Pioneer communities in the forefields of retreating glaciers: how microbes adapt to a challenging environment

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1. Introduction

Glacier forefields are the landscape formed by retreating glaciers. As a glacier melts down, bare rock and gravel that have been covered by the glacier are released. This material is then subjected to weathering and to soil forming processes. The young soils characterising glacier forefields are generally scarcely vegetated and low in nutrients such as C, N, P and S. Bedrock properties determine the physico-chemical properties of the corresponding soils such as texture, pH, nutrient concentrations, heat and humidity retention, water and gas fluxes. Moreover, they are characterized by strong physical extremes, in particular concerning temperature and water regimes. Such conditions may also vary locally. Site morphology and slope orientation, for example, may be related to different precipitation and irradiation. The climate at glacier forefields is generally highly variable: for example, precipitation tends to be more frequent in spring and in autumn; winters are instead characterized by heavy snowfall, which melts relatively fast in spring. Strong day-night fluctuations in soil temperatures are also often observable [1].

The colonization of the exposed bedrock by pioneer microorganisms which can well adapt to the low nutrient conditions and climatic fluctuations is the first step in soil development (Fig. 1). Pioneer microorganisms are involved in all the major biogeochemical cycles, contribute to mineral weathering and subsequent release of nutrients [2], which in turn favours the establishment of more complex and efficient microbial communities and plants [3]. Microbial communities are therefore vital for the initiation and maintenance of nutrient cycling and ecosystem productivity. In soil, 80-90% of soil function is provided by microbes [4].

![Fig. 1. Schematic representation of components (white boxes) and processes (black boxes) the forefield of a retreating glacier. The white arrows represent possible origins of the pioneer communities (below glacier, above glacier, from the surroundings)](image-url)
In glacier forefields, microbial community structure and activity is strongly influenced by the interplay of the different physical and chemical environmental factors. For example, soil pH was related to microbial community structure in an array of different soils [5,6,7], influenced enzymatic activities [8] and is recognized as a major limiting factor for plant development [9]. However, the concentration of soluble nutrients and physical conditions of the sites rather than soil pH itself were identified as important factors related to bacterial community structure in different alpine glacier forefields [10]. Soil pH must therefore be considered a comprehensive parameter which includes soil-related properties, such as ion retention and base saturation, which are more meaningful for the soil microbial communities.

Seasonality plays a major role in influencing microbial community structure and activity. For example, a very important resource for microorganisms is the dissolved organic matter (DOC). In soil, it is present in several different chemical forms which include readily degradable substances to more recalcitrant compounds such as lignin. In glacier forefields, average DOC contents are low (Fig. 2), but represent an important source for microbial communities. Ratios of different kinds of organic matter vary in relation of soil type and of season, with a peak of accumulation of recalcitrant compounds during winter, under snow cover [11]. As a consequence, microbial groups possessing the specific exoenzymes for different organic types will fluctuate accordingly.

Critical periods for microbial communities are represented by the transition from autumn to winter and from spring to summer. In late autumn, temperatures decreases, while precipitation increases. At the end of autumn, if the temperatures drop below 0°C, in absence of snow, the water in the soil may freeze and negatively affect bacteria and fungi. During freezing and thawing aggregates are broken and, shearing forces lyse cells and hyphae, resulting in the selection of tolerant microbes [18] and in increased nutrient fluxes.

Therefore, snow cover plays a crucial role, as it forms a protective layer which maintains temperatures in the soil above freezing point, permitting the conservation of liquid water and the survival of the belowground microbial communities even at soil temperatures approaching -15°C [19]. Soil temperature monitoring at glacier forefields (Fig. 3) showed average winter temperatures which never dropped below -5°C. Snowmelt in spring produces a flush of water which causes loss of nutrients by leaching and limiting aeration in the soils. Microbial communities have to respond accordingly to such changes resulting in another selection pressure.

In conclusion, glacier forefields represent a unique platform for research on primary productivity and ecosystem development [20]. They offer in addition new insights on the role and stability of pioneer microbial communities under changing environmental conditions. The objective of this review is to underline the importance of these pioneer communities by outlining recent investigations regarding composition, activity and adaptation to extreme environmental conditions.
2. Structure of glacier forefield microbial communities

Assessing diversity in environmental samples is a general challenge in microbial ecology, as the existing techniques still do not manage to adequately detect the extreme complexity of microbial diversity [21]. Community structure in glacier forefields has been assessed through different approaches ranging from cultures [22] to clone libraries [23] and Phospholipid Fatty Acid or PCR-based profiling techniques such as denaturing gradient gel electrophoresis [1] and T-RFLP [10]. Bacterial, archaeal and fungal communities are often characterized by the profiling of their rRNA genes. The majority of studies involved the analysis at the DNA level; active communities may be alternatively profiled through the analysis of rRNA [24]. Such approach however may be limited by the instability of RNA and coextraction of DNA from the samples [25].

