Active microbial ecosystem in glacier basal ice fuelled by iron and silicate comminution-derived hydrogen

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Significance statement

The subglacial ecosystem is globally significant because glaciers and ice sheets cover ~10% of the Earth's land surface, yet remains poorly understoodThis study provides a detailed characterisation of the geochemistry and bacterial community inhabiting the basal ice layer of three Icelandic glaciers. Our data show that a thriving chemolithitrophic-powered community exists within basal ice, fuelled by oxidation of hydrogen and iron. Although low compared to other ecosystems, we report the highest carbon concentration in basal ice to date.

Summary

Basal ice forms in the lowermost part of glaciers, where ice and substrate are in intimate contact with one another, yielding unique physical, chemical and biological properties. Here, we report a detailed study of the bacterial community and geochemistry of distinctive basal ice facies at three Icelandic glaciers, and find notable differences between facies. Overall, carbon and nitrogen concentration were very low in the included sediment, which is dominated by silicates and iron-containing particles. Nonetheless, we report the highest carbon concentration of basal ice to date. Analysis of the bacterial community using 16S showed a high abundance of hydrogen (e.g. *Polaromonas* and *Rhodoferax*) and iron oxidisers (e.g. *Thiobacillus* and *Gallionella*), which can fix carbon for heterotrophic communities to proliferate. Network analysis showed significant correlations between heterotrophic and chemolithotrophic genera, furthermore supporting this hypothesis. Functional prediction based on 16S rDNA genes showed a high abundance of potential C-fixation pathways and methane oxidation pathways. Despite not being targeted specifically, 16S rDNA affiliated with methanogenic hydrogenotrophic archaea. We conclude that the basal ice in these glaciers is an active oligotrophic environment, in which iron and hydrogen chemolithotrophy plays a fundamental role.

Abstract

The basal zone of glaciers is characterised by physicochemical properties that are distinct from firnified ice because of strong interactions with underlying substrate. Basal ice ecology and the roles that the microbiota play in biogeochemical cycling, weathering, and proglacial soil formation, remains poorly known. We report bacterial diversity and potential ecological roles at three temperate Icelandic glaciers. We sampled three physically distinct basal ice facies (stratified, dispersed, debris bands) and found biological similarities and differences between them; basal ice character is therefore an important sampling consideration in future studies. High abundance of silicates and Fe-containing minerals could sustain the basal ice ecosystem, in which chemolithotrophic bacteria (~23%), especially Fe-oxidisers and hydrogenotrophs, can fix C, which can be utilised by heterotrophs. Methanogenic-affiliated detected sequences showed that silicate comminution-derived hydrogen can also be utilised for methanogenesis. Metabolism predicted by 16S rRNA diversity revealed that methane metabolism and C-fixation are the most common pathways, indicating the importance of these metabolic routes. Carbon concentrations were low compared to other ecosystems, but we report the highest carbon concentration in basal ice to date. Carbon release from melting basal ice may play an important role in promoting pioneering communities establishment and soil development in deglaciating forelands.

Keywords

Extremophiles, cryosphere, microbial ecology, environmental microbiology, glaciers

Introduction

Glaciers represent important ecosystems (e.g. (Hodson *et al.*, 2008; Anesio *et al.*, 2009; Hotaling *et al.*, 2017)). Glacial microbiology research has mostly been focused on understanding the supraglacial ecosystem, in large part because of its relevance to albedo, and hence the surface mass balance of glaciers and ice sheets (Edwards, Douglas, *et al.*, 2013; Edwards, Rassner, *et al.*, 2013; Lutz *et al.*, 2014, 2015; Bradley *et al.*, 2016; Kaczmarek *et al.*, 2016). The subglacial environment has received much less attention, but is thought to be important for substrate weathering and the release of nutrients into the proglacial environment as mineral-bearing debris melts out from the ice (Cook, Graham, *et al.*, 2011; Rime *et al.*, 2016). Subglacial environments are characterised by low temperature, absence of light, oligotrophic conditions, and high mineral content (Yde *et al.*, 2010; Bakermans and Skidmore, 2011; Montross *et al.*, 2014), which suggests that subglacial ecosystems are dominated by microbial chemolithotrophic metabolisms (Mitchell *et al.*, 2013; Boyd *et al.*, 2014; Christner *et al.*, 2014).

The focus of this work is the microbial diversity of subglacially-derived basal ice (BI) and its broader significance within glacial and deglaciating systems. BI inherits physical and chemical characteristics from its close interaction with the glacier substrate, which differ from those of atmospherically-derived (i.e. firnified) englacial ice (Hubbard *et al.*, 2009; Swift *et al.*, 2018). Specifically, BI is commonly characterised by high sediment content, enrichment in certain ions, and low bubble content (Knight, 1997; Hubbard *et al.*, 2009). Differences in BI physical and chemical characteristics are often interpreted to indicate formation by distinct subglacial processes (Knight and Knight, 1994; Hubbard and Sharp, 1995; Knight, 1997; Cook *et al.*, 2007; Swift *et al.*, 2018).

BI is commonly split into different facies based on distinct physical and chemical characteristics (Knight, 1997; Hubbard *et al.*, 2009). Three such facies are relevant to this study: stratified, dispersed, and debris bands**.** Stratified facies (Fig 1a) is stratigraphically the lowermost layer of basal

ice, and is found mainly in the southern part of Svínafellsjökull, in both western and eastern parts of Skaftafellsjökull, and in the southern part of Kviárjökull (locations shown in Fig. 2), but is absent around much of the glacier margins (Ebert, 2003; Cook *et al.*, 2010). It is characterised by its layered appearance and high debris content (5-80% by volume) (Cook *et al.*, 2007, 2010; Swift *et al.*, 2018). Dispersed facies (Fig 1b) appears ubiquitously around glacier margins in sections of 3.5 m thickness on average, and is characterised by low debris content (0.2-2% by volume) and massive structure (Cook, Swift, *et al.*, 2011). Debris bands (Fig 1c) are characterised by discrete layers of debris-laden stratified ice between bubble-rich and debris-poor firnified englacial ice or debris-laden dispersed facies. Debris bands commonly exhibit high clast content. Depending on the nature of the subglacial environment and the sediment that exists there, clasts may exhibit evidence of glaciofluvial or subglacial wear, or both (i.e. sub-angular to well-rounded clasts with striations and/or facets) (Swift *et al.*, 2018). Ice of different origin, composition, and provenance is hypothesised here to harbour distinct microbial communities as a result of natural selection and ecological processes. Knowledge of these communities in turn can contribute to understanding of the evolution and biochemical functionality of glaciated landscapes.

