ABSTRACT  Glaciers are rapidly receding under climate change. A melting cryosphere will dramatically alter global sea levels, carbon cycling, and water resource availability. Glaciers host rich biotic communities that are dominated by microbial diversity, and this biodiversity can impact surface albedo, thereby driving a feedback loop between biodiversity and cryosphere melt. However, the microbial diversity of glacier ecosystems remains largely unknown outside of major ice sheets, particularly from a temporal perspective. Here, we characterized temporal dynamics of bacteria, eukaryotes, and algae on the Paradise Glacier, Mount Rainier, USA, over nine time points spanning the summer melt season. During our study, the glacier surface steadily darkened as seasonal snow melted and darkening agents accumulated until new snow fell in late September. From a community-wide perspective, the bacterial community remained generally constant while eukaryotes and algae exhibited temporal progression and community turnover. Patterns of individual taxonomic groups, however, were highly stochastic. We found little support for our a priori prediction that autotroph abundance would peak before heterotrophs. Notably, two different trends in snow algae emerged—an abundant early- and late-season operational taxonomic unit (OTU) with a different midsummer OTU that peaked in August. Overall, our results highlight the need for temporal sampling to clarify microbial diversity on glaciers and that caution should be exercised when interpreting results from single or few time points.

IMPORTANCE  Microbial diversity on mountain glaciers is an underexplored component of global biodiversity. Microbial presence and activity can also reduce the surface albedo or reflectiveness of glaciers, causing them to absorb more solar radiation and melt faster, which in turn drives more microbial activity. To date, most explorations of microbial diversity in the mountain cryosphere have only included single time points or focused on one microbial community (e.g., bacteria). Here, we performed temporal sampling over a summer melt season for the full microbial community, including bacteria, eukaryotes, and fungi, on the Paradise Glacier, Washington, USA. Over the summer, the bacterial community remained generally constant, whereas eukaryote and algal communities temporally changed through the melt season. Individual taxonomic groups, however, exhibited considerable stochasticity. Overall, our results highlight the need for temporal sampling on glaciers and that caution should be exercised when interpreting results from single or few time points.

KEYWORDS  alpine glacier, snow algae, glacier biology, cryosphere, biological albedo reduction, alpine
FIG 1  (a) Location of our study site on the Paradise Glacier of Mount Rainier, Washington, USA. Imagery from Google Earth. (b) Sentinel-2 satellite imagery of the study site from late May to September 2019. A fresh snowfall occurred between the final two sampling time points (Continued on next page)
Chlorophyta), perhaps the most in see also Fig. 1d). In contrast, Proteobacteria were abundant in all samples. Algal taxa (phylum and Actinobacteria were most abundant in July and less abundant in early September; Fig. 2a;}

level.

and

Filobasidium abundant OTUs. These 6 OTUs were af

Cyanidiales. Basidiomycota OTUs were prevalent in the fungal data, including 6 of the 10 most

OTUs assigned to

phenoliferia abundant OTUs. These 6 OTUs were af

Ferruginibacter and Solitalea were most abundant and OTUs assigned to

Pseudomonas (Gammaproteobacteria) and

Exiguobacterium (Bacillota) were also common. For

eukaryotes, OTUs assigned to green algae were abundant, including four Chlorophyta OTUs;

three were assigned to the snow algae genus

Chlainomonas, while the fourth belonged to

Cyanidiales. Basidiomycota OTUs were prevalent in the fungal data, including 6 of the 10 most

abundant OTUs. These 6 OTUs were affiliated with the Microbotryomycetes, including

Phenoliferia and Filobasidium as well as OTUs that could not be classified below the class level.

