

Are recently deglaciated areas at both poles colonised by the same bacteria?

del Moral Álvaro^{1,2}; Garrido-Benavent Isaac¹; Durán Jorge³; Lehmann Jan R⁴, Rodríguez Alexandra³, Heiðmarsson Starri⁵; de los Ríos Asunción^{1,*}.

¹Department of Biogeochemistry and Microbial Ecology, National Museum of Natural Sciences (MNCN), CSIC, E-28006 Madrid, Spain.

²AstrobiologyOU, School of Environment, Earth and Ecosystem Sciences, STEM Faculty, The Open University, MK7 6AA, Milton Keynes, United Kingdom.

³Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal.

⁴Remote Sensing and Spatial Modelling, Institute of Landscape Ecology, University of Münster, Münster, Germany.

⁵Icelandic Institute of Natural History, Akureyri, Iceland.

Abstract

Polar glacier forefields offer an unprecedented framework for studying community assembly processes in regions that are geographically and climatically isolated. Through amplicon sequence variant (ASV) inference, we compared the composition and structure of soil bacterial communities from glacier forefields in Iceland and Antarctica to assess overlap between communities and the impact of established cryptogamic covers on the uniqueness of their taxa. These pioneer microbial communities were found to share only 8% of ASVs and each taxonomic group's contribution to the shared ASV data subset was heterogeneous and independent of their relative abundance. Although the presence of ASVs specific to one glacier forefield and/or different cryptogam cover values confirms the existence of habitat specialist bacteria, our data show that the influence of cryptogams on the edaphic bacterial community structure also varied also depending on the taxonomic group. Hence, the establishment of distinct cryptogamic covers is probably not the only factor driving the uniqueness of bacterial communities at both poles. The structure of bacterial communities colonising deglaciated areas seems also conditioned by lineage-specific limitations in their dispersal capacity and/or their establishment and persistence in these isolated and hostile regions.

INTRODUCTION

Shrinking glaciers and polar ice sheets are among the most noticeable consequences of climate change (Fitzharris, Lawson and Owens 1999; Oerlemans *et al.* 1998). A side effect of glacier retreat is the generation of wide expanses of land, which after centuries or millennia covered by ice, are once again exposed to the environment and therefore susceptible to colonisation (Matthews 1992; Brown and Jumpponen 2014; Cicazzo *et al.* 2016). Pioneer colonisers are suggested to be psychrophilic or psychrotolerant organisms that must cope with the near absence of organic matter (Deming 2002; Sigler and Zeyer 2004; Lee *et al.* 2018). Later on, the activity of pioneering microbial communities modifies the physicochemical properties of the soil (Frey *et al.* 2010) and drives ecological succession by favouring the development of more complex communities including cryptogams, i.e. mosses and lichens (Breen and Levesque 2008; Knelman *et al.* 2012; Donhauser and Frey 2018). In polar regions, the establishment of cryptogams results in the development of the so-called soil cryptogamic covers, which harbour specific bacterial communities (Belnap *et al.* 2001; Arróniz-Crespo *et al.* 2014; Juottonen *et al.* 2020). These multi-organism structures play a key role in soil biogeochemical cycling, allowing for the subsequent development of plant communities (Belnap and Harper 2001; Fernández-Martínez *et al.* 2016). Accordingly, polar glacier forefields offer an unprecedented framework for exploring soil microbial diversity and community assembly processes in a scenario of climate change (e.g. Kim *et al.* 2017; Garrido-Benavent *et al.* 2020).

