Minireview

Microbial ecology of mountain glacier ecosystems: biodiversity, ecological connections and implications of a warming climate

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Summary

Glacier ecosystems are teeming with life on, beneath, and to a lesser degree, within their icy masses. This conclusion largely stems from polar research, with less attention paid to mountain glaciers that overlap environmentally and ecologically with their polar counterparts in some ways, but diverge in others. One difference lies in the susceptibility of mountain glaciers to the near-term threat of climate change, as they tend to be much smaller in both area and volume. Moreover, mountain glaciers are typically steeper, more dependent upon basal sliding for movement, and experience higher seasonal precipitation. Here, we provide a modern synthesis of the microbial ecology of mountain glacier ecosystems, and particularly those at low- to mid-latitudes. We focus on five ecological zones: the supraglacial surface, englacial interior, subglacial bedrock–ice interface, proglacial streams and glacier forefields. For each, we discuss the role of microbiota in biogeochemical cycling and outline ecological and hydrological connections among zones, underscoring the interconnected nature of these ecosystems. Collectively, we highlight the need

to: better document the biodiversity and functional roles of mountain glacier microbiota; describe the ecological implications of rapid glacial retreat under climate change and resolve the relative contributions of ecological zones to broader ecosystem function.

Introduction

Mountain glaciers are globally distributed, developing where the accumulation of snowfall exceeds seasonal melting, which is often above treeline in the alpine biome. At low- to mid-latitudes, mountain glaciers tend to be warm-based with temperatures throughout generally equal to or exceeding the ice melting point. Like their polar counterparts, mountain glaciers are host to diverse microbial communities carrying out important ecosystem processes (e.g., carbon fixation; Anesio *et al.*, 2009). However, in contrast to polar ice sheets, mountain glaciers are often characterized by steep elevational gradients (and high ice velocities), elevated levels of hydrological connectivity from surface to base (via fractures, crevasses and moulins), and greater snow accumulation (and seasonal melting), particularly in maritime environments (Hodson *et al.*, 2008; Anesio and Laybourn-Parry, 2012).

Glacier ecosystems contain diverse habitats that we have divided into five ecological zones: supraglacial snow and ice (where sunlight penetrates); interior englacial ice; subglacial sediments at the bedrock–ice–meltwater interface; proglacial streams derived from supra- and subglacial runoff, as well as possible groundwater inputs and glacier forefields where the recession of glacier ice exposes historically ice-covered soils to atmospheric processes (Figs 1 and 2). Supraglacial snow and ice, subglacial sediments, proglacial streams and glacier forefields support a diverse array of life, dominated by bacteria and eukarya (Anesio and Laybourn-Parry, 2012; Boetius *et al.*, 2015). In contrast, the englacial zone appears to contribute negligibly to broader ecosystem function and diversity (Boetius *et al.*, 2015),
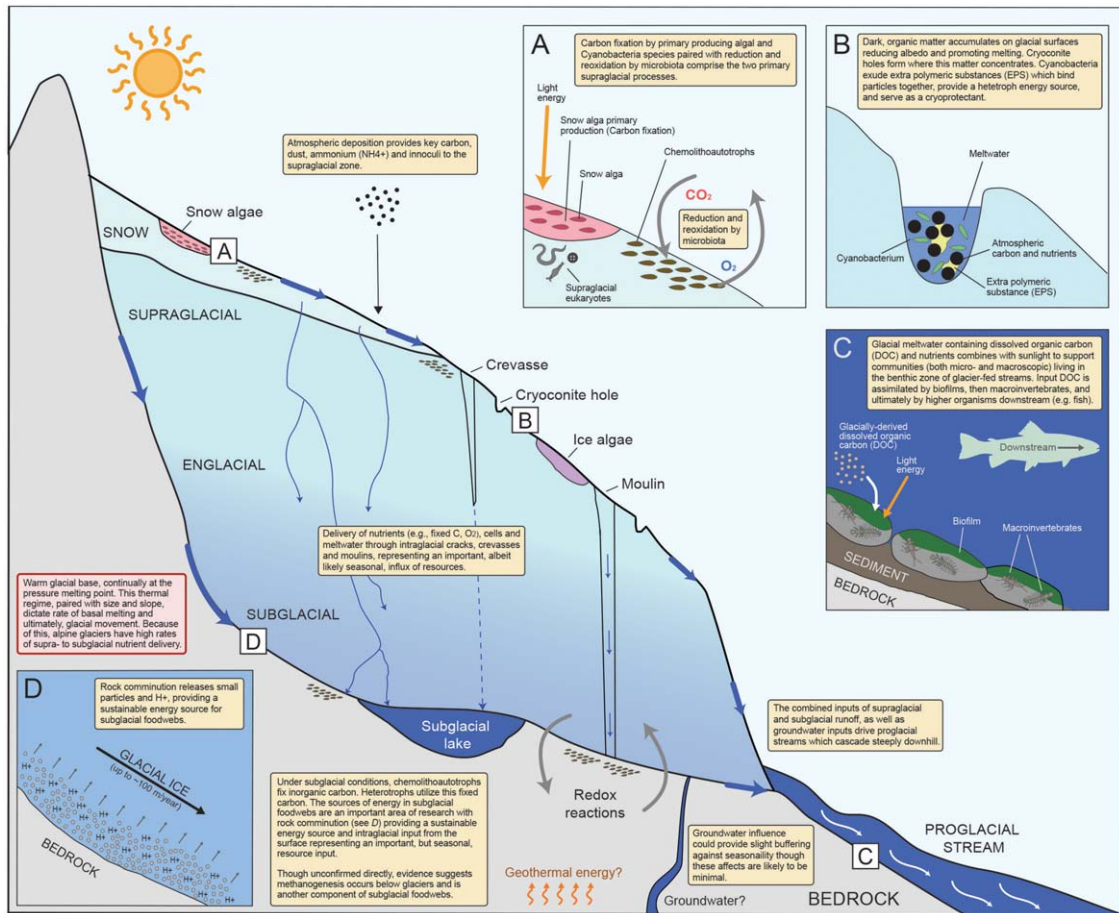
Fig. 1. A schematic of mountain glacier ecology, hydrology and geomicrobiology. Insets represent zoomed in perspectives of (A) supraglacial snow, (B) cryoconite holes, (C) proglacial streams, and (D) subglacial rock comminution. Black arrows represent atmospheric deposition, orange arrows (A, C) indicate solar radiation, blue arrows indicate meltwater moving on, through, and under the glacier, eventually emanating from the glacier terminus into a proglacial stream, and the black arrow (D) shows the flow of glacier ice downhill. This figure is based on Fig. 2A in Boetius *et al.* (2015), and has been extended to reflect mountain glaciers specifically.

and primarily functions as a conduit delivering nutrients from supra- to subglacial habitat, and eventually, into proglacial streams (Hodson *et al.*, 2008; Anesio and Laybourn-Parry, 2012).

