phenoliferia* and *Filobasidium* as well as OTUs that could not be
87 classified below the Class level.

88

89 The abundance of most major bacterial groups fluctuated through time (e.g., Bacteroidetes and
90 Actinobacteria were most abundant in July and less abundant in early September, Fig. 2a). In
91 contrast, Proteobacteria were abundant in all samples. Algal taxa (phylum Chlorophyta),
92 perhaps the most influential eukaryotes on glaciers [9], were recovered in all samples from all
93 months (Fig. 2c) but were least abundant in June. Algal community composition shifted
94 throughout the summer: abundant *Chlainomonas* OTUs in May and late September were
95 distinct from those recovered in July-September samples (Fig. 2d). For fungi, the relative
96 abundance of sac fungi (Ascomycota) increased in late summer, peaking after the first
97 significant snowfall in September (Fig. 2e). Conversely, the highest abundances of
98 Basidiomycota (the other division that comprises the subkingdom Dikarya alongside
99 Ascomycota) were observed in May with lower levels from June-September (Fig. 2f).

100

101 Broadly, our results support dynamism in both taxonomic composition and abundance of
102 microbial communities on mountain glaciers during the summer melt season. For many groups
103 (e.g., *Ferruginibacter*, Fig. 2b), abundance trends appeared stochastic, or at least not linked to
104 any seasonal dynamics, while others (e.g., Pucciniomycota) exhibited clear directionality across
105 the melt season. Given the resource-poor nature of glacier ecosystems [13], we expected to
106 observe an early-season wave of primary producers followed by an increase in heterotrophs
107 later in the season. Contrary to our expectation, OTUs for snow algal primary producers,
108 particularly *Chlainomonas* within the Chlorophyta, were abundant in all samples except June.
109 Because the same May *Chlainomonas* OTUs increased in abundance in late September, May
110 samples could reflect cells buried from previous years. In contrast, the July—September
111 samples contain *Chlainomonas* OTUs that are distinct from this “resident” community and are
112 perhaps the product of atmospheric input. However, since physical and chemical snow
113 conditions can impact snow algae composition and pigment content [12] which vary seasonally
114 [11], it is also possible that both algal communities are present and local conditions drive the
115 differences we observed. We did observe a decrease in Microbotryomycetes (in the
116 Basidiomycota) and an increase in Ascomycota, fungi which typically favor nutrient-rich niche
117 space [2], in later season samples. Shifts in fungal taxa in response to temperature and
118 nutrients [10] have been linked to resource availability selecting for specific taxa.
119
120 With widespread interest in microbial diversity in the cryosphere to better understand carbon
121 cycling, biological albedo reduction, and community ecology of glacier ecosystems [1, 6, 13], it
122 is clear that one or a few estimates of abundance may not reflect broader trends. Thus, our data
123 underscore the need for temporal sampling to ultimately uncover higher level links between
124 biology and the cryosphere in the mountain cryosphere [9]. To realize this potential, such efforts
125 should ideally occur across multiple locations within and among montane regions.

126

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133 **Author contributions:**

134 S.H. and T.L.H. conceived of the study. S.H. collected samples. T.L.H. performed analyses.
135 S.H. and T.L.H. wrote the manuscript with support from T.P. All authors read and approved the
136 final version.

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138 **Competing interests:** The authors declare no competing financial interests.

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140 **References:**

141 1. Anesio AM, Laybourn-Parry J (2012). Glaciers and ice sheets as a biome. *Trends Ecol Evol*
142 27;219-225.

143 2. Crowther TW, Boddy L, T Jones H (2012). Functional and ecological consequences of
144 saprotrophic fungus–grazer interactions. *The ISME Journal* 6;1992-2001.