Although culture-dependent procedures for surveying edaphic bacterial communities are useful to characterize the physiological potential of the isolates, they offer only a partial view of community diversity and structure (Rappé and Giovannoni 2003; Janssen 2006; Makhalanyane *et al.* 2015) owing to the challenges of cultivating a substantial number of edaphic microorganisms (Handelsman 2004; Thompson *et al.* 2017). In the past few decades, metabarcoding strategies have revolutionized microbiology by enabling the detection of a wider range of microorganisms in a culture-independent manner (Hebert 2005; Taberlet *et al.* 2012). The use of these techniques along with improving bioinformatics means that thousands of generated sequence reads can be now analysed in a relatively short amount of time using computationally intensive software (Segata *et al.* 2013; Gweon *et al.* 2015; Bolyen *et al.* 2019). These strategies have prompted diversity surveys of highly complex soil microbial communities and sparked renewed interest in microbial biogeography (Martiny *et al.* 2006; Tedersoo *et al.* 2014; Delgado-Baquerizo *et al.* 2018). In early studies, biogeographic patterns were interpreted in the context of the old tenet of microbiology ‘*Everything is everywhere, but, the environment selects*’ (Baas Becking 1934). Reports of certain groups thriving under similar conditions in geographically distant regions were considered evidence of the exceptional dispersal ability of microorganisms (Smith *et al.* 2013; Finlay and Clarke 1999; Finlay 2002). However, this older view has been gradually refuted owing to recent metabarcoding approaches revealing varying levels of endemic and cosmopolitan prokaryotic and eukaryotic microorganisms (Tedersoo *et al.* 2014; Cox *et al.* 2016; Zhang *et al.* 2016a). By clustering bacterial sequence reads into operational taxonomic

units (OTUs) (Blaxter *et al.* 2005), Kleinteich *et al.* (2017) observed that terrestrial and lacustrine biofilms at both Earth's poles were compositionally more similar to each other than to those found in geographically closer temperate habitats. These authors proposed a model of global dispersal of bacterial species controlled by selective pressures in cold environments (global ubiquity hypothesis, Baas Becking 1934) in combination with the effect of distance in decreasing exchange rates between very distant localities (distance-decay theory, Nekola and White 1999; Morlon *et al.* 2008). At present, however, the clustering of sequence reads into OTUs has evolved to avoid the use of arbitrary thresholds to delimit species (e.g. Callahan *et al.* 2016; Edgar 2018) and some biogeographical patterns could change. In particular, the analysis of amplicon sequence variants (ASVs), which differ from each other at least by a single nucleotide (Callahan *et al.* 2016, 2017), is a promising tool to gain insight into the global distribution of microorganisms because it could reveal patterns hidden by the OTU-based approach. This approach could be complemented by high-throughput metagenomics technologies to delve into the functional potential of the microbial community.

Herein, we use inference of ASVs to examine the composition and structure of soil bacterial communities from both polar regions to assess the extent to which these communities overlap and the impacts of the establishment of cryptogamic covers on the uniqueness of their taxa. Our working hypothesis is that the edaphic communities of these distant polar regions share some habitat generalist bacteria with bipolar distribution, whereas each glacier forefield sustain its own habitat specialist bacterial lineages associated with different states of specific cryptogamic cover development. Our ultimate aim was to provide useful information on microbial diversity and community assembly for regions significantly threatened by global warming.

MATERIALS AND METHODS

Study area and experimental design

Soil samples were collected from the Breiðamerkurjökull glacier forefield (SE Iceland) in July 2017 and from a glacier forefield located in the Sally Rocks tongue of the Hurd Glacier (Livingston Island, South Shetland Islands, maritime Antarctica) in February 2018 (Fig. 1). These sites are hereafter referred to simply as IGF and AGF, respectively. The sampling procedure was designed to evaluate the edaphic microbial communities found in soils experiencing increasing development of cryptogamic cover. Thus, a gradient of cover 0 to 100% was established in the field according to the observed percentage of moss cover. Then, 20 and 18 sampling plots (1m × 1m) were selected in IGF and AGF respectively, representing circa 5% cover increases (Fig. 1). Accurate Cryptogamic cover values were calculated following Durán *et al.* (2020).