Ice sheets and glaciers are host to significant biodiversity despite cold temperatures, limited available water for cellular processes (particularly seasonally), and low nutrient availability (Fountain and Walder, 1998; Hodson *et al.*, 2008; Anesio and Laybourn-Parry, 2012; Boetius *et al.*, 2015). The ecology and productivity of microbial life on, in, and beneath mountain glaciers is largely dictated by a combination of proximity to sources of allochthonous organic matter (OM) and nutrients (e.g., an urban area, forest or desert; Stibal *et al.*, 2012aa), interactions between bedrock, ice and subglacial sediments, solar radiation and the magnitude of annual melting, which influences the scale of hydrological connectivity across ecological zones. While available

sunlight is the most important component of supraglacial productivity (Hodson *et al.*, 2008; Anesio *et al.*, 2009; Chuvochina *et al.*, 2011; Boetius *et al.*, 2015; Lutz *et al.*, 2015), the supraglacial zone is also influenced, though perhaps marginally, by wind deposition of dust, ash and particles from nearby bedrock (Chuvochina *et al.*, 2011). This limited influence of bedrock and elevated importance of solar radiation as an ecological control contrasts starkly with the subglacial zone where, in the absence of light, variation in ecological processes depends directly upon bedrock lithology, because different rock compositions vary in their ability to facilitate abiogenic chemical energy production (Mitchell *et al.*, 2013; Telling *et al.*, 2015) and influence basal sliding (Sharp, 1988). The connection between hydrology and the supraglacial–englacial–subglacial axis is an important but underexplored component of mountain glacier ecology, as variation in water flowpaths – e.g., the extent
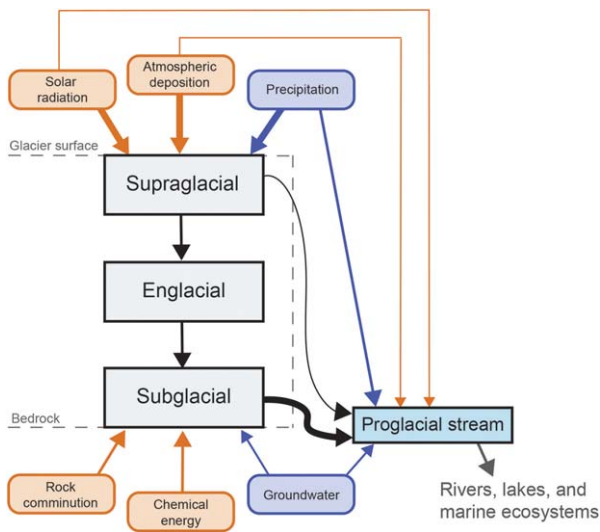
Fig. 2. A schematic of energy and nutrients (orange) and hydrologic (blue) inputs and flow through a mountain glacier. Black arrows indicate combined flow of both. Differential flow, up to ~2 orders of magnitude, and variance in atmospheric deposition and solar energy input, are shown by darker versus lighter arrows between the glacier-proglacial connections.

of crevasses, moulins or finer-scale fissures – within the supraglacial or englacial zones can greatly influence microbial activity in the subglacial zone. This high degree of influence stems from the vital role flowing water plays in linking the supraglacial to subglacial zones, through the englacial intermediary. Downstream, the microbial ecology of proglacial streams is influenced by basal and supraglacial meltwater in summer (when stream channels are most active) and by basal melting in concert with any groundwater inputs the rest of the year. Seasonal variation of basal meltwater discharge typically spans approximately two orders of magnitude (Fountain and Walder, 1998). Taken together, geomicrobiological and ecological activity anywhere on, within, or beneath a glacier can directly influence proglacial stream and lake ecosystem function (Fig. 1). This includes carbon use across trophic levels (e.g., Fellman *et al.*, 2015), and these collective influences are likely highly seasonal.

Mountain glaciers respond strongly to climate change (Dyurgerov and Meier, 2000; Hall and Fagre, 2003; Barnett *et al.*, 2005; Wrona *et al.*, 2006; Bolch *et al.*, 2012), and the implications of their ongoing recession are far-reaching. These impacts range from changes in the amount of available habitat (and taxonomic diversity) to both short- and long-term effects on the global carbon cycle (Wadham *et al.*, 2008; Anesio *et al.*, 2009; Jacobsen *et al.*, 2012; Hood *et al.*, 2015). Despite slow doubling times and decreased rates of biogeochemical transformations (Anesio *et al.*, 2010), microbially

mediated biogeochemical cycling is an important aspect of glacier ecology (Hodson *et al.*, 2008) and has been an emerging focus of glacier geomicrobiological research. Traditionally, the difficulty of culturing glacier microbes with slow doubling times and remote research locations has hindered geomicrobiological studies of mountain glaciers. Advances in sequencing technology (e.g., next-generation sequencing or NGS), genomic data collection (e.g., microscale metagenomics, Rinke *et al.*, 2016), microcosm and isotope studies (e.g., single cell stable isotope probing, Wang *et al.*, 2016), and improved cultivation methodologies (e.g., microfluidic cultivation, Jiang *et al.*, 2016) hold significant potential for improving understanding of microbial diversity and function, as well as biogeochemical interactions in mountain glacier ecosystems. However, a comprehensive framework linking microbial ecology of mountain glaciers with the hydrological and ecological connections, both within, nearby and downstream of their influence, has not been developed.