The advantage of the rRNA (DNA and RNA) molecules as phylogenetic markers is that a wide range of primers for PCR, targeting single phylogenetic groups as well as more complex communities exists [26]. The resolution of profiling techniques based on this phylogenetic marker may be however limited to only the dominant members of a community (>1% of total [27]). Next-generation sequencing is currently being implemented and may permit a higher resolution and sensitivity [28]. The approach however is still in its infancy and requires complex bioinformatic-biostatistical tools, as the large number of sequences which can be generated in a single analysis requires a thorough interpretation.

In one of their first works on glacier forefields, Sigler & Zeyer [22] found a dominance of culturable heterotrophic bacteria at two sites in the swiss Alps. More recent studies permitted to obtain a broader overview of other important microbial groups inhabiting these environments (Table 1); these range from autotrophs such as Cyanobacteria [23] and free-living N-fixers [29] to S-oxidizers [30] and heterotrophs [31].

Fungal communities appear to be common at all stages of soil development, ranging from facultative ectomycorrhizae in the scarcely vegetated young soils [32] to species living in strong association with plants in the later stages of succession, further away from the glacier front [33]. The bacterial: fungal ratio appears therefore to decrease along the chronosequence, as the increasing amount of organic material in more developed soils favours the establishment and growth of decomposing communities [32]. However, the richness of macrofungal species tended to decrease as the vegetation cover became more established, suggesting that the niches available are reduced [34].

Just a few studies investigated archaeal communities on top of glaciers [35] and in glacier forefields [36]. These studies generally evidenced a majority of Crenarchaeota, which may be represented by psychrophilic groups: little is however still known on their role in these primary succession environments, although recent studies may suggest their involvement in subglacial methanogenesis [37].
Table 1. Approaches and occurrences of microbial groups in glacier forefields

<table>
<thead>
<tr>
<th>Group</th>
<th>Trophic level</th>
<th>Method</th>
<th>region</th>
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<tbody>
<tr>
<td><strong>Bacteria</strong></td>
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</tr>
<tr>
<td>α-Proteobacteria</td>
<td>Varia</td>
<td>Clone library [31]</td>
<td>Subglacial sediments</td>
</tr>
<tr>
<td>β-Proteobacteria</td>
<td>Sulfur-oxidizers</td>
<td>Clone library [23, 31] Microscopy [38]</td>
<td>Subglacial sediments</td>
</tr>
<tr>
<td>γ-Proteobacteria</td>
<td>Clone library [31]</td>
<td>Clone library [31]</td>
<td>Subglacial sediments</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>Clone library [31]</td>
<td>Clone library [23, 31]</td>
<td>Subglacial sediments</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Clone library [31]</td>
<td>Clone library [23, 31]</td>
<td>Subglacial sediments</td>
</tr>
<tr>
<td>Citopha</td>
<td>Clone library [31]</td>
<td>Clone library [23, 31]</td>
<td>Subglacial sediments</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>Photoautotrophs</td>
<td>Clone library [39]</td>
<td>Glacier forefield</td>
</tr>
<tr>
<td>Rhizobia</td>
<td>N-fixers</td>
<td>Clone library [40] T-RFLP [40]</td>
<td>Glacier forefields</td>
</tr>
<tr>
<td>Nitrosomonas</td>
<td>Ammonia oxidizers</td>
<td>Clone library [40] T-RFLP [40]</td>
<td>Glacier forefields</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
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<tr>
<td>Mycorrhiza</td>
<td>Heterotrophs</td>
<td>Microscopy [32]</td>
<td>Glacier forefield</td>
</tr>
<tr>
<td>General fungal community</td>
<td>Heterotrophs</td>
<td>PLFA [33]</td>
<td>Glacier forefield</td>
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<tr>
<td><strong>Archaea</strong></td>
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3. Functions of glacier forefield microbial communities

A key question in the microbial ecology of glacier forefields is the relationship between microbial community structure and function. The activity of pioneer microorganisms of glacier forefields is a crucial parameter to assess nutrient cycling and ecosystem development.

General microbial activity can be assessed by measuring soil respiration rate [43]. Specific microbially mediated functions measured include photosynthesis [44], decomposition of organic material and potential activities of enzymes involved in the major biogeochemical cycles (e.g., nitrogenase, acid and alkaline phosphatase, sulfatase, β-glucosidase, chitinase, urease, ammonium monoxygenase) [45, 46, 47].

