

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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132

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.

137

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

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140 **References:**

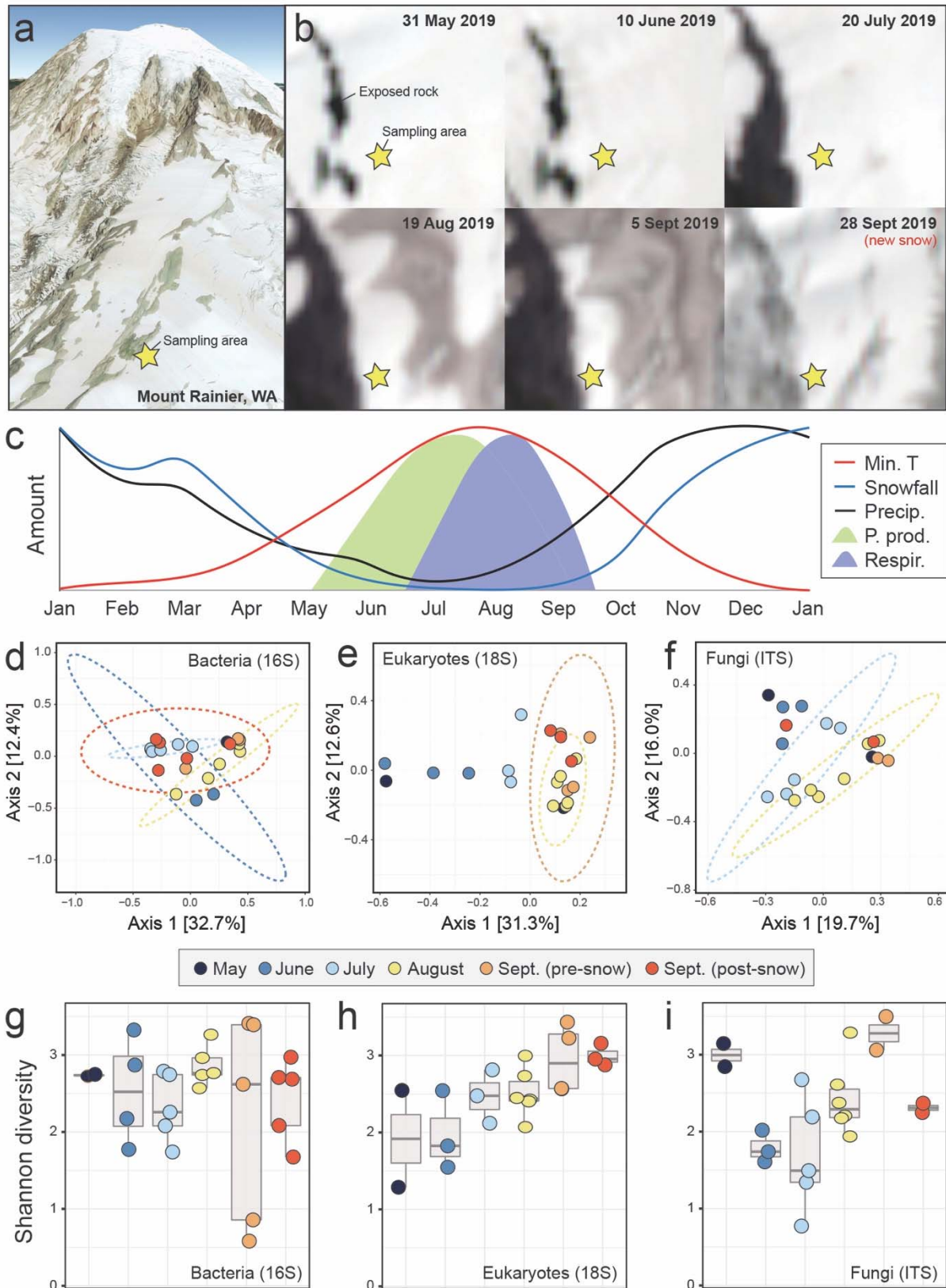
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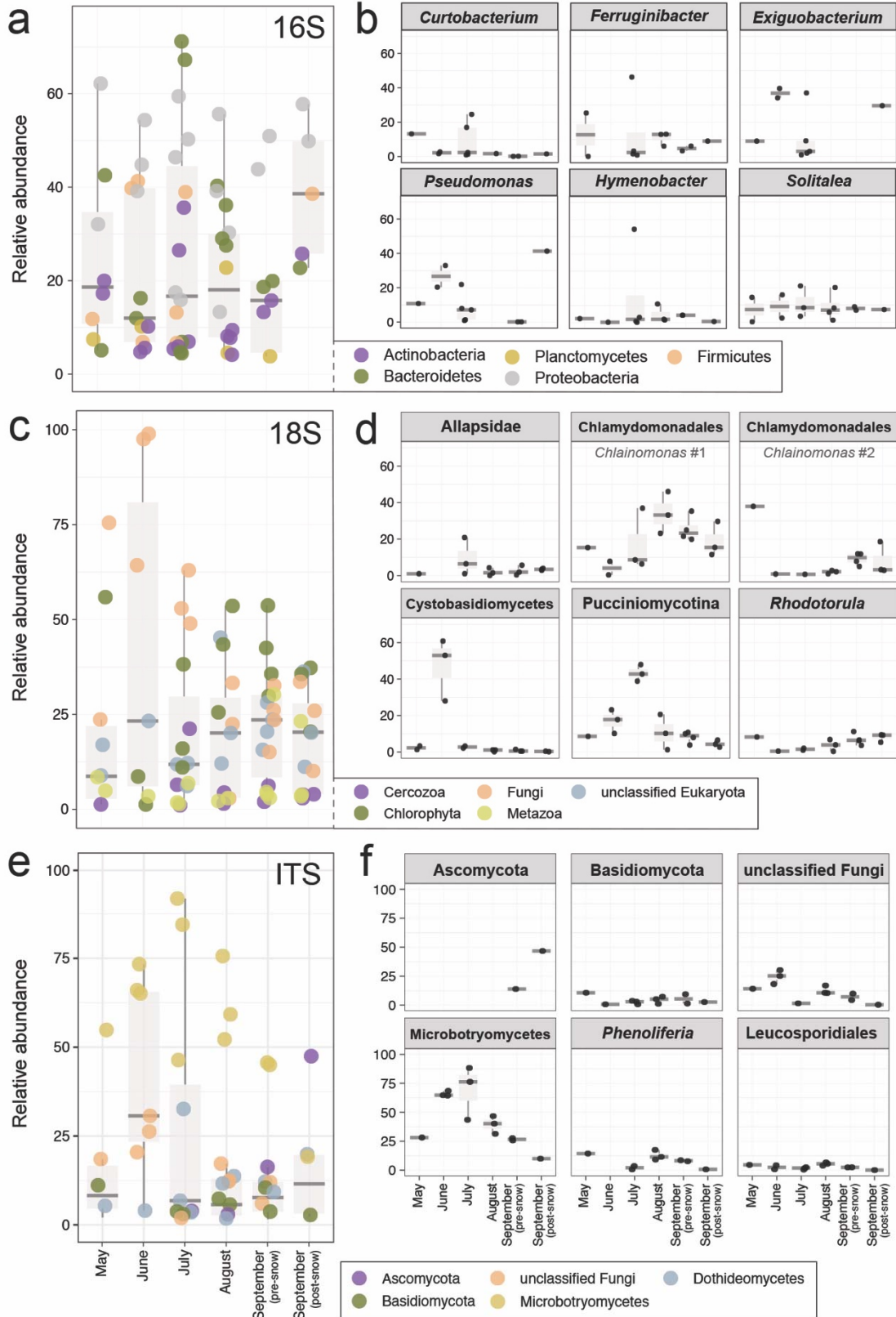


187 **Figure:**



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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.