Here, we provide a modern synthesis of microbial ecology of low- to mid-latitude mountain glacier ecosystems with a focus on biodiversity and function. Specifically, we extend previous efforts that focused on the ecology and biodiversity of glaciers or ice generally (Hodson *et al.*, 2008; Anesio and Laybourn-Parry, 2012; Boetius *et al.*, 2015) and complement existing reviews centered upon the microbial ecology of specific components of the global cryosphere, including sea ice (Boetius *et al.*, 2015) and permafrost (Jansson and Taş, 2014). We also integrate broad perspectives on the interconnected nature of the mountain glacier ecosystem and highlight the inextricable ecological linkages between mountain glaciers and their proglacial aquatic habitats. We begin by synthesizing existing knowledge across five ecological zones – supraglacial, englacial, subglacial, proglacial streams and glacier forefields. Next, we discuss the relevant ecological, hydrological and geological connections between our four ecological zones that are connected by hydrology – supraglacial, englacial, subglacial and proglacial streams – and their influences on microbial ecology. We conclude by identifying areas for future research, possibilities for greater integration of emerging tools, and discuss the implications of a rapidly changing climate on mountain glacier ecosystems through the lens of microbial ecology.

Ecological zones

Supraglacial zone

Encompassing the uppermost, sun-lit layer of ice and snow, as well as surface streams, ponds, cryoconite holes and moraines, the supraglacial zone is heavily influenced by solar radiation (Figs 1A and 2) and

inoculated with microbes and essential nutrients via atmospheric deposition (reviewed by Xiang *et al.*, 2009; Rime *et al.*, 2016). When conditions are conducive to ice-melt, water becomes available and facilitates microbially mediated biogeochemical processes (Anesio and Laybourn-Parry, 2012). A significant portion of biological activity on glacier surfaces occurs in cryoconite holes – small, water-filled depressions (Fig. 1B) – that form when dark material (e.g., soil or dust) is deposited on the glacier surface. The low albedo of the dark material (versus highly reflective snow and ice surrounding it) causes increased absorption of solar radiation and locally accelerated melting. Cryoconite holes are rich in OM, host diverse communities, and are an important habitat for glacial carbon cycling and fixation (Nylen *et al.*, 2004; Nkem *et al.*, 2006; Anesio *et al.*, 2009; 2010; Edwards *et al.*, 2014).

Surface life on mountain glaciers can be substantial with algal cell concentrations ranging from 4.4×10^4 to 9.9×10^5 cells per ml^{-1} of meltwater (Takeuchi, 2001). The pigmented cells of eukaryotic algae and cyanobacteria colour snow and ice during the melt season, contributing up to 1.2 kg km^{-2} of biomass to the ecosystem (as observed in Alaskan mountains, Takeuchi *et al.*, 2006). Though dominated by autotrophic and heterotrophic bacteria, the supraglacial community also includes a number of eukaryotes: protozoa, diatoms, rotifers, fungi and, in rare cases, even annelid ice worms (Tynen, 1970; Hodson *et al.*, 2008; Anesio and Laybourn-Parry, 2012). The most commonly observed heterotrophic bacteria are affiliated with Actinobacteria and Proteobacteria, but members of other phyla (e.g., Bacteroidetes) are also present (Simon *et al.*, 2009; Chuvochina *et al.*, 2011; Franzetti *et al.*, 2016). The bulk of supraglacial heterotrophic activity is supported by allochthonous carbon sources. In cryoconite holes for instance, microbial assemblages are equipped to degrade many different exogenous organic carbon sources (Simon *et al.*, 2009; Stibal *et al.*, 2012a; Edwards *et al.*, 2013; Musilova *et al.*, 2015) as well as autochthonous extracellular polymeric substances (EPS) resulting from *in-situ* primary production. These EPS likely represent an important, high-density energy source for heterotrophs and light-limited photoautotrophs (Takeuchi *et al.*, 2010; Laybourn-Parry *et al.*, 2012; Cook *et al.*, 2016). Though photoautotrophic communities of snow algae and cyanobacteria drive the majority of primary production on mountain glaciers, the recovery of 16S rRNA genes and transcripts affiliated with methanogens and ammonia-oxidizing archaea suggests that chemoautotrophic carbon fixation also occurs in the supraglacial zone (Hamilton *et al.*, 2013; Lutz *et al.*, 2015; Hamilton and Havig, 2016). Moreover, recent metagenomic evidence suggests that anoxygenic phototrophy may also play a vital role in mountain glacier primary production (Franzetti *et al.*, 2016).

Primary productivity in supraglacial ecosystems occurs at rates that are relevant on the scale of the global carbon cycle (e.g., Anesio *et al.*, 2009). However, the balance between autotrophic and heterotrophic activity, and ultimately whether glaciers are net CO_2 sources or sinks, remains largely unknown. For polar glaciers, autotrophic production tends to exceed respiration (Anesio *et al.*, 2009; Telling *et al.*, 2011; Yallop *et al.*, 2012), and the same is likely true for mountain glaciers, but few data points exist. Indeed, significant algal and bacterial primary production have been observed on alpine snowfields and glaciers (Thomas and Duval, 1995; Hamilton and Havig, 2016), but respiration and heterotrophic activity have not been quantified for these habitats. In contrast to studies of surface blooms, observed rates of autotrophic production were lower than respiration rates in cryoconite holes in the Alps where microbial communities likely depend on the delivery of exogenous carbon (Edwards *et al.*, 2013). However, considerable variation in primary productivity measurements (differences of more than two orders of magnitude) have been reported for cryoconite holes in the Austrian Alps (Anesio *et al.*, 2009; Edwards *et al.*, 2013). Though intriguing, this wide variation was likely observed because one glacier, Stubacher Sonnblickes, was heavily influenced by the Chernobyl accident in 1984 through an accumulation of radionucleotides in the cryoconite (Anesio *et al.*, 2010). Regardless, the contributions of snow algal blooms and OM-rich cryoconite holes on mountain glaciers to the global carbon cycle remain poorly constrained. This is further complicated by the fact that the role of meiofauna in the carbon balance of mountain glacier ecosystems is not well understood. In general, respiration rates decrease with decreasing temperature (Clarke and Fraser, 2004) and observed grazing rates of sea ice meiofauna are reported to be much lower than algal production (Grandinger, 1999; Nozais *et al.*, 2001). Still, meiofauna are regularly observed in supraglacial ecosystems including cryoconite holes, but studies to characterize the contribution of these heterotrophs to carbon mineralization are lacking.