Presence and abundance of certain functional groups may be assessed by DNA-based approaches ranging from quantitative PCR and microarrays to metagenomics. These are sensitive and accurate techniques which allow the simultaneous analysis of a large sample number [48], and have a great potential to increase our knowledge on microbial diversity and function [28]. Microarrays, for example, have been successfully used for the detection of bacterial species [49], or for the expression of specific functional genes [50].

An attractive additional target is the investigation of the functional genes at the mRNA level, because mRNA is very sensitive to environmental variations [51]; such approach however is not void of technical difficulties such as low mRNA yields and short half-life [52] and must be considered in combination with DNA-based analysis.

In glacier forefields, it has generally been observed that microbial community enzymatic activity patterns are correlated to the successional stage, and in particular to the C and N content of the soils [53]. This is certainly due to the influence of plants, which become more abundant and uniform at later successional stages, and form “hotspots” for microbial activity [54].

In the very young soils, microorganisms are able to initiate ecosystem development by fixing C and N from the atmosphere and recycling them in the environment, in available forms for plants and successive organisms. In particular, recent investigations [23, 29, 55, 56] suggest that major dominant active groups in the glacier forefields are photosynthetic diazotrophs such as *Firmicutes* and *Cyanobacteria*. As they are able to perform simultaneously photosynthesis and N-fixation, photosynthetic diazotrophs provide C and N inputs at the early stages of succession and increase soil nutrient status [55, 57].

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Table 2. Target microbial functions and methodological approaches in glacier forefields and other high-altitude environments

<table>
<thead>
<tr>
<th>Target</th>
<th>Methods</th>
<th>Regions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N-cycle</strong></td>
<td></td>
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<tr>
<td>N fixation</td>
<td>Acetylene reduction</td>
<td>High arctic [58, 59]</td>
</tr>
<tr>
<td></td>
<td>RFLP of nifH</td>
<td>Glacier forefields [1, 29, 60, 63, 64]</td>
</tr>
<tr>
<td></td>
<td>Microarray of nifH</td>
<td>Subarctic soils [61]</td>
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<tr>
<td></td>
<td>qPCR</td>
<td>Tibetan Plateau [62]</td>
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<tr>
<td></td>
<td>Enrichment cultures</td>
<td></td>
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<td></td>
<td>Stable isopes</td>
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<tr>
<td></td>
<td>Enzyme activity</td>
<td></td>
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<tr>
<td>Nitrate reduction</td>
<td>RFLP of narG</td>
<td>Glacier forefield [65, 66]</td>
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<tr>
<td></td>
<td>qPCR on narG</td>
<td></td>
</tr>
<tr>
<td>Denitrification</td>
<td>qPCR on nirS, nirK, nosZ</td>
<td>Glacier forefield [64, 65, 66]</td>
</tr>
<tr>
<td>Nitrification</td>
<td>qPCR on amoA</td>
<td>Glacier forefield [64]</td>
</tr>
<tr>
<td><strong>C-cycle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiration</td>
<td>Soil respiration</td>
<td>Glacier forefield [22, 43]</td>
</tr>
<tr>
<td>Photosynthesis</td>
<td>CO2 gas exchange rates</td>
<td>Glacier forefield [67]</td>
</tr>
<tr>
<td><strong>P-cycle</strong></td>
<td></td>
<td></td>
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<tr>
<td>P solubilisation</td>
<td>Enzymes</td>
<td>Glacier forefield [1]</td>
</tr>
<tr>
<td><strong>S-cycle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S\textsubscript{0}-reduction</td>
<td>T-RFLP of asfA</td>
<td>Glacier forefield [30]</td>
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</tbody>
</table>

4. Adaptation of glacier forefield microbial communities to changing environmental conditions

Environmental factors may all represent a stress if they fluctuate away from the normal range observed in nature [68]. The consequences of environmental changes on microbial community structure and activity involve disturbances of functions and variations in community composition, because organisms which can tolerate and better adapt to the new environmental conditions will grow and outcompete other organisms. Cells are able to respond to environmental changes by adjusting their physiology and metabolism. Some microbial responses have evolved in relation to predictable environmental changes, such as seasonal or circadian cycles [69]. Unpredictable changes instead involve specific gene expression which may lead to long-term adaptation.

The type and extent of the responses of microbial communities to disturbances are an index of stability of the community, and can be defined by the concepts of resistance and resilience [70, 71]. Resistance defines the capacity of a system to maintain functions throughout a disturbance, while resilience has been used to define the time for a system to return to equilibrium after a disturbance ([72, 73], Fig. 4).