Previous studies have demonstrated an abundance of microbial cells within BI. At Taylor Glacier, Antarctica, a BI cell content of 2.5 x 10^2 to 1.2 x 10^4 g ml^{-1} was found, and 7.9 x 10^6 cells g^{-1} existed within basal sediment (Stibal, Hasan, *et al.*, 2012; Montross *et al.*, 2014); 8.7 x 10^5 cells g^{-1} were found in Russell Glacier (Greenland) (Stibal, Hasan, *et al.*, 2012); 1.7 - 6.8 x 10^5 cells g^{-1} were found in Finsterwalderbreen, Svalbard (Lawson *et al.*, 2015); and 1.3 - 1.4 x 10^7 cells g^{-1} were found in Svínafellsjökull, Iceland (Toubes-Rodrigo, S J Cook, *et al.*, 2016). Subglacial environments have been shown to foster an abundance of chemolithotrophic-associated microorganisms (Yde *et al.*, 2010; Boyd *et al.*, 2014). For example, Mitchell et al. (2013) reported that bacterial Fe- and S- oxidisers were abundant in subglacial meltwater discharge from Robertson Glacier, Canada, and that microorganisms were more abundant in minerals that could be easily oxidised, such as pyrite. Chemolithotrophic communities are not only important in subglacial environments (Boyd *et al.*,

2014), but also play important roles in proglacial systems once released from the glacier (Frey *et al.*, 2010). The amount of available C in freshly exposed proglacial sediments is a limiting factor for soil formation (Brankatschk *et al.*, 2011). Until recently, it was thought that the main driver for the evolution from bare sediment into soil was the deposition of microorganisms from atmospheric sources, such as wind, marine aerosols, or precipitation (Womack *et al.*, 2010; Chuvochina *et al.*, 2011; Temkiv *et al.*, 2012). Nevertheless, recent research by Rime et al. (2016) indicated that BI has a substantial microbial input to the development of new soils. Our work builds upon these observations of the subglacial environment microbiota by characterising the microbial communities and geochemical characteristics of the major ice types, enabling finer-scaled interpretation of ecological interactions and biologically driven functionality within glaciers.

The potential microbial differences between BI facies and the relationship between geochemistry and microbial community in the BI are yet to be studied/are poorly understood. To address these gaps, the primary aim of this study is to characterise the microbial diversity within distinct BI facies at three glaciers in southern Iceland. We hypothesise that distinctive ice facies, formed through different processes, will yield different microbial content and community compositions. The second aim of this study is to elucidate the potential microbiota-mineralogy relationship based on microbial diversity and geochemical analysis of the BI layer.

Results

Geochemistry of BI sediment

C was consistently low across all facies, but ranged from 82 ppm in a debris band sample (B6-16) to 2833 ppm in a dispersed facies sample (D3-16). On average, C concentrations were lowest in debris bands (1046.5 ± 668.6 ppm; n=5), followed by dispersed facies (1748.0 ± 637.2 ppm; n=6), and stratified facies (2102.3 ± 874.0 ppm; n=4).

N concentrations were very low in all samples and ranged from 8 ppm in debris band sample B1-16, to 486 ppm in dispersed facies sample D3-16. On average, N concentrations were lowest in debris

bands (189.5 ± 109.3 ppm), followed by dispersed facies (265.5 ± 152.2 ppm), and stratified facies (302.8 ± 26.9 ppm). Fe concentration ranged from 0.6 ppm in debris band sample B6-16, to 14.8 ppm in stratified facies sample S1-15. On average, Fe values were lowest in dispersed facies (2.9 ± 0.8 ppm), followed by debris bands (3.3 ± 2.6 ppm), and stratified facies (5.1 ± 4.3 ppm). S values were very low, ranging from 4.8 x 10^{-3} ppm in debris band sample B6-16, to 0.7 ppm in debris band sample B4-15. On average, S concentration values were lowest in dispersed facies (0.09 ± 0.05 ppm), followed by stratified facies (0.13 ± 0.12 ppm), and debris bands (0.14 ± 0.20 ppm). Although Fig 3 illustrates a consistent pattern in elemental concentrations being lowest in debris bands, followed by dispersed and stratified facies, no statistically significant differences (p-value>0.05) were found between the concentrations of elements in different BI types.

The glaciers in this study drain the Öræfajökull ice cap, which covers a stratovolcano. The bedrock is dominated by basaltic lavas, olivine porphyritic lavas, with minor components of hyaloclastite, and subglacially erupted cube jointed basic to intermediate volcanic rock with hyaloclastic layers (Helgason and Duncan, 2001). The SPA, performed following the chemical boundaries defined in Kandler et al, (Kandler *et al.*, 2007) (Fig. 4), showed a sediment mineralogy dominated by silicates (50.2 % in debris bands, 52.1 % in dispersed, and 53.8 % in stratified). Carbonaceous particles – which encompasses all C-rich particles - appeared consistently amongst the analysed samples (5.6% in debris bands, 5.6% in dispersed, and 6.7% in stratified). Fe-rich and Fe-oxides particles were abundant in the SPA (2.2% in debris bands, 3.9% in dispersed, and 1.5% in stratified). Although not as abundant, sulphate particles also appeared amongst the samples (3.3% in debris band, 1.4% in dispersed, and 3.6% in stratified). No statistically significant differences were observed between samples. (p-value > 0.05) Mixed particles, which represent those particles that could not be resolved to a specific mineralogy, represented a significant proportion of the analysed particles (4.7-5.9% of the total).

Silicates were dominated by olivine, feldspars (e.g. sanadine, oligoclase, anorthotite) and quartz. Fe-rich particles analysed by micro-Raman corresponded to martite, haematite, magnetite, and pyrrhotite. Carbonates identified by micro-Raman were calcite, aragonite, and rhodochrosite. Some of the minor minerals corresponded to apatite, titanite, and libethenite.

Bacterial diversity of BI sediment

BI facies-specific bacterial 16S based phyla abundance is presented in Figure 5. The most abundant phyla were Proteobacteria (debris bands 46.6 %, dispersed 62.9 %, stratified 58.0 %), Acidobacteria (debris bands 11.0%, dispersed 3.5%, stratified 10.6%), Chloroflexi (debris band 6.2%, dispersed 7.6%, stratified 5.2%), Actinobacteria (debris band 5.7%, dispersed 5.0%, stratified 4.7%), Nitrospirae (debris bands, 6.9%, dispersed 6.2%, stratified 1.6%). Acidobacteria showed significant differences between ice types (Kruskal-Wallis p-value < 0.05), being significantly less abundant in dispersed facies than in stratified facies or debris bands, although no differences were observed between debris bands and stratified ice.

Table 1 shows genera of an abundance over 1%. A Tukey test revealed that the only statistically significant difference observed was for stratified facies, where *Thiobacillus* was more abundant (7.5 ± 3.7%) than in dispersed facies.

Network analysis

Network analysis showed two main groups: one containing the majority of taxa, and a smaller group that contained only minor groups (Fig 7). Two taxa showed no significant relationships (r-coefficient > 0.5): the family mb242 and *Thermomonas*. None of the chemolithotrophic-affiliated taxa occupied terminal positions in the network analysis, and in general they were linked to heterotrophic genera (e.g *Thiobacillus* – *Leeia*, *Gallionella* – *Maritimonas*).