Even less is known regarding other biogeochemical cycles (e.g., N, Fe, S and P) in supraglacial ecosystems. Debris, rock flour and volcanic ash can be important sources of essential nutrients to the ice and snow surface (Lutz *et al.*, 2015) as well as the delivery of fixed nitrogen via atmospheric precipitation. Metagenome sequencing of cryoconite microbial communities recovered abundant evidence for efficient nutrient acquisition including genes for assimilating Fe, inorganic and organic S, and ammonia (Edwards *et al.*, 2013). Moreover,

the recovery of nitrifying archaea (Hamilton *et al.*, 2013) and *nifH* sequences, a gene encoding a structural protein necessary for nitrogen fixation (Boyd *et al.*, 2011), suggests active nitrogen cycling occurs in surface microbial assemblages. The observation of nitrate production, presumably via nitrification, within a supraglacial stream reach in coastal Alaska (Scott *et al.*, 2010) also supports the notion that N is actively cycled in supraglacial ecosystems.

Englacial zone

Located in the interior of the glacier where light does not penetrate, the englacial zone is characterized by high pressure (Price, 2000), limited interstitial space in the ice matrix, a near absence of available water for cellular processes, and few energy sources (Priscu *et al.*, 2006). Since biological activity in the englacial is likely negligible compared with other glacial habitats, meltwater channels (i.e., moulins, crevasses and any small interstitial spaces) are the englacial zones' most important contribution to the broader glacial ecosystems (Hodson *et al.*, 2008). Specifically, through these meltwater channels, englacial zones provide an important, albeit strongly seasonal (both in scale and structure), conduit for delivering nutrients, water, atmospheric gases and viable cells from the glacier surface to bed, and eventually into proglacial streams and lakes. This delivery of resources is greatly dependent on several factors: incidence of crevasses and moulins on the glacier surface (and their respective depth), seasonal snow coverage, ambient temperature, and solar radiation. Mountain glaciers experience significant variability in all of these factors, and especially the scale of seasonal melt; thus the englacial network of nutrient delivery pathways may be especially important in these ecosystems. Additionally, though discussions of viable microorganisms entombed in glacial ice is beyond the scope of this review, see Hodson *et al.* (2008), Anesio and Laybourn-Parry (2012) and Boetius *et al.* (2015) for general discussions stemming from polar studies (e.g., Lanoil *et al.*, 2009) that likely also apply to mountain glaciers.

Subglacial zone

Temperate glacial ice is permeated by a network of hydraulically linked fractures (Fountain *et al.*, 2005). Water percolates and flows through these fractures and cavities from the supraglacial surface, through the englacial zone and accumulates beneath glaciers at the ice–bedrock interface when mean subglacial temperatures reach the pressure melting point (Nye and Frank, 1973). For polar glaciers, this mix of available water and nutrients accumulates in saturated sediments, often forming subglacial lakes. The steep elevational gradients that typify mountain ecosystems often preclude the

development of subglacial lakes, though under the right conditions, they can form, often at glacier margins (Capps *et al.*, 2010; Livingstone *et al.*, 2016). Therefore, meltwater streams and sediments at the glacier–bedrock interface are the most common subglacial habitat in montane habitats. Energy sources in subglacial ecosystems are both diverse and abundant, with basal melting, rock comminution, supraglacial input, and, in some cases, geothermal energy, all contributing to ecosystem function and associated services (Hodson *et al.*, 2008; Hamilton *et al.*, 2013; Boyd *et al.*, 2014; Boetius *et al.*, 2015). Moreover, redox potential is a key control on microbial community structure and function in the subglacial zone, and depends primarily on hydrological flow-paths and connectivity between chemical weathering and oxygen sources (Tranter *et al.*, 2005).

Diverse, active communities of archaea, bacteria and even fungi live beneath mountain glaciers with observed cell counts ranging from $\sim 10^6$ to 10^7 cells mL^{-1} of meltwater (Sharp *et al.*, 1999; Skidmore *et al.*, 2000; Foght *et al.*, 2004; Price and Sowers, 2004; Montross *et al.*, 2013). While viable bacteria, archaea and eukarya have been recovered and cultured from subglacial sediments (Sharp *et al.*, 1999; Foght *et al.*, 2004; Margesin *et al.*, 2005; Turchetti *et al.*, 2008; Buzzini *et al.*, 2012; Hamilton *et al.*, 2013), NGS technologies have greatly clarified the scale of diversity and functions of subglacial microbes (Hamilton *et al.*, 2013). Specifically, 16S rRNA sequences affiliated with chemolithoautotrophs and autotrophic methanogens have been recovered from alpine subglacial sediments suggesting primary production within the subglacial system (Skidmore *et al.*, 2005; Boyd *et al.*, 2010; Hamilton *et al.*, 2013). Further evidence for lithotrophic primary production in subglacial sediments includes the recovery of RuBisCO mRNA transcripts (Boyd *et al.*, 2014) and the isolation of *Thiobacillus* sp. RG5 (a member of the Betaproteobacteria subphyla) from subglacial sediments in the Canadian Rockies (Harrold *et al.*, 2016). In polar and subpolar regions, chemolithoautotrophic activity can fix several micrograms of carbon per m^2 per day (Gaidos *et al.*, 2004; Mikucki and Priscu, 2007; Christner *et al.*, 2014). While similar measurements are not available for mountain glaciers, the recovery of abundant transcripts affiliated with chemolithoautotrophs and autotrophic methanogens from mountain glacier subglacial sediments (Boyd *et al.*, 2010; Hamilton *et al.*, 2013) suggests this activity is likely significant enough to be highly relevant to broader ecosystem function.