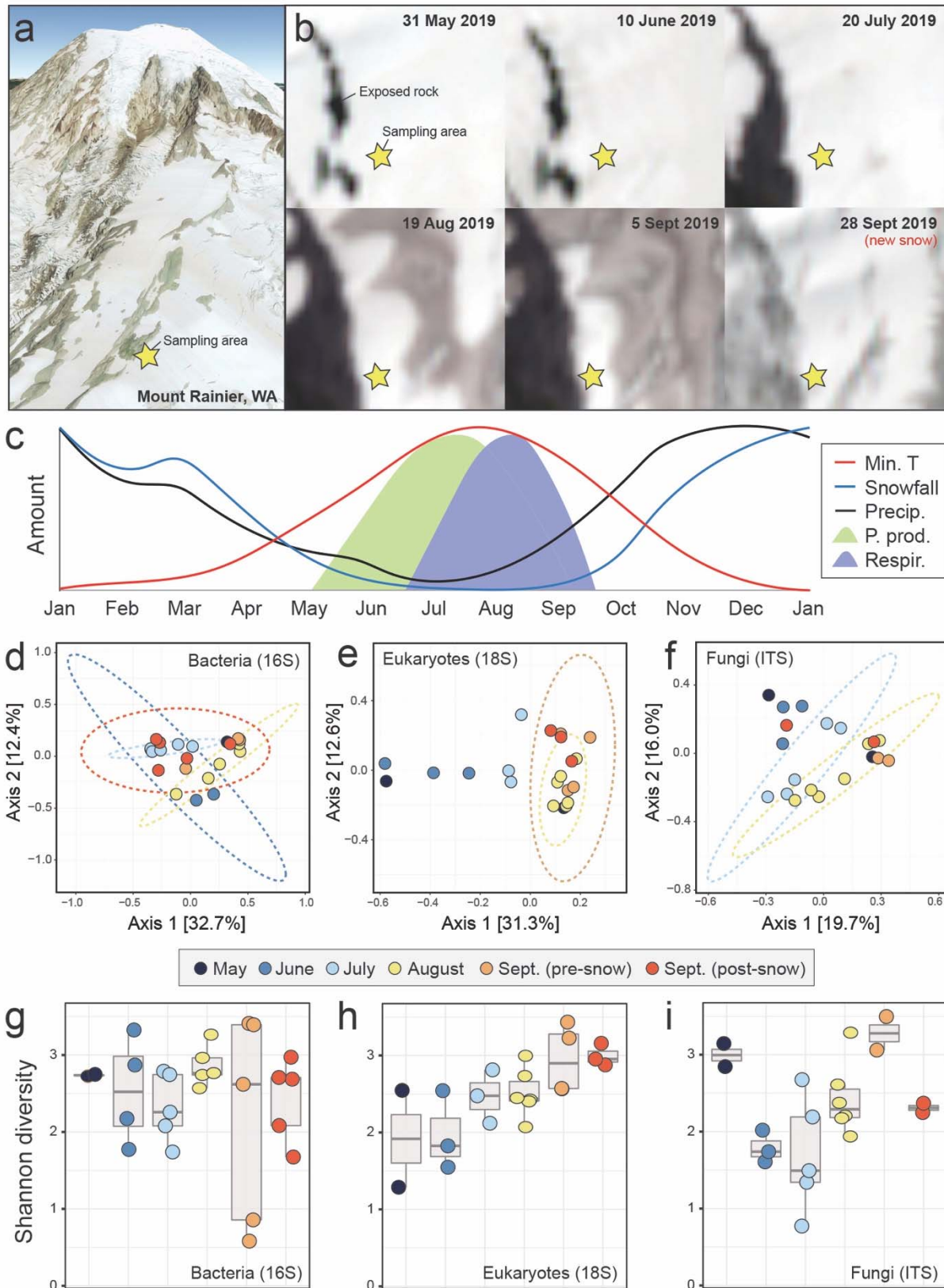
145 3. Dial RJ, Becker M, Hope AG, Dial CR, Thomas J, Slobodenko KA *et al* (2016). The role of
146 temperature in the distribution of the glacier ice worm, *Mesenchytraeus solifugus*
147 (Annelida: Oligochaeta: Enchytraeidae). *Arctic, Antarctic, and Alpine Research* 48;199-
148 211.

149 4. Edwards A, Pachebat JA, Swain M, Hegarty M, Hodson AJ, Irvine-Fynn TDL *et al* (2013). A
150 metagenomic snapshot of taxonomic and functional diversity in an alpine glacier
151 cryoconite ecosystem. *Environmental Research Letters* 8;035003.