Potential metabolic pathways in glacier BI

The PICRUSt KEGG Orthologue (KO) analysis identified a high abundance of diverse metabolism-related metagenomes in BI, together with genetic information and environmental information processing. The most abundant functional groups predicted were “Metabolism” representing ~ 50% in all cases, followed by “Genetic Information Processing” and “Environmental information processing”. Due to the oligotrophic nature of the BI environment, analysis of pathways for obtaining energy was undertaken. The most abundant pathways were oxidative phosphorylation, carbon fixation, and methane metabolism, and in less relevance, nitrogen metabolism and sulphur metabolism.

Discussion

The aim of this paper was to elucidate two crucial geomicrobiological properties of BI in our target temperate Icelandic glaciers, namely, (i) to characterise the BI bacteriome to identify whether there are significant differences amongst physically-distinctive ice facies; and (ii) knowing that BI is an active ecosystem (Kayani *et al.*, 2018), to elucidate the likely biogeochemical pathways that fuel that ecosystem.

Microbial differences among BI facies

Previous research on BI has classified ice facies based on physico-chemical characteristics with the aim of ascertaining their respective origins under the premise that different characteristics are likely to represent different origins (e.g. (Hubbard and Sharp, 1995; Knight, 1997; Hubbard *et al.*, 2009; Cook *et al.*, 2010; Cook, Swift, *et al.*, 2011; Swift *et al.*, 2018)). Such work, typically undertaken at the ice margin, provides important information about the otherwise inaccessible subglacial environment, crucial to our understanding of glacier ecology. Our prediction was that different BI facies would host different microbial communities and abundances because of their different physico-chemical properties.

One phylum (Acidobacteria) and one bacterial genus (*Thiobacillus*) showed significant differences between facies. Acidobacteria were more abundant in both stratified facies and debris bands than in dispersed facies, and *Thiobacillus* was more abundant in stratified facies than dispersed facies, but not more abundant than in the debris bands (Table 1). These results show that there are microbiological differences between ice types, especially between stratified facies and dispersed facies, that can be linked to their different origins, agreeing with observations by Swift et al., (2018). It is generally acknowledged that stratified facies forms by the freeze-on of subglacial meltwaters beneath the glacier terminus and, specifically in the case of Icelandic glaciers, through glaciohydraulic supercooling (Cook *et al.*, 2007, 2010; Hubbard *et al.*, 2009) . Dispersed facies, on the other hand, is thought to be derived from further up-glacier through tectonic and strain-induced metamorphism of the lower parts of ogive (debris) bands (Cook, Swift, *et al.*, 2011).Our data lend some support to these hypotheses that stratified and dispersed facies are formed through very different processes. Several studies have hypothesised that debris bands are related in some way to stratified ice based on their similar physical and chemical characteristics (Hubbard *et al.*, 2009; Cook *et al.*, 2010; Swift *et al.*, 2018), and again our data lend support to that idea.

Iron and hydrogen fuel the BI ecosystem

Total carbon concentration in the sediment was very low (debris band: 1046 ppm, dispersed: 1748 ppm, stratified: 2102 ppm) (Fig 3) compared to soils of other liquid water-limited ecosystems (e.g. hyperarid deserts (Valdivia-Silva *et al.*, 2012)). Nonetheless, we report the highest carbon concentrations found for BI, or in any glacial ecosystem (dispersed: 31.5 mg L^{-1}, debris bands: 434.9 mg L^{-1}, stratified: 818.4 mg L^{-1})(Hodson *et al.*, 2015). C concentration in the recently deglaciated soils of the surrounding area has been previously reported to be on the same order of magnitude as our results (~500 ppm) (Vilmundardóttir *et al.*, 2015).

Two possibilities for the presence of C in basal ice are feasible: Ives (2007) illustrated how the terminus of Svínafellsjökull was ~2 km further up-valley between the 14th and 19th centuries. The

former glacier foreland, now covered by ice, was used for agricultural purposes, which implies that soil must have been rich in C. It was also reported that the ice margin of Skaftafellsjökull was mined for birch wood by early settlers in Iceland. In both instances, the glacier termini advanced over these carbon-rich landscapes to reach their Little Ice Age maximum extents in the 19th Century (Helgason and Duncan, 2001). These carbon-rich agricultural soils and woodland would have become entrained into the BI layer (Cook *et al.*, 2007, 2010). The same process was used to explain the high cell counts in BI sediment observed by Toubes-Rodrigo et al (2016). The other possibility is that C-fixation via chemolithotrophy occurs over time, leading to an increase in C linked to the oxidation of minerals. Oxidised minerals were detected by both Raman (Fe-oxides, such as pyrrhotite or magnetite) and SPA (Fe-oxides and sulphates).

Acknowledging that PICRUSt is not exempt from caveats, and that this simulation is not substitute of metatranscriptomics, we decided to use it in order to build a hypothesis about the potential metabolisms in BI. Fig 7 shows that BI is dominated by chemosynthesis over photosynthesis (16.2% vs 7.3% of energy-related KOs; Welch t-test p-value < 0.01), which is not surprising given the lack of light in the subglacial environment (Yde *et al.*, 2010). Recent research has shown a high abundance of genes associated with ribulose bisphosphate carboxylase (RuBiSCO) in BI, which supports our hypothesis that BI is driven by chemolithotrophy (Kayani *et al.*, 2018). Based on the low abundance of N and S compared to Fe-rich particles and silicates, and the abundance of microorganisms with Fe-oxidising capabilities (*Thiobacillus*, *Gallionella*) and H-oxidising capabilities (*Polaromonas*, *Rhodoferax*), we suggest that the BI ecosystem is sustained by the oxidation of Fe-particles, and oxidation of silicate comminution-derived H_2 (Telling *et al.*, 2015), especially when reduced Fe is depleted. Interestingly, analysis of the microbiome in Matanuska glacier also showed a high abundance of members of presumably active families (analysed by RNA/DNA ratio) that have been identified in this work, namely: *Comamonadaceae* (which includes *Polaromonas* and *Rhodoferax*), *Gallionellaceae* (which includes *Gallionella*), *Methylophilaceae* (including *Methylotenera*) (Kayani *et al.*, 2018).

Whereas Fe- and S- oxidation have been previously reported as potential metabolism supporting subglacial environments, results from this work indicate that H-oxidation could be of vital importance in subglacial environments, as suggested by Telling et al., (2015). H_2 oxidation is known to support life in subsurface ecosystems (Chapelle *et al.*, 2002; Bagnoud *et al.*, 2016) and has been proposed as the main reaction to support life in extra-terrestrial planets and moons, such as Mars (McMahon *et al.*, 2016) or Enceladus (Seewald, 2017).