Evidence for phylogenetically diverse heterotrophic bacteria and eukarya has also been recovered from mountain subglacial sediments including sequences affiliated with Proteobacteria, Bacteroidetes, ciliates (Strichotrichida) and amoebae (Tectofilosida; Hamilton *et al.*, 2013).

Subglacial heterotrophs are most likely supported by a diversity of sources: labile carbon deposited in pre-glacial times, organic outputs from the activity of chemolithoautotrophs, ancient OM in sediments, and organic carbon delivered from the supraglacial zone (Mikucki and Priscu, 2007). While conditions in subglacial sediments – specifically, anoxia (Bottrell and Tranter, 2002; Wadham *et al.*, 2004; Wynn *et al.*, 2006) and the availability of labile carbon substrates (Wadham *et al.*, 2008) – are often referenced as favourable for organic carbon degradation via methanogenesis, recent evidence suggests the majority of subglacial methanogenesis may be hydrogenotrophic (autotrophic; Boyd *et al.*, 2014; Telling *et al.*, 2015). Evidence for active cycling of Fe, fixed N and S has been observed in mountain subglacial sediment flowpaths where variable redox conditions are common. Specifically, sulfate reduction and sulfide oxidation (Bottrell and Tranter, 2002; Wadham *et al.*, 2004; Lanoil *et al.*, 2009), ferric iron reduction (Foght *et al.*, 2004) and nitrification and denitrification (Hodson *et al.*, 2005; Wynn *et al.*, 2006; 2007; Boyd *et al.*, 2011) all occur however, the spatial extent and magnitude of the cycling for these elements remains poorly constrained.

Glacial bedrock comminution, or the grinding of bedrock into particles by a glacier, generates fresh mineral surfaces capable of sustaining chemotrophic microbial communities including autotrophic mineral-based metabolism with reduced Fe and S as key electron donors (Mitchell *et al.*, 2013; Boyd *et al.*, 2014; Fig. 1D). In the Canadian Rockies, chemical energy generated during the oxidation of pyrite (FeS_2) and nitrification likely fuels a significant portion of primary productivity in the subglacial zone (Boyd *et al.*, 2011; 2014). The oxidation of pyrite produces hydrogen ions – key drivers in chemical weathering – that promote dissolution of bedrock calcite and dolomite, thereby contributing to microbially mediated weathering (Raiswell, 1984; Schlesinger and Jiang, 1991; Fairchild *et al.*, 1993; Tranter *et al.*, 1994). Subglacial abiotic hydrogen production may also support microbial metabolism of both autotrophic bacteria (Boyd *et al.*, 2014) and methanogenic archaea (Boyd *et al.*, 2010; Stibal *et al.*, 2012b; Dieser *et al.*, 2014; Telling *et al.*, 2015).

To date, the breadth of functional and phylogenetic diversity beneath mountain glaciers has likely been underestimated due to multiple factors: undersampling of the full range of bedrock lithologies that drive habitat conditions, limited spatial variability in study sites at individual glaciers, and relatively few studies have incorporated NGS approaches to characterize diversity. Moreover, the potential for bedrock comminution to provide a sustainable energy source to subglacial ecosystems has broad implications for not only the present-day landscape of biodiversity, but also Earth's ancient past and the possibility of life on other planets. This is supported by stable climatic conditions provided by glacial beds that may have enabled biodiversity to persist during periods of inhospitable climatic, atmospheric or geologic conditions (Skidmore *et al.*, 2005; Hodson *et al.*, 2008).

Proglacial streams

Proglacial streams and lakes are a prominent component of glacierized ecosystems, exerting strong controls on the geomorphology and ecology of these systems, while also serving as a link between glacial processes and downstream habitats, both freshwater and marine (Battin *et al.*, 2003; 2004; Besemer *et al.*, 2009; Wilhelm *et al.*, 2013; 2014; O'Neel *et al.*, 2015). Though not explicitly discussed in this review, proglacial lakes form between glacial ice and downstream habitats, significantly influencing abiotic stream conditions (Jacobsen *et al.*, 2012; Freimann *et al.*, 2014). Proglacial lakes are an important habitat in need of future study, particularly in the context of anthropogenic climate change (Peter and Sommaruga, 2016). Still, streams are the overarching constant in high-elevation freshwater ecosystems, yet despite their importance as biogeochemical conduits, microbial diversity and function in proglacial streams has not been well studied (Hotaling *et al.*, 2017). To date, most research on proglacial stream ecosystems has focused on macroinvertebrate species and genetic diversity (Brown *et al.*, 2007; Jacobsen *et al.*, 2012; Giersch *et al.*, 2016; Tronstad *et al.*, 2016). In contrast to general research in cryobiology which has largely focused on polar latitudes, much of our existing understanding of proglacial streams stems from mountain glacier ecosystems.

The microbial ecology of proglacial streams is geographically diverse with studies from the Austrian Alps (Wilhelm *et al.*, 2013; 2014), Swiss Alps (Freimann *et al.*, 2013; 2014) and North America (Sheik *et al.*, 2015; Fegel *et al.*, 2016). These studies have collectively revealed that proglacial streamwater and biofilms tend to be dominated by Proteobacteria, Bacteroidetes, Actinobacteria, Cyanobacteria and algae (Wilhelm *et al.*, 2013; Fegel *et al.*, 2016). Microbial alpha (within site) diversity in streamwater and biofilms decreases with elevation, possibly due to harsher higher elevation habitat conditions, less diverse sources of microorganism input (i.e., aside from the glacier itself), or a combination of the two (Wilhelm *et al.*, 2013). In contrast, beta (between site) diversity decreases with increasing streamwater temperature (and correspondingly lower elevation), which indicates that as glaciers recede, a more homogenous microbial community will shift to higher elevations (Wilhelm *et al.*, 2013). This predicted change in microbial diversity of proglacial streams adds

a third layer to a larger, more ominous, trend in alpine stream biology. That is, there is combined evidence that as glaciers recede, gamma (regional) and beta (between site) diversity will decline in proglacial headwaters for macroinvertebrate species diversity (Jacobsen *et al.*, 2012), macroinvertebrate genetic diversity (Finn *et al.*, 2013; Jordan *et al.*, 2016) and microbial diversity (Wilhelm *et al.*, 2013). Bacterial diversity and richness of glacier-fed streams appears to depend heavily upon microhabitat (sediment, streamwater or biofilms) and hydrological composition, whether purely glacier-fed or also influenced by other hydrological sources (e.g., groundwater, Wilhelm *et al.*, 2013). However, other stream characteristics, particularly Ca, Fe, SiO_2 concentrations, appear to be specific to local geology and bedrock regardless of source contributions and may be largely dictated by basal geochemical processes rather than surface inputs (Fegel *et al.*, 2016). From a temporal standpoint, microbial communities in proglacial streams have been observed as being relatively stable across summer months (May-September) with only fine-scale day-to-day variation, likely in response to shifting geochemistry and water residence times (Sheik *et al.*, 2015).