Soil Sampling, DNA extraction, PCR amplification and high-throughput sequencing

Five samples of the upper soil layer (0-5 cm) were randomly collected at each sampling plot using a diameter stainless steel corer. Soil samples were sieved (2 mm mesh) and thoroughly mixed to create one homogeneous composite sample per plot which was immediately preserved in *RNAlater*TM until further processing. Genomic DNA was extracted from each composite soil sample using the PowerSoil[®] DNA Isolation Kit (MOBIO) according to the

manufacturer's protocol. For DNA amplification, we followed the bacterial 16S rRNA Illumina Amplicon Protocol recommended by the Earth Microbiome Project (<http://www.earthmicrobiome.org/protocols-and-standards/16s/>) and used the primer pair 515F-806R (Caporaso *et al.* 2011). An amplicon library was then generated at the ASU Genomics Core (Arizona State University) and loaded in MiSeq Illumina and run using the version 2 module, 2 × 250 pair-end, following the manufacturer's instructions. Raw reads were demultiplexed and barcode sequences were removed by the sequencing centre. The datasets generated for this study can be found in the NCBI Sequence Read Archive with PRJNA693562 BioProject number

Bioinformatics data processing

Bacterial 16S rRNA Illumina amplicon data were processed to infer ASVs (sequences differing at least by a single nucleotide) using the R package *dada2* v.1.8.0 (Callahan *et al.* 2016) and following the DADA2 workflow for Big Data 1.4 (more details in Supplementary material). Alternatively, the same bacterial Illumina amplicon dataset was used to assess how estimates of unique and shared bacteria between both forefields would be affected by the use of the Operational Taxonomic Unit (OTU) approach compared to the ASV method. QIIME v.1.9.1 (Caporaso *et al.* 2010) was used to define OTUs based on recommendations of the Microbiome Helper workflow (Comeau *et al.* 2017). The same steps and scripts as in Garrido-Benavent *et al.* (2020) were used, although a threshold of 220 bp was set for initial truncation of reads in the present work.

Data analysis and statistics

Venn diagrams calculated in the R package *VennDiagram* were used to compare the number of ASVs and OTUs shared between both forefields. To further examine the contribution of different taxa to the shared data subset, a presence/absence data matrix was built to calculate the percentage of net change between the relative abundance of a particular taxon in the total and shared datasets using the formula $Y = [(X_i - X_0)/X_0] * 100$, where X_0 is the proportion of ASVs corresponding to a particular taxon in the total dataset and X_i the proportion of ASVs corresponding to this particular taxon in the shared subset. Net changes close to 0 mean that the subset is stochastically selected. In addition, alpha-diversity indices (Chao1, Shannon and Pielou's evenness) for both glacier forefields were calculated with the *phyloseq* package, and differences tested using the function *kruskal.test*. All plots were built with the package *ggplot2*. Subsequently, a non-metric multidimensional scaling (NMDS) ordination using a Bray-Curtis dissimilarity matrix was performed with the R package *phyloseq* to illustrate differences in the composition of microbial communities across sample categories (geographic location and cryptogamic cover). NMDS also displayed vectors indicating the direction and magnitude of influence of the top ten phyla having a major impact on community structure, which were inferred on the basis of a permutational multivariate analysis of variance (PERMANOVA) implemented in the R package *Vegan* with the *adonis* function (Anderson 2001). Phylogenetic beta-diversity was also quantified using unweighted and weighted UniFrac distance matrices (Lozupone and Knight 2005) and visually represented through NMDS. Finally, ASV ribbon maps were generated using the R package *circlize* (Gu 2014) to visualise the relative abundance of unique and shared ASVs as well as

shared patterns between soils of IGF and AGF with different cryptogamic cover values, using the soil categories established in the NMDS.

RESULTS

The number of bacterial ASVs and OTUs obtained from 38 soil samples were 20865 and 6491. Of these, 10801 ASVs (3377 OTUs) belonged to the Antarctic (AGF) and 10064 ASVs (3114 OTUs) to the Icelandic (IGF) forefields (Fig. 1). The number of estimated ASVs was therefore markedly higher than the number of OTUs. However, only 7.9 % of the ASVs were shared between AGF and IGF (1648), in contrast to the 72.6 % of OTUs (2730) shared between them. The ASVs clustered into 158 unique orders, which were grouped into 52 phyla while 1357 sequence variants could not be assigned to any known bacterial phylum.