Several clusters of chemolithotrophic and heterotrophic bacteria were identified in the network analysis (Fig 7), suggesting that BI is a cooperative environment in which C fixed by autotrophic bacteria can then be utilised by heterotrophic organisms (e.g. *Lysobacter*, *Kaistobacter*). A very tightly clustered group, comprising bacteria and archaea, albeit at low abundance, was identified in the network analysis (Fig 7). Although archaea were not the main focus of this study, 16S rDNA genes affiliated with two archaeal methanogens - *Methanolinea* and *Methanospirillum* – were recovered. These two genera co-occurred in the same cluster with bacteria presenting different metabolisms: aerobic (*Dokdonella*, *Flavobacterium*, *Sphingorhabdus*), facultative anaerobic (*Thauera*), anaerobic (*Geobacter*), fermentative (*Geothrix*) (Coates *et al.*, 1999). The presence of fermenting bacteria can generate substrates used by methanogens, such as acetic acid for acetoclastic methanogenesis (Stibal, Wadham, *et al.*, 2012). On the other hand, the presence of H_2 from fermentative processes and silicate comminution can be utilised by hydrogenotrophic methanogens as a substrate (Telling *et al.*, 2015). In addition, methane metabolism was predicted amongst the most abundant metabolic pathways from PICRUSt data, which not only related to methanogenesis, but also to methanotrophy. A high abundance of methanotrophic bacteria was identified in BI, such as *Methylotenera* (Table 1) (Mustakhimov *et al.*, 2013). The presence of tightly associated clusters of anaerobes and aerobes could be indicative of the presence of biofilms whereby aerobic species form the outermost part of the biofilm where they consume oxygen, which in turn allows the proliferation of anaerobic organisms in the inner part of the biofilm; this also results in mineralogical heterogeneity of the biofilm (Brown *et al.*, 1994).

Conclusions

BI from southern Icelandic glaciers contains a diverse community of bacteria. Different ice facies were analysed separately and results indicate that BI types support distinctively different bacterial communities: stratified and dispersed facies being two end members, and debris bands occupying an intermediate position. This is consistent with similar differences found based on chemical and physical composition of the BI (Swift *et al.*, 2018). Toubes-Rodrigo et al. (2016) estimated that 10^{16} cell yr^{-1} were released from the BI at Svínafellsjökull, and demonstrated the capacity of some of these microorganisms to proliferate at low temperatures. To sustain such a high cell number, we suggest that BI sediment is enriched in C as a consequence of subglacial chemolithotrophy, strongly linked to the mineralogy of the environment as previous research has shown (Mitchell *et al.*, 2013; Boyd *et al.*, 2014; Kayani *et al.*, 2018). Although poor in C, the BI of the glaciers studied in this work contain the highest abundance of C ever reported in glacial ecosystems (Hood *et al.*, 2015). Dispersed facies showed the highest abundance of silicates compared to debris bands and stratified facies. Silicates can produce H_2 in subglacial ecosystems (Telling *et al.*, 2015), which could support a microbial ecosystem based on hydrogenotrophy, and the abundance of H-oxidising *(Polaromonas)* bacteria was greatest in dispersed facies, suggesting that silicate comminution could act as fuel for microorganisms, upon depletion of other reduced substrates, such as Fe (II), that are more abundant in stratified and debris bands, and could be used by Fe-oxidisers, such as *Thiobacillus*. Network analysis revealed a tight co-occurrence of these C-fixing bacteria with heterotrophs (e.g. *Lysobacter*, *Kaistobacter*), which can feed on the C fixed by mineral oxidisers. The presence of an active mineral-oxidising microbiota is likely to increase the weathering rates in BI, releasing nutrients, but in the process increasing the fixed organic C content due to chemolithotrophy. The melt-out of BI at the ice margin will release carbon, other nutrients and active microbiota to the ice-marginal environment, thereby promoting soil formation as glaciers undergo recession (Rime *et al.*, 2016).

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Experimental Procedures

Study site and BI samples

The main focus of this study was Svínafellsjökull (n=16) (Fig. 2a), and additional samples were also taken from nearby Kvíárjökull (n=2; Fig. 2b) and Skaftafellsjökull (n=4; Fig. 2c) to determine whether basal ice layers of different glaciers have similar or different microbiological properties. All three locations are temperate valley glaciers. On the first sampling campaign (April 2015), neither Skaftafellsjökull nor Kvíárjökull had accessible basal ice facies, but they were sampled during the second field campaign (May 2016). Sampling at each glacier targeted three distinct BI facies that have been identified in previous studies, namely debris band, dispersed, and stratified facies (Swift *et al.*, 2006, 2018; Cook *et al.*, 2007, 2010; Cook, Graham, *et al.*, 2011; Cook, Swift, *et al.*, 2011) (Fig 1). Accessible BI facies at Svínafellsjökull were sampled aseptically in April 2015 and May 2016 following procedures outlined by Toubes-Rodrigo *et al.*, (2016). The first 20-30 cm of BI was removed with an ice axe to prevent surface melt-derived cross-contamination. Sampled BI blocks were carved using flame-sterilised chisels and carefully triple-bagged in sterile plastic bags to prevent cross-contamination from potential piercing (Toubes-Rodrigo, Simon J Cook, *et al.*, 2016). Individual BI samples were melted at ~4°C in the bags (Stibal *et al.*, 2015). Samples were allowed to settle, and soil and liquid fractions were separated by decanting. Samples for chemical analysis were oven dried at 60°C overnight, whilst sediment samples for DNA extraction were processed immediately after decanting the liquid fraction.

Sediment chemical analysis

Carbon (C), nitrogen (N), sulphur (S), and iron (Fe) were analysed due to their relevance in chemolithotrophic metabolism (Madigan *et al.*, 2010). Sediment samples were vigorously shaken and mixed thoroughly in order to increase their homogeneity ahead of analysis. For total carbon and nitrogen, 0.2 grams of oven-dried (60°C) BI sediment was subjected to dry combustion elemental analysis at 950˚C in a Leco TruSpec™ instrument (Thermo Scientific, UK) (Macreadie *et al.*, 2012).

Fe and S analysis was performed using an iCAP 6000 series ICP-OES (Thermo Scientific, UK) and microwave digestion. In short, 25 ml of an aqua regia were added to a pre weighted sediment sample (0.5 g) and acid digested using a two-step cycle at 90 and 170 °C. Filtered samples were diluted to 50 ml and analysed (Wavelengths and concentration ranges were: Fe, 240.4 nm (Gomez *et al.*, 2007) and 10-500 ppm; S, 180.7 nm (Santelli *et al.*, 2008) and 0.5-50 ppm. Calibration standards were matrix matched and method blanks were used to correct data.

Sediment Single Particle Analysis (SPA)

Dried BI-derived sediment (25 mg) aliquots were suspended in pure methanol and subjected to ultra-sonication using an S-Series Table Top ultrasonicator (Sonicor Inc. USA) at the highest power rating until a homogenous dispersion was observed. Fifty microlitre aliquots were transferred to Leit adhesive Carbon Tabs 12 mm (Agar Scientific, UK) mounted on aluminium SEM Stubs 12 mm diameter, 6 mm pin (Agar Scientific, UK) in such a way that particles were separated from each other. Mounted stubs were subjected to computer-controlled scanning electron microscopy coupled with energy dispersive X-ray spectrometry (SEM-EDX) using a Supra 40VP microscope controlled by SmartSEM software (Carl Zeiss Ltd, UK). For SEM, the acceleration voltage of 25kV was applied and samples viewed at a magnification of 2500X. For *in situ* elemental analysis, the SEM is equipped with a Backscattered Electron Detector (Apollo 40 SDD. EDAX Inc. USA) controlled by Genesis software (Laskin *et al.*, 2006; Williams *et al.*, 2013); optimisation of the scanning time was performed and the best results were obtained using a scanning time of 15 seconds per particle. Can you add how many particles were analysed per sample?