![Fig. 4. Schematic representation of the concepts of resistance and resilience. The 3 curves (dotted, dashed and full) represent different parameters (e.g., biomass, diversity, activity, including natural fluctuations) which diverge after the change of an environmental factor (t\textsubscript{0}) indicating possible responses; In gray, area of normal range; for clarity, only a negative deviation from the normal range is depicted [74].](image-url)
These indices can be quantified through simple equations [70], which are based (Table 3) on the differences between control parameter $C_0$ (measured just before the disturbance) and disturbed parameter $S_t$ (measured at a defined time). Conversely, if the sampling time corresponds to the maximum extent of the response ($t_x$), then the calculations indicate resistance. The sampling takes place when the system is returning to a new equilibrium ($t_x$), then it will be an indication of resilience. The definition of time required to recovery is often arbitrary, limited by the experimental system, as recovery times may range from hours to years [75].

Table 3. Calculation of stability (adapted from [70])

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Equation</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance (RS)</td>
<td>$RS(t_1) = 1 - 2* D_1/(C_0 + D_1)$</td>
<td>1 = full resistance; -1 = full sensitivity</td>
</tr>
<tr>
<td>Resilience (RL)</td>
<td>$RL(t_x) = 2* D_1/(D_1 + D_x) - 1$</td>
<td>1 = full recovery</td>
</tr>
<tr>
<td>Stability (ST)</td>
<td>$ST = RS + RL$</td>
<td></td>
</tr>
</tbody>
</table>

$t_1$ = time of maximum response; $t_x$ = time of recovery; $C_0$ = control parameter; $S_t$ = sample at time $t$; $D_1 = C_{r-S_0}$; $D_x = C_{r-S_x}$

Generally, microorganisms are considered to be resistant and resilient due to their high degree of metabolic flexibility, high growth rates and physiological tolerance to changing environmental conditions [76]. These properties depend strongly on microbial composition, but also on the nutrient turnover time [77]. For example, a positive relationship between availability of C and N resources with resistance and resilience has been proposed by Wardle [78]. In alpine environments such as glacier forefields, which are strongly oligotrophic, and where microbial diversity is strongly adapted to the harsh conditions characterizing these sites, resilience could be low [79].

The interactions between external environmental factors and soil microbial communities can be tested with transplantation experiments where soil from one site (origin) is transferred to a receiving site. This setup has often been adopted to analyze the adaptive capacity of plants [80], or to investigate the effects of climatic parameters on microfauna [81]. Field transplantations have also been used to investigate adaptation of microbial communities under different temperature ranges [82] or under different vegetation [83].

Laboratory and field studies aiming at investigating effects of environmental factors such as soil properties on microbial diversity and functions involve the extraction of microbial assemblages from soils, and their inoculation in a foreign system in the laboratory or in the field [84, 85]. Laboratory-based inoculations allow to manipulate and isolate single variables, and to assess their effects on model communities. However, this type of approach may not be representative of the situation in nature [86, 87].

Common strategies involve mild extractions or centrifugation to obtain a native soil community inoculum to use in experiments [85, 88], or by directly diluting native soil in the experimental soil sample [89]. While the former approach may not be representative of the total microbial diversity of the soil sampled, in terms of species extracted and also of relative abundance [88], the latter may cause excessive disturbance to the inoculated soil.

Resilience studies either use incubations of simplified model systems (reductionist approach, [90]) or may take into account the complex interaction between factors which can be observed in nature (holistic approach) [71]. A challenge in microbial ecology studies involves the manipulation of single environmental factors in the field. This approach allows to examine processes taking into account the whole set of interplaying factors found in nature [71]. Experimental field-based investigations of climate effects have been performed by Ineson et al [91] with reciprocal transplantation of lysimeters in environments with different altitudes. Temperature effects have also been assessed in the field, by using screening and heating [92]. However, these approaches are limited as they involve excessive disturbance to the soils, which could lead to a misinterpretation of the results, and they are not feasible for all environments. Among the soil-related properties, experimental field manipulations to examine the effects of a change in pH often involve large-scale artificial liming [79], or reciprocal inoculation experiments of allochtonous communities in local or limed soils [84] and soil transplantations [93].

5. Conclusion

A wide array of methodologies, ranging from enzymatic assays to molecular-based techniques permits to characterize microbial community structures and activities in extreme environments such as glacier forefields. Different experimental approaches are used to assess the dynamic responses to changing environmental conditions both in laboratory and field. However, due to the complexity of factors which may influence the soil microbial communities, lab-to-field extrapolation is often not possible. Future research should strongly focus in obtaining a comprehensive
overview of the relationships of microbial community structure and functions under natural physical and chemical conditions.

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