Despite supraglacial melt acting as a significant hydrological source for proglacial ecosystems, microbiota from supraglacial environments contribute only marginally to biofilm and streamwater community composition (Wilhelm *et al.*, 2013). Instead, microbial communities in proglacial streamwater and subglacial sediments are most similar, a finding which is consistent with the majority of suspended sediment in glacier-fed streams being derived from subglacial comminution (Mitchell *et al.*, 2013). This limited supraglacial microbial footprint in proglacial stream ecosystems is likely twofold in origin. First, as described above, potential differences in routing of meltwater stemming from supraglacial versus subglacial environments dictates the amount of input contribution for each source. For temperate glaciers, most meltwater is routed through glacial fractures (Fountain and Walder, 1998; Fountain *et al.*, 2005), and therefore passes through the subglacial environment before exiting into proglacial streams. Second, differences in the physical and chemical characteristics of ice, streamwater and/or biofilms are significant and likely promote specialization by microbiota for each, thereby limiting colonization across habitats, particularly from ice to streamwater.

An additional link exists between glaciers and the physicochemistry of downstream ecosystems which correlates with changes in microbial community composition in both streamwater and biofilms (Wilhelm *et al.*, 2013). Glacial meltwater, which scales with catchment glacier coverage, exerts a strong control on the environmental properties of mountain streams, manifested in variations of streamwater electrical conductivity, pH and temperature (Hood and Berner, 2009; Wilhelm *et al.*, 2013; Fellman *et al.*, 2014). All three significantly influence biofilm community composition (Wilhelm *et al.*, 2013), and stream microbial assemblages are also sensitive to warming, even at small magnitudes. This sensitivity will be exacerbated by future decreases in glacial influence and increasing fragmentation of the alpine stream landscape, which will expose more stretches of streams to flowing over bedrock (and associated solar radiation). In glacial floodplains, structure and function of microbial communities is again linked to ecosystem physicochemistry, with a prevalence of specialist taxa in harsh glacier-fed streams versus a greater abundance of generalists in more stable, warmer groundwater-fed springs (Freimann *et al.*, 2013; 2014).

While studying microbial diversity in mountain glacier streams has been gaining interest, the functional role of these organisms remains understudied; however, this gap is also narrowing, albeit more slowly. For example, when relative abundances of rRNA versus rDNA are compared, some relatively rare taxa (i.e., comprising a small amount of rDNA abundance) are significantly more abundant in rRNA samples, suggesting that rare taxa play a disproportionate functional role in mountain proglacial streams (Wilhelm *et al.*, 2014). However, given the range of metabolic states that microbes exist in, results of this comparative approach should be interpreted with caution (Blazewicz *et al.*, 2013). While seasonality affects physical conditions in the other glacier zones discussed above, none are subject to the scale of variability inherent to proglacial streams. This variability spans both daily and seasonal temporal scales, and includes changes in volume of stream discharge, temperature, sediment load and redox conditions, all of which have important implications for resident biota (Hannah *et al.*, 2007; Milner *et al.*, 2010; Sheik *et al.*, 2015; Hotaling *et al.*, 2017). The possibility of observed variation in metabolic activity (rRNA abundance) versus total diversity (rDNA abundance) in hyper variable proglacial streams supports the idea that a microbial 'seed bank' (Pedrós-Alió, 2006) may provide community-level functional plasticity with different taxa being transcriptionally active in response to different sets of environmental parameters (Wilhelm *et al.*, 2014).

As mountain glaciers recede and contribute less to streamflow, there will be corresponding shifts in the export of basal resources and nutrients (i.e., C, N, P and Fe) from glaciers to proglacial streams (Hood and Scott, 2008; Saros *et al.*, 2010; Hood *et al.*, 2015). This is in part because glaciers can act as an important source of rock-derived elements such as P and Fe (Hodson *et al.*, 2004; Schroth *et al.*, 2011). In addition, OM

derived from glacier ecosystems is compositionally unique – it is N-rich and highly bioavailable compared with OM derived from terrestrial ecosystems that are dominated by higher plants (Hood *et al.*, 2009; Singer *et al.*, 2012). Glacier OM can function as a source of C and energy for heterotrophs and ultimately higher trophic level consumers including macroinvertebrates and fish (Fig. 1C; Fellman *et al.*, 2015; Guillemette *et al.*, 2017). Future changes in treeline elevation and vegetation succession in glacier forelands will impact the magnitude and character of OM inputs to proglacial streams with implications for the structure of in-stream microbial communities. To this end, recent evidence suggests biofilm bacteria below treeline tend to be more specialized, possibly in response to the higher diversity of allochthonous and autochthonous organic carbon inputs to mountain streams below treeline versus above (Wilhelm *et al.*, 2015). Thus, shifts in microbial community assemblages in proglacial streams may occur as treelines creep higher and new vegetation communities develop in glacier foreland riparian zones where they have not existed, at least since prior glacial cycles.