Raman analysis

Small amounts of sample were mounted on double-sided carbon tape fixed onto glass microscope slides, removing excess by tapping it on the side of the microscope slide. At least fifty particles per sample were analysed using a Renishaw InVia Raman microscope fitted with a Peltier-cooled charge-coupled device detector. The source of excitement was a 514.5 nm Ar^{+} laser. The instrument was

calibrated at the beginning of each set of analysis using a silicon chip. The instrument was used in extended mode obtaining spectral data ranging from 100 to 2200 wavenumbers. The number of acquisitions varied between 1 and 4 with 10-second exposures to ensure an acceptable S/N ratio. The power density varied between 2 – 8 mW at the sample. Data acquisition was carried out with the WireTM and Spectracalc software packages from Renishaw. Spectral identification was done using an in-house spectral library for the iron oxides, the RRUFF database, and a commercially available spectral library via Spectracalc software (GRAMS, Thermo Fischer, UK).

DNA extraction and sequencing

Total genomic DNA was extracted using MoBio kits. For the 2015 samples, the PowerSoil® kit was used to extract DNA on 0.25g (wet weight) samples of BI sediment. However, as relatively low DNA yields were achieved, the PowerMax® Soil DNA Isolation kit was employed in the 2016 campaign allowing DNA extraction from up to 10 g of wet sediment (Elser *et al.*, 2015; Fernández-Martínez *et al.*, 2016). DNA extracts were maintained at -20ºC prior to analysis. Samples were amplified for sequencing in a two-step process. The forward primer was constructed with the Illumina i5 sequencing primer (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3') and the 357F primer (5'-CCTACGGGNGGCWGCAG-3'). The reverse primer was constructed with the Illumina i7 sequencing primer (5-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3') and the 785R primer (5'-GACTACHVGGGTATCTAATCC-3') (e.g. (Diao *et al.*, 2017)). Amplifications were performed in 25 µl reactions with the Qiagen HotStar Taq master mix (Qiagen Inc, US), 1 µl of each 5uM primer, and 1 µl of template. Reactions were performed on ABI Veriti thermocyclers (Applied Biosytems, US) under the following thermal profile: 95˚C for 5 min, then 10 cycles of 94˚C for 30 secs, 50˚C for 40 secs (+0.5˚C per cycle), 72˚C for 1 min, followed by 25 cycles of 94˚C for 30 secs, 54˚C for 40 secs, 72˚C for 1 min, and finally, one cycle of 72˚C for 10 min and 4˚C hold.

Amplification products were visualised with eGels (Life Technologies, Grand Island, New York). Products were then pooled equimolar and each pool was size selected in two rounds using

Agencourt AMPure XP (BeckmanCoulter, Indianapolis, Indiana) in a 0.75 ratio for both rounds. Size selected pools were then quantified using the Quibit 2.0 fluorometer (Life Technologies, Thermo Fisher, UK) and loaded on an Illumina MiSeq (Illumina, US) 2x300 flow cell at 10pM. The average number of reads per sample was 10K.

Sequence classification

Raw sequence reads (FastQ) were trimmed, aligned, and filtered using Mothur (Kozich *et al.*, 2013). Sequences that diverged from the sequence length median were removed. Chimeras were removed using uchime included in mothur. Sequences were aligned and processed using Parallel-META 3.4.1 pipeline (Jing *et al.*, 2017) to analyse the paired end 16S sequences. Phylum and Genera were the taxonomic levels analysed and reported. For the operational taxonomic unit (OTU) clustering, a threshold of 97% homology was chosen (Jing *et al.*, 2017). Taxonomic identification of OTUs was performed using the SILVA database built in Parallel-META 3.4.1 (v123). The Phyla, Genera and OTU tables generated were used for further analysis. The minimum sequence count threshold was 2; abundance thresholds were fixed for maximum 0.1% and minimum 0%, minimum no-zero abundance threshold 10% and minimum average abundance threshold 0.1% (Jing *et al.*, 2017).

Functional prediction from community data

The PICRUSt v1.1.13 pipeline was used to predict the functional potential of the communities (Langille *et al.*, 2013) inhabiting BI. OTUS were classfified against the Green Genes database (version 13_5) (DeSantis *et al.*, 2006). Copy number was normalised, and the metagenome functional profiles were predicted, generating a table of Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthologs (KOs). The resulting table was collapsed at KO level 1, in order to give a broad assessment of the general functionality of the metagenome, and level 3, filtering for metabolism (Wang *et al.*, 2016).

Statistical analysis

All data analysis was performed using R (version 4.0.2) combined with ggplot2(Wickham, 2009) and igraph (Csardi and Csardi, 2007) packages for graphical visualisation. Samples were assumed to not

be normally distributed, and Kruskal-Wallis test was performed using the pgrimess package (Giraudoux, 2018). Samples were considered to differ significantly when p-value < 0.05.

To visualise potential relationships between bacteria genera a correlation matrix was produced. In order to calculate the correlation coefficients the package Hmisc (Harrell Jr, 2013) was used to generate a matrix that was imported into the package igraph (Csardi and Csardi, 2007). Network visualisation of highly correlated taxa (r > 0.5) (Csardi and Nepusz, 2006; Csardi and Csardi, 2007) was produced. The length of the edges connecting the nodes of the plot is inversely proportional to the r-coefficient and the size of the nodes is logarithmically proportional to the abundance of the taxon. Different colours were given to the different genera based on their potential metabolisms based on literature search.

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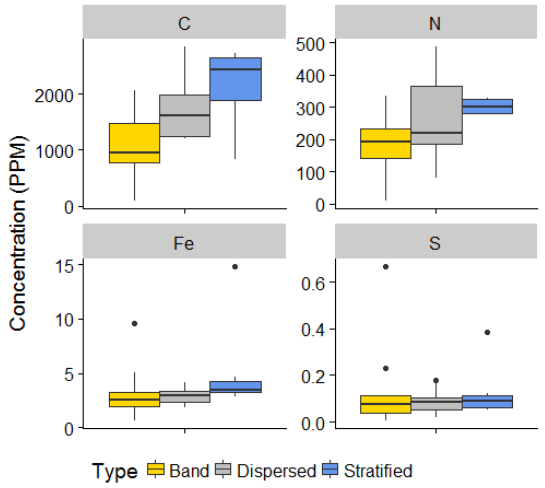
Fig 1. Examples of basal ice (BI) facies collected from Svínafellsjökull: a) stratified facies (S), rich in fine (clay/silt) sediment, with sediment arranged in angular aggregates; at a centimetre to decimetre scale, stratified facies appears layered, as the name suggests; b), dispersed facies (D) comprising dispersed aggregates of polymodal sediment; c), debris band (B), composed of sub-vertically layered alternations between clear, bubble-free ice and polymodal sediment – note also the white and bubble-rich englacial ice to the right.