When net changes in taxon relative abundances between the total and shared datasets were quantified, we observed heterogeneous phylum-specific contributions to the shared ASV subset (Fig. 2). The greater net change values were detected for taxonomic groups showing low relative abundances (Supplementary Fig. 1) with the exception of *Actinobacteria* and *Cyanobacteria* (Fig. 2). Some phyla were more abundant in the shared subset than in the total dataset (red bars in Fig. 2, positive net change), whereas others were less abundant (blue bars in Fig. 2, negative net change).

Differences in alpha-diversity metrics between the total dataset and shared subset were detected for both glacier forefields, indicating the non-stochastic nature of the shared dataset. Bacterial abundance (i.e. Chao1), diversity (i.e. Shannon), and evenness (i.e. Pielou) indices were significantly lower in the shared than in the total dataset ($P < 0.001$, $P < 0.001$, and $P < 0.05$, respectively; Fig. 3). However, differences in alpha-diversity could not be attributed to different cryptogamic cover values.

According to the NMDS ordination of Bray-Curtis (Fig. 4) and UNIFRAC (data not shown) distance matrices, bacterial communities from both glacier forefields formed two well-delimited groups. Within each glacier forefield, samples also grouped together depending on their cryptogamic cover percentage, especially for AGF where two clearly separate groups of low (LC = 0–50%) and high cover (HC >50%) emerged. *Actinobacteria* appeared clearly associated with the LC category in both forefields, while *Proteobacteria* and *Bacteroidetes* were associated with HC, especially in AGF. In addition, some ASVs assigned to different taxonomic groups appeared only in one of these two categories at both glacier forefields (Fig. 5; Supplementary Fig. 2A). In general, more ASVs were shared between bacterial communities from soils with different cryptogamic cover percentages within each glacier forefield (red and blue ribbons in Supplementary Fig. 2B) than between both glacier forefields (green ribbons in Supplementary Fig. 2B) in agreement with the NMDS ordination results.

Ribbon maps of the taxonomic groups showing greater contributions to NMDS ordination revealed phylum-specific patterns of ASV distributions with respect to geographic region (AGF and IGF) and cryptogamic cover category (HC and LC) (Fig. 5). Firstly, these maps show that unique ASVs (present only in one of the four groups) remarkably showed different relative abundances across different taxonomic groups. Specifically, *Firmicutes*, *Planctomycetes* and *Cyanobacteria* showed the highest relative abundance of unique ASVs (Fig. 5, Supplementary Fig. 2A). Also, for some taxonomic groups such as *Proteobacteria*,

Cyanobacteria and *Firmicutes*, the relative abundance of unique ASVs in both forefields was higher in HC than in LC plots (Fig. 5; Supplementary Fig. 2A). Secondly, the abundance of ASVs shared between glaciers differed also between taxonomic groups. Thus, while *Actinobacteria*, FBP and *Gemmatimonadetes* were the taxonomic groups sharing most ASVs between both glacier forefields, *Cyanobacteria*, *Planctomycetes* and *Firmicutes* shared the least. Thirdly, the relative abundance of ASVs shared between soils with a different degree of cryptogamic cover within the same glacier forefield depended not only on the taxonomic group but also on the geographic origin. For instance, *Cyanobacteria*, *Firmicutes* and *Bacteroidetes* shared more ASVs within AGF than within IGF. These phyla also displayed the highest relative abundances of total and unique ASVs in this glacier forefield (Fig. 5). Finally, we also found a trend towards more ASVs shared between LC categories than between HC categories in the two forefields (e.g. *Gemmatimonadetes*, *Actinobacteria*, *Chloroflexi*, FBP) (Fig. 5; Supplementary Fig. 2B).