The abundance of most major bacterial groups fluctuated through time (e.g., Bacteroidetes and Actinobacteria were most abundant in July and less abundant in early September; Fig. 2a; see also Fig. 1d). In contrast, Proteobacteria were abundant in all samples. Algal taxa (phylum Chlorophyta), perhaps the most influential eukaryotes on glaciers (2), were recovered in all samples from all months (Fig. 2c) but were least abundant in June. Algal community composition

FIG 1 Legend (Continued)
in September. (c) A conceptual image of primary production and heterotroph activity on a temperature mountain glacier over the course of 1 year. Overlaid on this conceptual framework are monthly averages of minimum temperature, average precipitation, and average snowfall for the nearby Paradise Ranger Station (1,655 m) from 1916 to 2016 (data from the Western Regional Climate Center). (d to f) Principal coordinate analysis of community composition based on Bray-Curtis dissimilarity for (d) bacteria, (e) eukaryotes, (f) and fungi (g to i). Shannon diversity through time for the same sampling points and communities: (g) bacteria, (h) eukaryotes, and (i) fungi. Data were binned by sample collection month for visualization (see Table S1 in the supplemental material), and each circle in d to i represents one replicate. After processing, our final sample sizes were the following: 16S rRNA, May: n = 2, June: n = 3, July: n = 4, September: n = 2; 18S: n = 4; ITS: n = 3, August: n = 4; ITS: n = 3, September: n = 3, late September: n = 3. For ITS, May: n = 2, June: n = 3, July: n = 4, August: n = 4, September: n = 2, late September: n = 2.  

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FIG 2. Temporal abundance of common taxonomic groups for each data set overall and broken down for select taxa: (a and b) 16S rRNA, (c and d) 18S rRNA, and (e and f) fungal ITS. Each circle represents one replicate. Taxonomic groups comprising the largest percent relative abundance in each library are shown in a, c, and e. The most abundant operational taxonomic units (OTUs) in each data set are shown in b, d, and f, where taxonomy has been assigned to each OTU at the highest resolution possible (see detailed methods in the supplemental material). Box plots show mean percent relative abundance of the group (a, c, and e) or OTU (b, d, and f). Data sets are binned by month of sample collection except for early and late September, where the first seasonal snow occurred between sampling efforts.
shifted throughout the summer: abundant Chlainomonas OTUs in May and late September were distinct from those recovered in July–September samples (Fig. 2d). For fungi, the relative abundance of sac fungi (Ascomycota) increased in late summer, peaking after the first significant snowfall in September (Fig. 2e). Conversely, the highest abundances of Basidiomycota (the other division that comprises the subkingdom Dikarya alongside Ascomycota) were observed in May, with lower levels from June to September (Fig. 2f).

Broadly, our results support dynamism in both taxonomic composition and abundance of microbial communities on mountain glaciers during the summer melt season. For many groups (e.g., Ferruginibacter; Fig. 2b), abundance trends appeared stochastic, or at least not linked to any seasonal dynamics, while others (e.g., Pucciniomycota) exhibited clear directionality across the melt season. Given the resource-poor nature of glacier ecosystems (6), we expected to observe an early-season wave of primary producers followed by an increase in heterotrophs later in the season. Contrary to our expectation, OTUs for snow algal primary producers, particularly Chlainomonas within the Chlorophyta, were abundant in all samples except June. Because the same May Chlainomonas OTUs increased in abundance in late September, May samples could reflect cells buried from previous years. In contrast, the July–September samples contain Chlainomonas OTUs that are distinct from this "resident" community and are perhaps the product of atmospheric input. However, since physical and chemical snow conditions can impact snow algae composition and pigment content (12), which vary seasonally (13), it is also possible that both algal communities are present and local conditions drive the differences we observed. We did observe a decrease in Microbotryomycetes (in the Basidiomycota) and an increase in Ascomycota, fungi which typically favor nutrient-rich niche space (14), in later season samples. Shifts in fungal taxa in response to temperature and nutrients (15) have been linked to resource availability selecting for specific taxa.

With widespread interest in microbial diversity in the cryosphere to better understand carbon cycling, biological albedo reduction, and community ecology of glacier ecosystems (6, 11, 16), it is clear that one or a few estimates of abundance may not reflect broader trends. Thus, our data underscore the need for temporal sampling to ultimately uncover higher-level links between biology and the cryosphere in the mountain cryosphere (2). To realize this potential, such efforts should ideally occur across multiple locations within and among montane regions.

SUPPLEMENTAL MATERIAL
Supplemental material is available online only.

**TEXT S1**, DOCX file, 0.02 MB.

**TABLE S1**, DOCX file, 0.02 MB.

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

We declare no competing financial interests.

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