Figure 2. Map of the sampling points. Three glaciers were analysed in this study: Svínafellsjökull (A), Kviárjökull (B), Skaftafellsjökull (C). Different shapes indicate different ice facies sampled.



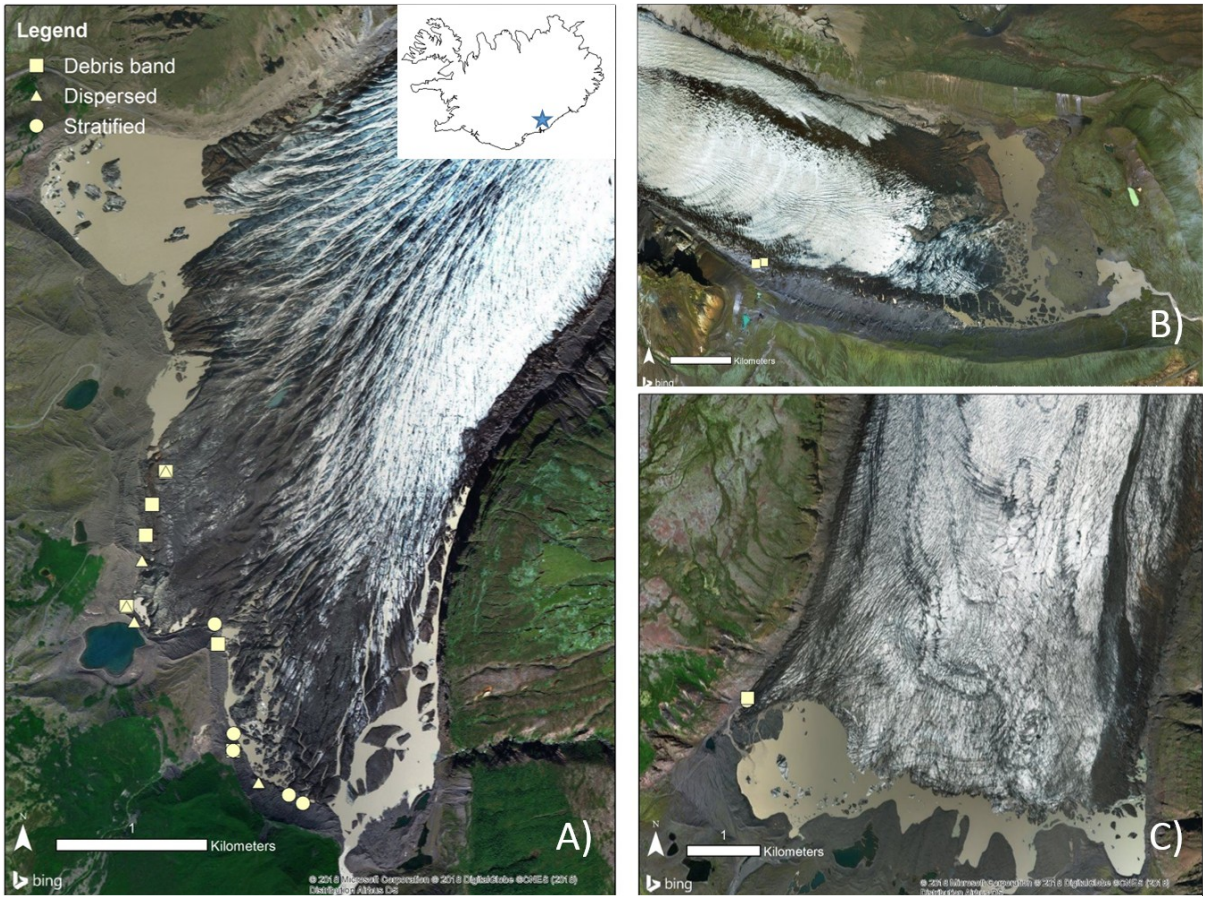
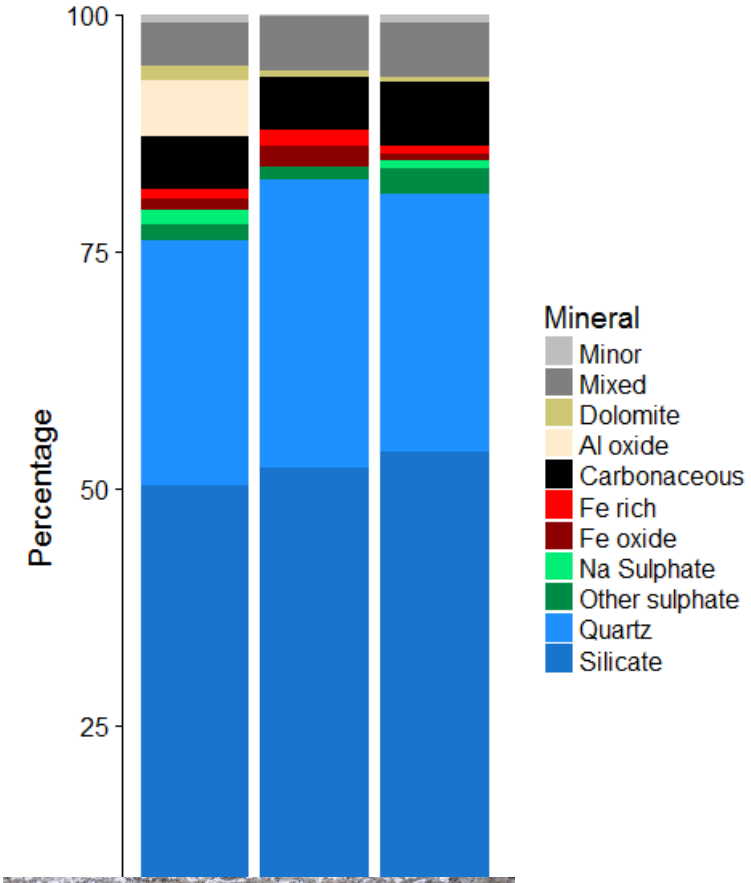
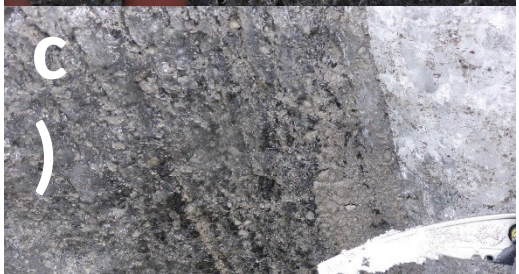


Figure 3. Box plot showing the concentrations (in ppm) of the different elements analysed from sediment entrained in basal ice (BI). C and N were analysed by Leco TruSpec, whereas Fe and S were analysed by ICP-OES. Lines represent the median and the upper and lower limit of the box represent the 75 and 25 percentiles respectively. Dots represent the outliers.