Glacier forefields

As mountain glaciers continue to retreat on a global scale (Roe *et al.*, 2017), retreating ice fronts will expose newly deglaciated forefields to colonization. The sequence of colonization, scale and sources of energy inputs, and how these forefields transition from newly deglaciated terrain to supporting higher-level organisms, remains underexplored (Bradley *et al.*, 2014). Based on comparative sequencing of microbial communities from the Damma glacier in the Swiss Alps, atmospheric input (through snow, rain or aeolian dust) of microorganisms to mountain glacier forefields is not a significant factor driving colonization patterns (Rime *et al.*, 2016). Instead, bacterial community composition of newly exposed soils is more similar to endogenous supra- and subglacial habitats, indicating that microbiota from the existing glacial ecosystem are the initial constituents in newly exposed soils (Rime *et al.*, 2016). However, the same pattern is not present for fungal communities, suggesting differential dispersal abilities between bacteria and fungi (Rime *et al.*, 2016), likely due to differences in size and physiological capabilities between the groups (Schmidt *et al.*, 2014). Although atmospheric deposition is not a significant source of microorganisms in the Damma glacier forefield, it was an important source of carbon, nitrate and ammonium to these ecosystems. Thus supra- and subglacial habitats may play a large role in seeding glacier forefields while atmospheric deposition may be important source of nutrients for these ecosystems. Both fungi and bacteria have been identified as important in primary succession of glacier forefields, establishing organic carbon and nitrogen pools prior to vegetation (Brown and Jumpponen, 2014). As mountain glaciers continue to recede, understanding the biological and nutrient dynamics of glacier forefields, particularly in the context of global nutrient cycles, will directly inform our collective capability to anticipate ecosystem-level changes.

Ecological linkage among zones connected by hydrology

Mountain glacier ecosystems encompass a unique combination of hydrological sources, largely microbially mediated ecosystem services, and geologic activity dictating the structure and function of distinct yet fundamentally linked habitats. Essentially, the physical properties of glaciers influence the microbial communities that reside within them, and these communities drive ecosystem services, the products of which are exported into proglacial aquatic habitats (Fig. 2). Beyond immediate connections between supraglacial, englacial, subglacial and proglacial habitats, mountain glacier-fed streams flow from headwaters, through many biomes, and eventually into the world's oceans. While beyond the scope of this review, focused investigation and synthesis regarding the influence of mountain glaciers on downstream biomes, aquatic or otherwise, is urgently needed (but see Moore *et al.*, 2009; Slemmons *et al.*, 2013; O'Neel *et al.*, 2015; Arimitsu *et al.*, 2016; Hotaling *et al.*, 2017). In this section, we outline a framework describing the relevant hydrological and ecological connections in mountain glaciers upon which future syntheses can build.

The supraglacial–subglacial ecological connection depends upon the hydraulic configuration of glacial drainage. This connection is mediated by the englacial zone, and primarily the layout of fractures and caverns within, which dictate the flow of OM, nutrients and micronutrients throughout the ecosystem (Fountain *et al.*, 2005; Laybourn-Parry *et al.*, 2012). At the height of summer, meltwater either runs off the supraglacial surface directly into proglacial streams, or more likely, drains through a constantly evolving matrix of crevasses, moulins, fractures and other spaces within the ice. Depending on drainage dynamics, ephemeral ponds can form on the surface and margins of mountain glaciers. Under the right conditions, these ponds can drain rapidly, delivering a pulse of meltwater and nutrients to the subglacial zone (Laybourn-Parry *et al.*, 2012). Ice velocities, their variation from surface to base, and local topography largely determine the degree of crevassing, and represent an important control on intra-glacier hydrology (Sharp, 1988). For warm-based mountain glaciers, high

ice velocities yield the highest incidence of crevasses in the global cryosphere (Benn and Evans, 2014) and this high crevasse incidence can translate to ~100% delivery of surface water and nutrients to subglacial sediments (Fountain and Walder, 1998; Hodson *et al.*, 2008). This potential for high supra-to-subglacial delivery of nutrients contrasts starkly with the cold-based, valley glaciers of polar latitudes where almost no surface water or nutrients are delivered to the subglacial zone (Hodson *et al.*, 2008). The supraglacial–subglacial connection in mountain glaciers fluctuates on daily and seasonal time-scales. During the melt season, intraday increases in delivery of nutrients and water from the surface occurs in parallel with daily temperature fluctuations (and solar radiation) on ice and snow surfaces. Outside of the melt season, only basal melting and the possibility of ground-water input can provide water and nutrients to subglacial ecosystems and thereby to proglacial streams.

As the literal source of many riverine networks globally, mountain glaciers and the microbial communities they support hold significant potential for ecosystem influence both near (e.g., proglacial streams) and far (e.g., oceans). Accelerated input (and ultimate decline) of glacier-derived OM as glaciers recede will directly impact the availability of carbon in proglacially influenced stream ecosystems as warming proceeds (Hood *et al.*, 2009; Singer *et al.*, 2012; O'Neel *et al.*, 2015). And, while the Antarctica Ice Sheet is the major repository of organic carbon on a global scale, mountain glaciers are responsible for much of its release on an annual basis. By 2050, it is estimated that mass loss from mountain glaciers will release ~15 Tg of dissolved organic carbon (DOC; Hood *et al.*, 2015), equivalent to roughly half the annual DOC flux from the Amazon River (~27 Tg; Moreira-Turcq *et al.*, 2003). Much of this labile glacier-derived DOC will likely be respired in glacier-fed streams (Singer *et al.*, 2012), although in regions with abundant tidewater and near-marine terminating glaciers, DOC released from glaciers will contribute to heterotrophic productivity in coastal ecosystems. This finding underscores that importance of proglacial streams in modulating global land-to-ocean biogeochemical fluxes.