DISCUSSION

Deglaciation is an ongoing climate change-caused phenomenon that opens up new land areas, where microbial pioneer communities harbouring bacteria from endogenous (glacier habitats) and exogenous (aeolian dust, rain and snow) sources can gradually become established (Brown and Jumpponen 2014; Cicazzo *et al.* 2016; Rime *et al.* 2016). Prominent relationships of these pioneer microorganisms with later colonisers, such as cryptogams, and the soils that both of them inhabit, make these microbial communities keystone elements to understand the succession that takes place in both northern and southern polar regions. Little by little, we are unravelling the diversity and composition of these microbial communities. However, it remains unknown whether key species play similar roles in the colonisation of deglaciated areas in distant polar regions. In this study, we show for the first time that pioneer soil communities of Icelandic and Antarctic glacier forefields share only a small proportion of bacterial lineages that may therefore feature a bipolar distribution.

Genetically closely related bacteria that group together in the OTU clustering steps are clearly differentiated by their ASVs (Stackebrandt and Ebers 2006; Cox *et al.* 2016; Edgar 2018; Callahan, McMurdie and Holmes 2017; Martinson *et al.* 2019; Garrido-Benavent *et al.* 2020) allowing a more accurate assessment of the level of similarity between bacterial communities from both forefields. To the best of our knowledge, the ASV approach had not been previously used to evaluate the presence of the same edaphic bacterial lineages in disjunct polar areas, in spite of the fact that these geographically isolated, but environmentally similar ecosystems, are ideal sites to examine global-scale microbial dispersal (Martiny *et al.* 2006; Herbold *et al.* 2014). Our detection of identical edaphic bacterial ASVs in AGF and IGF communities served to confirm the presence of bacteria showing a bipolar distribution, although a proportion of them may correspond to globally dispersed species. Based on these evidences, we propose that most global microbial biogeographic assumptions should be reassessed by the ASV method to obtain a better overview of the spatial and temporal distributions of microorganisms. However, these reassessments should be preceded by well-designed sampling strategies that capture the variability of microbial communities at very small scales in order to take advantage of the high sensitivity offered by the ASV approach.

The differences detected in diversity indices between the general and shared datasets suggest that ASV distribution patterns and richness were not randomly established at the analysed glacier forefields. Significantly lower bacterial evenness and Shannon diversity indices were found for the shared data subset, indicating that some taxa had increased predominance in the shared subset while others were less abundant, coinciding with our net change calculations. As expected, Chao abundance was lower for the shared subset than for the total dataset because of its smaller size. Abundant bacterial lineages like *Actinobacteria*, which is predominant in glacier forefields (Zhang *et al.* 2016a), are likely to be more widely distributed (Nemergut *et al.* 2011). However, here, less abundant taxonomic groups like *Spirochaetae*, *FBP*, *Fibrobacteres* or *Chlorobi* were also enriched in the subset shared by these polar ecosystems, thus suggesting that other driving forces, influencing differently across taxa, such as local environmental factors (e.g. soil pH, organic matter, available ammonium and nitrate, soil moisture) could be involved in the observed distribution patterns (Mo *et al.* 2018; Ji *et al.* 2020). Indeed, distinct patterns of distribution for different taxonomic groups at the global and local scale have been revealed in the present study. Some ASVs, for example in the phyla *Firmicutes* and *Cyanobacteria*, only appeared in one glacier forefield. The presence of these two phyla in polar regions has been associated with their high tolerance to extreme conditions involving the formation of resistance structures (Tashyreva and Elster 2012; Ramos *et al.* 2019) which could be more successful at harsher climatic conditions. Microbial dispersal is presumably not limited because their constraints are scarce due to their high probability of transmission and ability to survive during long-distance dispersion using vectors such as animals and wind (Wilkinson 2001; Fenchel and Finlay 2004; Omalley *et al.* 2008). However, the spatial and climatic isolation of polar regions from the rest of the globe is thought to limit microbial dispersal and, as a consequence, promote endemism (Papke and Ward 2004; Sominen, McDonald and Hillebrand 2007; Vyverman *et al.* 2010; Archer *et al.* 2019). In fact, the bacterial evenness for AGF was significantly lower than for IGF, indicating a greater abundance of rare ASVs in the most geographically isolated area. In addition, the highest abundance of unique ASVs was observed for *Firmicutes* and *Cyanobacteria* in the Antarctic forefield. The heterogeneity of taxa distribution observed could be related to differences in dispersal capacities among bacterial lineages, but a significant level of stochasticity associated with the dispersal and establishment processes could also explain this heterogeneity. These factors may be even more relevant to explain observed distributions of less abundant groups (Jenkins *et al.* 2007; Tedersoo *et al.* 2014; Evans, Martiny and Allison 2017). Alternatively, even considering dispersal limitless, the specific environmental conditions at higher latitudes could restrict the later establishment of microbial communities (Omalley 2008; Cox *et al.* 2016). In addition, a variety of biotic (competition and facilitation) and abiotic factors (e.g. pH, soil moisture, redox level, nutrient availability) operating at the microscale may influence successful colonisation (Fierer 2008). In polar environments, some microbial taxa can thrive, or at least tolerate, a broad range of extreme environmental conditions and are more likely to be ubiquitous, but other taxa could only persist under a very specific set of environmental conditions available in particular microhabitats (Barberán *et al.* 2014). While the presence of unique ASVs at these glacier forefields confirms the existence of habitat specific microorganisms, the detection of microorganisms showing a bipolar distribution in soils with different proportions of cryptogamic cover suggests the existence of habitat generalist taxa.