ntrapped in basal ice (BI) using automated Single Particle Analysis h Energy Dispersive X-ray (SEM-EDX). Particles were classified using a ned by Kandler et al., (2011) and (Anaf *et al.*, 2012)

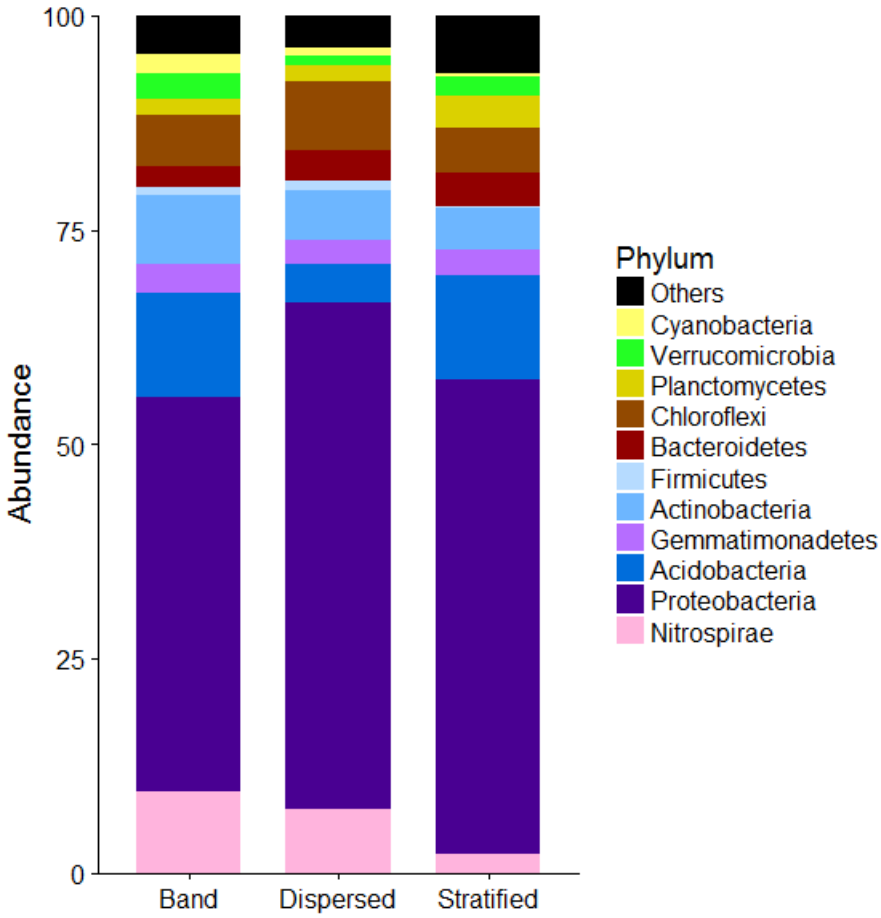
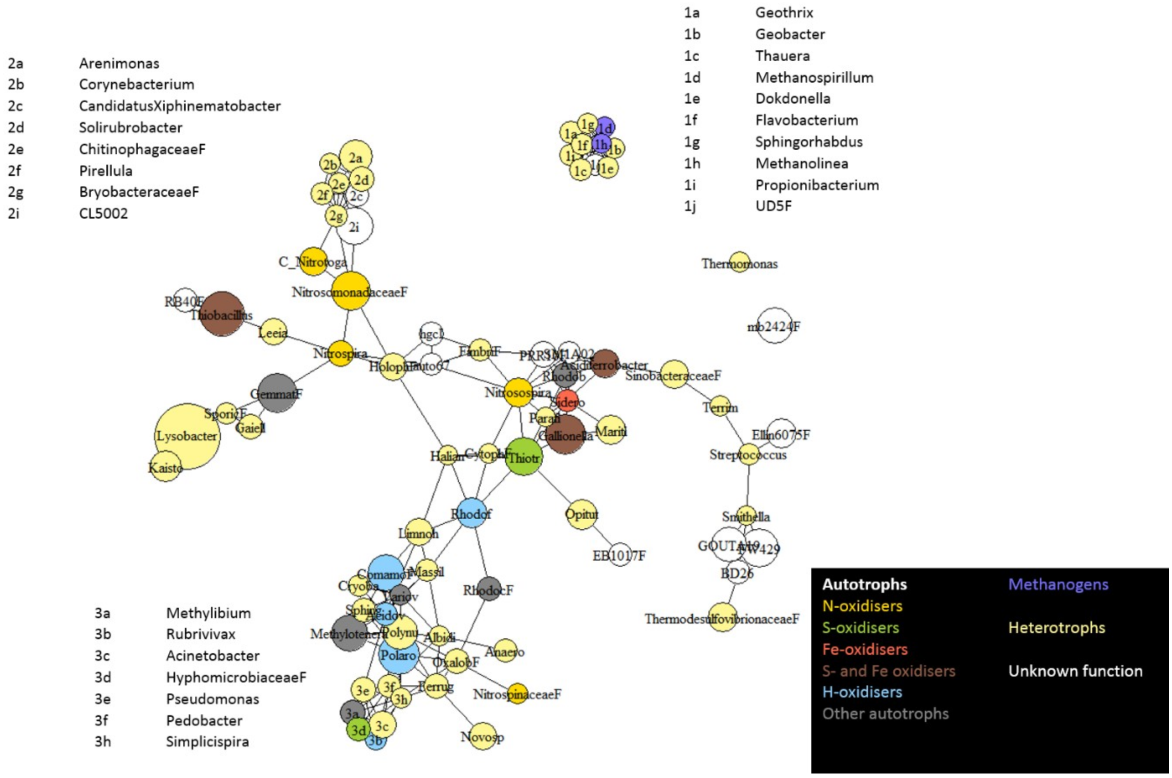


Fig 5. Relative abundance of 16S rDNA sequences classified to phylum level in the three basal ice (BI) types over the 2015 and 2016 sampling campaigns at Svínafellsjökull. Based on 16S rRNA gene sequence derived OTU table analysis in Parallel Meta 3.4.1. Phyla below 1% abundance were reported with genera below this threshold and designated as a single group termed "Others".

Table 1. Basal ice specific relative mean percentage abundance ± standard deviation of the major bacterial genera (>1% total abundance). P-values for different tests were calculated on the basis of sample distribution. When samples were normally distributed ANOVA was used in order to analyse for statistically significant differences; otherwise Kruskal-Wallis was utilised.