- 152 5. Els N, Greilinger M, Reisecker M, Tignat-Perrier R, Baumann-Stanzer K, Kasper-Giebl A *et al*
153 (2020). Comparison of bacterial and fungal composition and their chemical interaction in
154 free tropospheric air and snow over an entire winter season at Mount Sonnblick, Austria.
155 *Frontiers in microbiology* 11;980.
- 156 6. Ganey GQ, Loso MG, Burgess AB, Dial RJ (2017). The role of microbes in snowmelt and
157 radiative forcing on an Alaskan icefield. *Nature Geoscience* 10;754.
- 158 7. Hamilton TL, Havig JR (2018). Inorganic carbon addition stimulates snow algae primary
159 productivity. *The ISME journal*;1.
- 160 8. Hotaling S, Hood E, Hamilton TL (2017). Microbial ecology of mountain glacier ecosystems:
161 biodiversity, ecological connections and implications of a warming climate.
162 *Environmental microbiology* 19;2935-2948.
- 163 9. Hotaling S, Lutz S, Dial RJ, Anesio AM, Benning LG, Fountain AG *et al* (2021). Biological
164 albedo reduction on ice sheets, glaciers, and snowfields. *Earth-Science Reviews*
165 220;103728.
- 166 10. Li H, Yang S, Semenov MV, Yao F, Ye J, Bu R *et al* (2021). Temperature sensitivity of SOM
167 decomposition is linked with a K-selected microbial community. *Global Change Biology*
168 27;2763-2779.
- 169 11. Onuma Y, Takeuchi N, Tanaka S, Nagatsuka N, Niwano M, Aoki T (2020). Physically based
170 model of the contribution of red snow algal cells to temporal changes in albedo in
171 northwest Greenland. *The Cryosphere* 14;2087-2101.
- 172 12. Remias D, Karsten U, Lütz C, Leya T (2010). Physiological and morphological processes in
173 the Alpine snow alga *Chloromonas nivalis* (Chlorophyceae) during cyst formation.
174 *Protoplasma* 243;73-86.
- 175 13. Ren Z, Martyniuk N, Oleksy IA, Swain A, Hotaling S (2019). Ecological stoichiometry of the
176 mountain cryosphere. *Frontiers in Ecology and Evolution* 7;360.