The coexistence of cosmopolitan and endemic microorganisms has been previously suggested for different Antarctic areas (Jungblut, Lovejoy and Vincent. 2010; Herboldt *et al.* 2014).

Cryptogams were found to exert a clear effect on the structure of edaphic bacterial communities in the glacier forefields examined here, but this influence varied depending on the taxonomic group and differed between glacier forefields. The presence of *Actinobacteria* was associated with lower cryptogamic cover values, in agreement with abundance patterns of this phylum observed along a formation sequence of desert cyanobacteria-rich soil crusts (Abed *et al.* 2019). In contrast, the abundance of *Proteobacteria* and *Bacteroidetes* was greater under a high level of cryptogam cover, as similarly observed beneath soil crusts in the Gurbantunggut Desert (Zhang *et al.* 2016b). In consequence, the association of some ASVs to high cryptogam cover while rare or absent in soils with low cryptogamic cover states may suggest a link to the development of biological soil crusts. These effects could respond to the increase of organic matter accumulation and soil fertility associated to the development of cryptogamic cover in the studied areas (Durán *et al.* 2020). In addition, several studies have shown that the establishment of cryptogams increases the spatial heterogeneity of colonized soils, and consequently the amount of potentially distinct microbial niches (Breen and Levenske 2008; De los Ríos *et al.* 2011; Fernández-Martínez *et al.* 2017; Van Zuijlen *et al.* 2020). However, our findings indicate that cryptogamic cover development is not the only local environmental factor shaping the structure of these pioneer microbial communities. Local biogeochemical and microclimatic conditions could favour also the colonization of specific taxa uniquely adapted to these specific conditions (Fierer *et al.* 2009; Delgado-Baquerizo *et al.* 2018). Due to development of cryptogamic cover increase with exposure time since glacier retreat, the lower proportion of ASVs shared between HC than between LC categories from both glacier forefields also points to a potential increase in specificity of bipolar colonisers over time in response to environmental constraints and competition with more adapted microbial taxa (Garrido-Benavent *et al.* 2020).