Concluding remarks

Despite considerable progress, mountain glaciers remain one of the most understudied, yet arguably most imperiled and rapidly changing, ecosystems on Earth. Retreating ice directly translates to loss of habitat and species diversity, but beyond biodiversity implications lie myriad additional concerns. Chief among these is developing a better understanding of the role that microbial communities play in the cycling of carbon fixation and

nutrients on, within, and beneath glaciers, as well as how shifts in the release of these resources from glaciers may affect downstream ecosystems. And, these research foci should extend to the changing net area of glacier ice versus glacier forefields and how shifts in this ratio will affect many of the same large-scale processes. For example, if photosynthesis exceeds respiration on the surface of mountain glaciers (similar to conclusions drawn from polar ice sheets), then these habitats represent a contemporary sink for CO_2. If the opposite is true, then mountain glaciers are instead acting as net CO_2 sources. Both possibilities hold important climate change implications, particularly for atmospheric feedback loops driving the extension or retreat of glaciers themselves, and for any research efforts aimed at quantifying global CO_2 source-sink dynamics (Anesio *et al.*, 2009). Regardless, many factors linked to climate change may favour snow algae growth both temporally and spatially, ultimately decreasing surface albedo and accelerating both glacial melt and the release of stored carbon (Box *et al.*, 2012; Hood *et al.*, 2015; Lutz *et al.*, 2016). Retreating glaciers will also expose new stream channels, sediments and bedrock, significantly altering mountain landscapes, as well as the nutrients and cells transported via atmospheric deposition and precipitation. For subglacial environments, anoxia and darkness are defining habitat characteristics (Bradley *et al.*, 2014). Thus, exposure to light and oxygen will drastically shift energy inputs and selective pressures driving microbial assemblages, thereby altering diversity, ecosystem services and function (Wadham *et al.*, 2004; 2008).

Here, we have presented mountain glacier ecosystems as the product of five ecological zones, four of which are interconnected by hydrology. We also discussed the physical conditions, biotic makeup and linkages among them. This synthesis underscores several gaps in our basic knowledge of these difficult to study habitats. These include: the effects of large seasonal shifts in meltwater and nutrient delivery among zones; the role of bedrock composition in driving microbial community composition and function – to date, the majority of glacier geomicrobiological research has focused on glaciers that override primarily carbonate or granitic bedrock types, with little known of the processes that support microbial life for glaciers overriding volcanic terrains (e.g., basalt or andesite); and, the general microbial ecology of closely related mountain cryosphere habitats (i.e., snowfields and rock glaciers). With this in mind, we offer three aspects of mountain glacier microbial ecology that are ripe for future study.

First, for mountain glaciers, and particularly those below polar latitudes, there is a pressing need to improve understanding of how intra-annual shifts in hydrological flowpaths and sources impact microbial community

structure and biogeochemical function. The transport of water from supraglacial to subglacial environments exhibits extreme seasonality. Similarly, the melt season supports light-dependent primary producers for only a fraction of the year. In contrast, basal melting, rock comminution and geothermal energy sources should be less seasonally affected and, in most regions, snowfall covers the supraglacial surface and proglacial streams for large portions of the year. These colder ambient temperatures paired with filling of crevasses, moulins and other interstitial spaces should reduce supraglacial–subglacial–proglacial meltwater and nutrient connections to near zero. Therefore, seasonal shifts in intraglacial water transport, which alter both nutrient availability and redox potential (Tranter *et al.*, 2005), are likely key controls on the structure and function of microbial communities in mountain glacier ecosystems.

Second, the extent to which atmospheric dynamics and material deposition influence the microbial ecology of supraglacial and glacier forefield ecosystems deserves further attention. In particular, the degree to which exogenous material, particularly nutrients, is derived from local versus distant sources remains poorly constrained. While comminution and redox potential largely affect subglacial ecosystems, atmospheric particle deposition (e.g., black carbon, dust or volcanic ash) delivers key nutrients, organic matter and possibly microbiota to both zones, and ultimately this material can be released to downstream marine environments (Stubbins *et al.*, 2012; Doherty *et al.*, 2013; Dumont *et al.*, 2014; Gabbi *et al.*, 2015; Lutz *et al.*, 2015; O'Neel *et al.*, 2015).

Third, while the microbial ecology of mountain glaciers remains understudied compared with high-latitude glaciers and icefields, research into other components of the alpine cryosphere is acutely absent, especially when investigations beyond measures of biodiversity are considered. Indeed, perennial snowfields and rock glaciers are often contiguous with mountain glaciers; thus, a better understanding of their microbial dynamics has direct relevance to both glacier and mountain ecosystems. Moreover, snowfields and rock glaciers may provide important points of comparison. Snowfields do not exhibit rock comminution on the scale of glaciers and have much lower crevasse incidence, two key differences that could allow for better resolution of how those two contribute a wide array of variables that may be of interest. Similarly, rock glaciers and the debris-covered glaciers commonly found in the Himalaya have layers of organic and mineral debris, which provide insulation as well as a source of nutrients, thus serving as useful examples of naturally "nutrient-loaded" glaciers that may be less susceptible to anthropogenic warming.

Mountain glaciers are vital components of total biodiversity, carbon cycling, food web dynamics and ecosystem services on a global scale (Hotaling *et al.*, 2017), with microbial life acting as the dominant biogeochemical force in these extreme, ice-laden ecosystems. As they are among the most imperiled habitats on Earth, it is imperative that we continue to refine existing understanding of the role mountain glaciers play in geomicrobiological, biogeochemical and ecological processes. This understanding stands to be bolstered by continued incorporation of emerging technologies with established approaches. Though specific details and applications are beyond the scope of this mini-review, these technologies may include NGS tools for assessing biodiversity through metagenetic (single marker, many taxa; Wilhelm *et al.*, 2013) and metagenomic approaches (many markers, many taxa; Edwards *et al.*, 2013), transcriptional activity (Wilhelm *et al.*, 2014), particulate matter quantification through remote sensing (Di Mauro 2017), characterizing biodiversity via improved culture techniques (e.g., diffusion chambers, gel microdroplets and hollow-fibre membrane chambers, reviewed by Vester *et al.*, 2015) and measuring *in-situ* microbial activity (e.g., improved chemical sensors, Bagshaw *et al.*, 2016).

Given the links between microbial ecology, local bedrock and seasonal melting, individual data points from single glaciers or mountain ranges are useful but difficult to synthesize. Perhaps the field's most pressing need is that of collaborative globalization. Indeed, there is immense opportunity for researchers working in glacier ecosystems to establish standardized protocols, coordinate sampling efforts and integrate comparable data to build cross-continental perspectives. Similar calls have been raised for alpine streams (Hotaling *et al.*, 2017), and increased coordination between stream biologists, microbial ecologists and glaciologists could greatly enhance geographic sampling scopes with little added cost. Ultimately, our collective goal should be a more general understanding of the extant contributions of mountain glaciers to microbial biodiversity and ecosystem function, as well as how these patterns may be altered as climate change proceeds.

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