	Debris Band	Dispersed	Stratified	Kruskal-Wallis p-value
Lysobacter	9.7 ± 7.1	13.0 ± 10.3	12.1 ± 10. 8	0.808
Thiobacillus	3.0 ± 3.0	3.3 ± 3.3	7.5 ± 3.7	0.046*
Polaromonas	1.2 ± 1.4	6.2 ± 7.2	0.6 ± 0.6	0.236
Gallionella	1.6 ± 1.5	2.6 ± 2.2	5.5 ± 3.7	0.088
Methylotenera	0.8 ± 1.1	4.0 ± 4.3	1.1 ± 0.6	0.178
Polynucleobacter	1.0 ± 2.4	3.4 ± 4.8	0.1 ± 0.1	0.598
Arenimonas	2.8 ± 2.3	1.0 ± 1.6	1.0 ± 1.1	0.066
Kaistobacter	1.1 ± 1.0	1.3 ± 1.2	1.1 ± 1.3	0.981
Rhodoferax	1.4 ± 1.8	1.0 ± 0.7	1.0 ± 0.6	0.931

*Significant (p-value < 0.05)



1a Geothrix
1b Geobacter
1c Thauera
1d Methanospirillum
1e Dokdonella
1f Flavobacterium
1g Sphingorhabdus
1h Methanolinea
1i Propionibacterium
1j UD5F
2a Arenimonas
2b Corynebacterium
2c CandidatusXiphinematobacter
2d Solirubrobacter
2e ChitinophagaceaeF
2f Pirellula
2g BryobacteraceaeF
2i CL5002
3a Methylibium
3b Rubrivivax
3c Acinetobacter
3d HyphomicrobiaceaeF
3e Pseudomonas
3f Pedobacter
3h Simplicispira
C_Nitrotoga
NitrosomonadaceaeF
Thiobacillus
Nitrospira
Lysobacter
Kaisto
Thermomonas
mb2424F
Sinobacteraceae F
Ellin6075F
Streptococcus
Smithella
EB1017F
Thermodesulfovibrionaceae F
Nitrospinaceae F
Novosp
Autotrophs
N-oxidisers
S-oxidisers
Fe-oxidisers
S- and Fe oxidisers
H-oxidisers
Other autotrophs
Methanogens
Heterotrophs
Unknown function

1

Fig 6. Bacterial (16S) network analysis and inferred metabolic groupings associated with entrained sediment in BI facies. The size of the circles is proportional to the mean abundance of the bacterial taxonomic group. Colour-coding highlights inferred functional categories based on the literature.

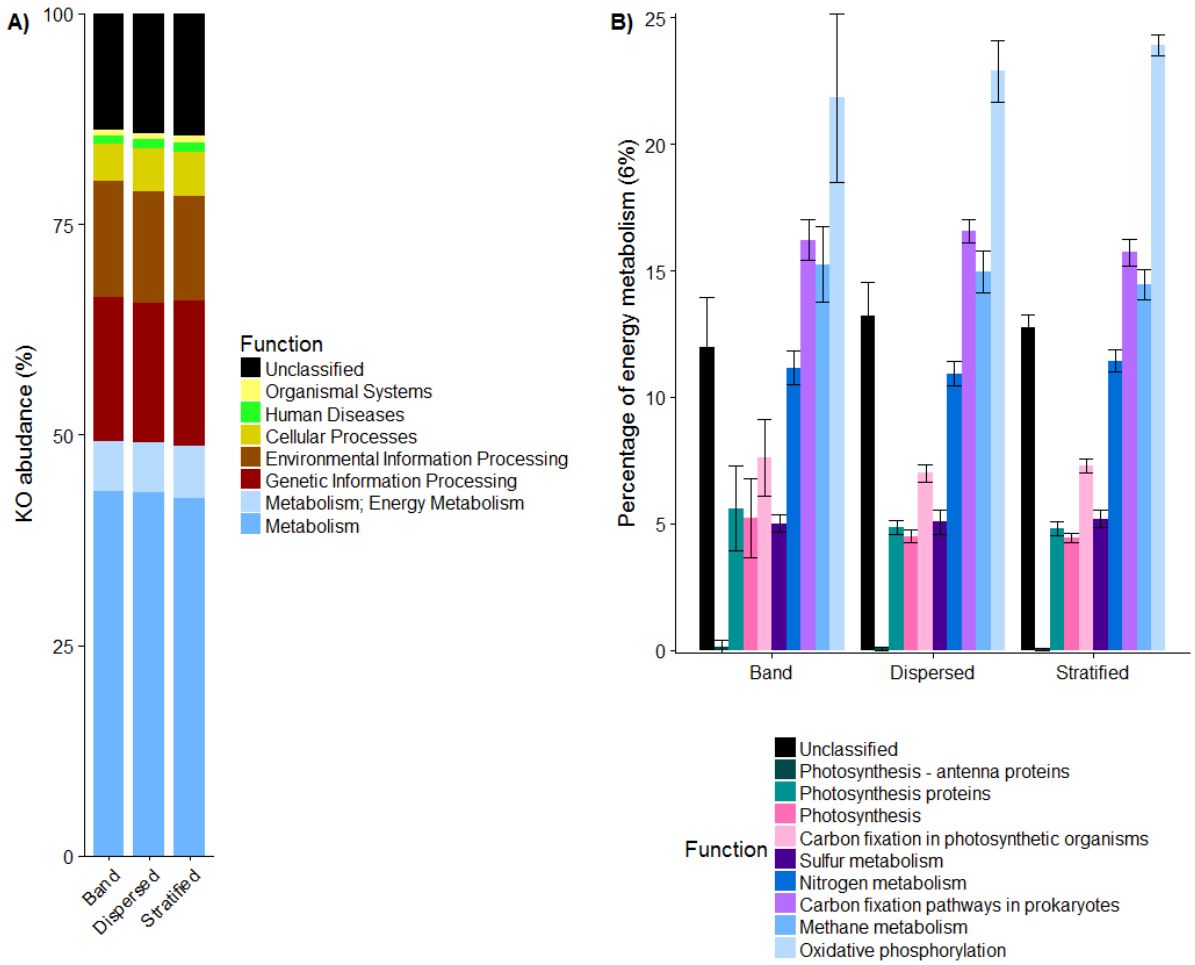


Fig 7. Potential functionality predicted by PICRUSt. A) General functions, B) Close-up to potential energy metabolic pathways, representing ~6% of the total of the KOs predicted.

Abstract

The basal zone of glaciers is characterised by physicochemical properties that are distinct from firnified ice because of strong interactions with underlying substrate. Basal ice ecology and the roles that the microbiota play in biogeochemical cycling, weathering, and proglacial soil formation, remains poorly known. We report bacterial diversity and potential ecological roles at three temperate Icelandic glaciers. We sampled three physically distinct basal ice facies (stratified, dispersed, debris bands) and found biological similarities and differences between them; basal ice character is therefore an important sampling consideration in future studies. High abundance of silicates and Fe-containing minerals could sustain the basal ice ecosystem, in which chemolithotrophic bacteria (~23%), especially Fe-oxidisers and hydrogenotrophs, can fix C, which can be utilised by heterotrophs. Methanogenic-affiliated detected sequences showed that silicate comminution-derived hydrogen can also be utilised for methanogenesis. Metabolism predicted by 16S rRNA diversity revealed that methane metabolism and C-fixation are the most common pathways, indicating the importance of these metabolic routes. Carbon concentrations were low compared to other ecosystems, but we report the highest carbon concentration in basal ice to date. Carbon release from melting basal ice may play an important role in promoting pioneering communities establishment and soil development in deglaciating forelands.

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