Taken together, our results provide empirical evidence that the soils of the analyzed glacier forefields of Antarctica and Iceland shared some bacterial lineages. Contrary to our hypothesis, these potential bipolar bacteria were not preferably habitat generalists. Further studies at other glacier forefields from both polar areas are necessary to properly estimate the level of endemism and bipolarity of these microbial communities. Cases of bipolar distributions have been extensively described among macroorganisms such as bryophytes (Biersma *et al.* 2017), lichens (Garrido-Benavent and Pérez-Ortega 2017) and vascular plants (Villaverde *et al.* 2017). The remarkable bacterial uniqueness inferred from our ASV data can be only partly explained by the presence of specific habitat specialists associated to the development of particular cryptogamic covers at both glacier forefields. This suggests that geographical and climatic isolation, as well as specific local microenvironmental conditions, could also be shaping the structure of these pioneer communities. Functional studies are now necessary to analyse if certain specific habitat specialists play comparable roles in the colonisation of deglaciated areas at both Earth's poles. As global warming expedites glacial melting in polar regions, glacier forefields will become increasingly important for both polar and global biogeochemical cycles. Understanding the mechanisms underlying patterns of microbial community assembly and distributions of cosmopolitan and endemic species is

therefore of utmost importance to better predicting the fate of these key polar ecosystems in future climate change scenarios.

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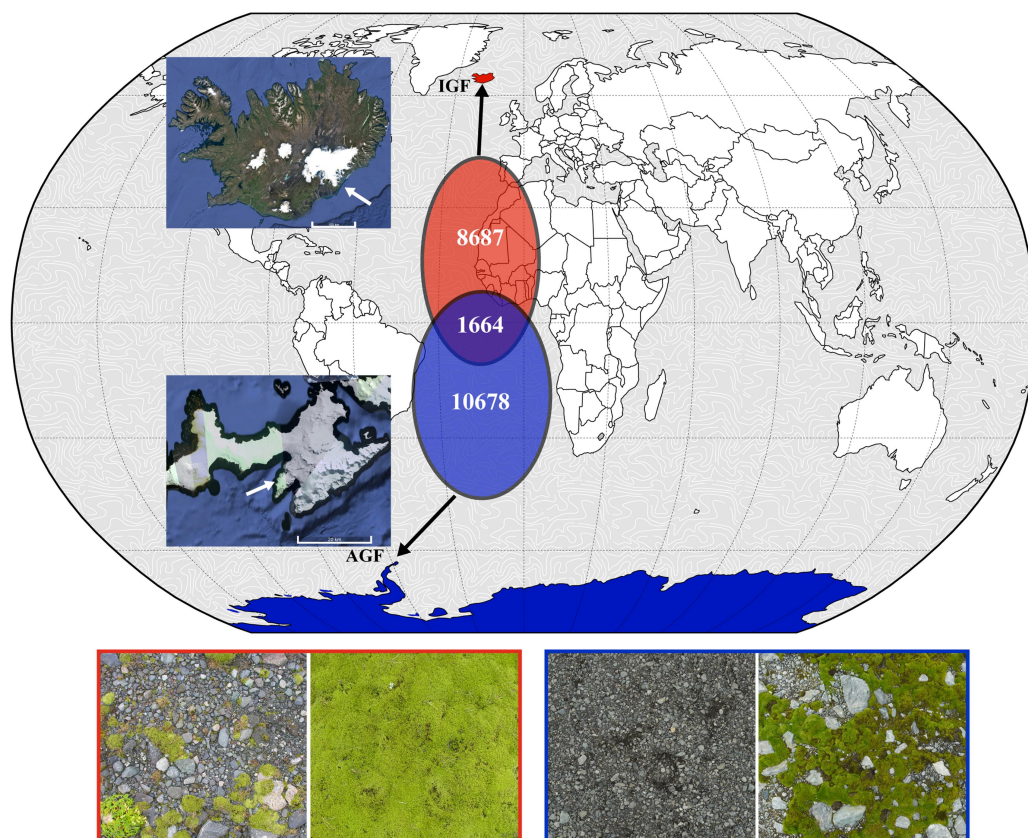


FIGURE 1

Figure 1. Map showing the sampling areas: red for the Icelandic glacier forefront (IGF) and blue for the Antarctic glacier forefront (AGF). Satellite images from google earth showing the soil sampling areas (marked with a white arrow): top image: Breiðamerkurjökull glacier forefield (SE Iceland); bottom image: Sally Rocks Tongue at the Hurd Glacier (Livingston Island, Antarctica). In red boxes: **a)** IGF plot with 27.2% cryptogamic cover **b)** IGF plot with 100% cryptogamic cover. In blue boxes: **c)** AGF plot with 0% cryptogamic cover. **d)** AGF plot with 65.5 % cryptogamic cover. Venn diagram showing the distribution of Amplicon Sequence Variants (ASVs) in soil samples from AGF (blue) and IGF (red). Overlapping regions contain the shared ASVs between both glacier forefields.