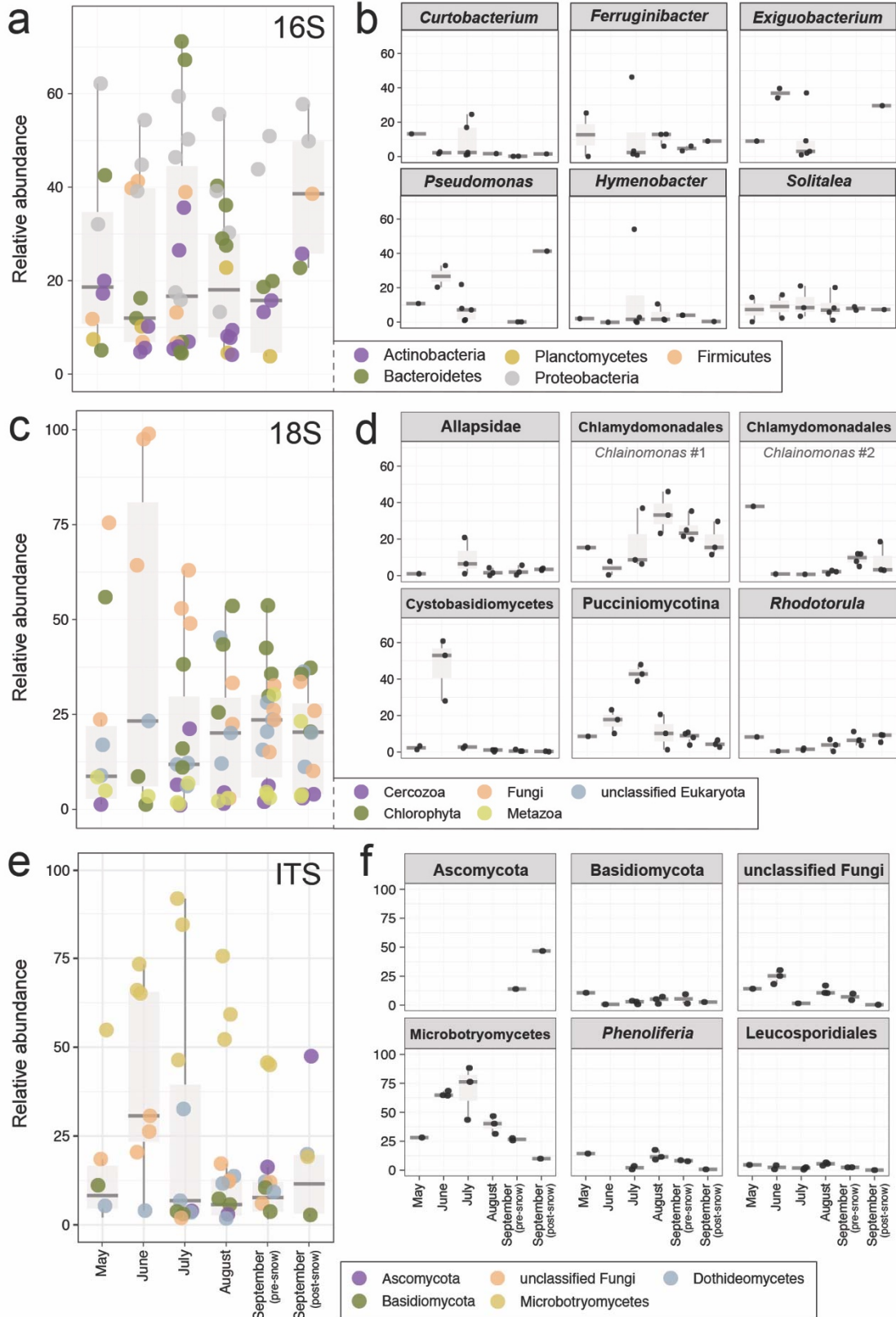
- 177 14. Sheik CS, Stevenson EI, Den Uyl PA, Arendt CA, Aciego SM, Dick GJ (2015). Microbial
178 communities of the Lemon Creek Glacier show subtle structural variation yet stable
179 phylogenetic composition over space and time. *Frontiers in microbiology* 6;495.
- 180 15. Stibal M, Bradley JA, Edwards A, Hotaling S, Zawierucha K, Rosvold J *et al* (2020). Glacial
181 ecosystems are essential to understanding biodiversity responses to glacier retreat.
182 *Nature Ecology & Evolution* 4;686-687.
- 183 16. Winkel M, Trivedi CB, Mourot R, Bradley JA, Vieth-Hillebrand A, Benning LG (2022).
184 Seasonality of Glacial Snow and Ice Microbial Communities. *Frontiers in*
185 *microbiology*;1760.
- 186

187 **Figure:**



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189 Figure 1. (a) Location of our study site on the Paradise Glacier of Mount Rainier, Washington, USA.
190 Imagery from Google Earth. (b) Sentinel-2 satellite imagery of the study site from late May to
191 September, 2019. A fresh snowfall occurred between the final two sampling timepoints in
192 September. (c) A conceptual image of primary production and heterotroph activity on a temperature
193 mountain glacier over the course of one year. Overlaid on this conceptual framework are monthly
194 averages of minimum temperature, average precipitation, and average snowfall for the nearby
195 Paradise Ranger Station (1655 m) from 1916-2016 (data from the Western Regional Climate
196 Center). (d-f) Principal coordinate analysis of community composition based on Bray-Curtis
197 dissimilarity for (d) bacteria, (e) eukaryotes, (f) and fungi. (g-i) Shannon diversity through time for the
198 same sampling points and communities: (g) bacteria, (h) eukaryotes, and (i) fungi.
199



201 Figure 2. Temporal abundance of common taxonomic groups for each data set overall and broken
202 down for select taxa: (a-b) 16S rRNA, (c-d) 18S rRNA, and (e-f) fungal ITS. Taxonomic groups
203 comprising the largest percent relative abundance in each library are shown in a, c, and e. The most
204 abundant operational taxonomic units (OTUs) in each data set are shown in b, d and f where
205 taxonomy has been assigned to each OTU at the highest resolution possible (see detailed methods
206 in the Supporting Information). Box plots show mean percent relative abundance of the group (a, c
207 and e) or OTU (b, d and f). Data sets are binned by month of sample collection except for early and
208 late September.