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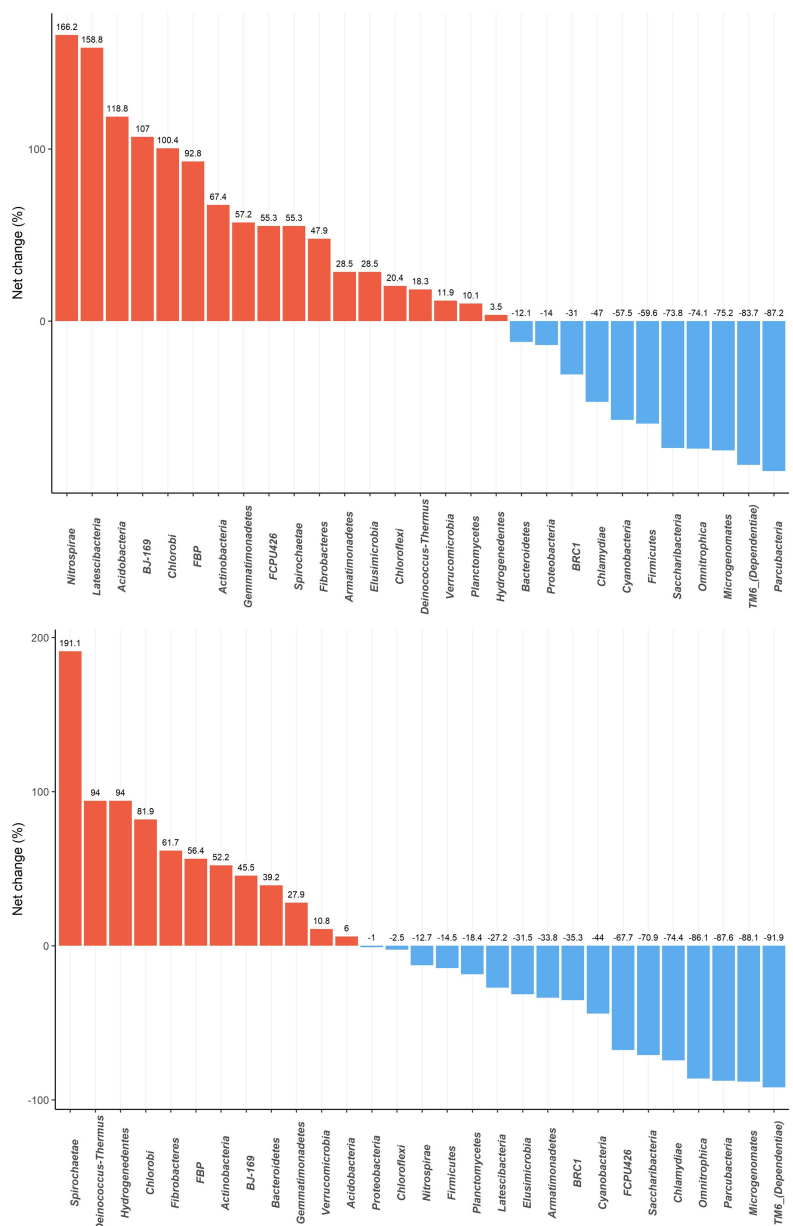


Figure 2. Barplots showing net changes in phylum relative abundances based on ASVs between the total dataset and the shared data subset for AGF (a) and IGF (b). Red bars indicate a positive net change (increase in phylum relative abundance in shared subset) and blue bars negative change (decrease in phylum relative abundance in shared subset).

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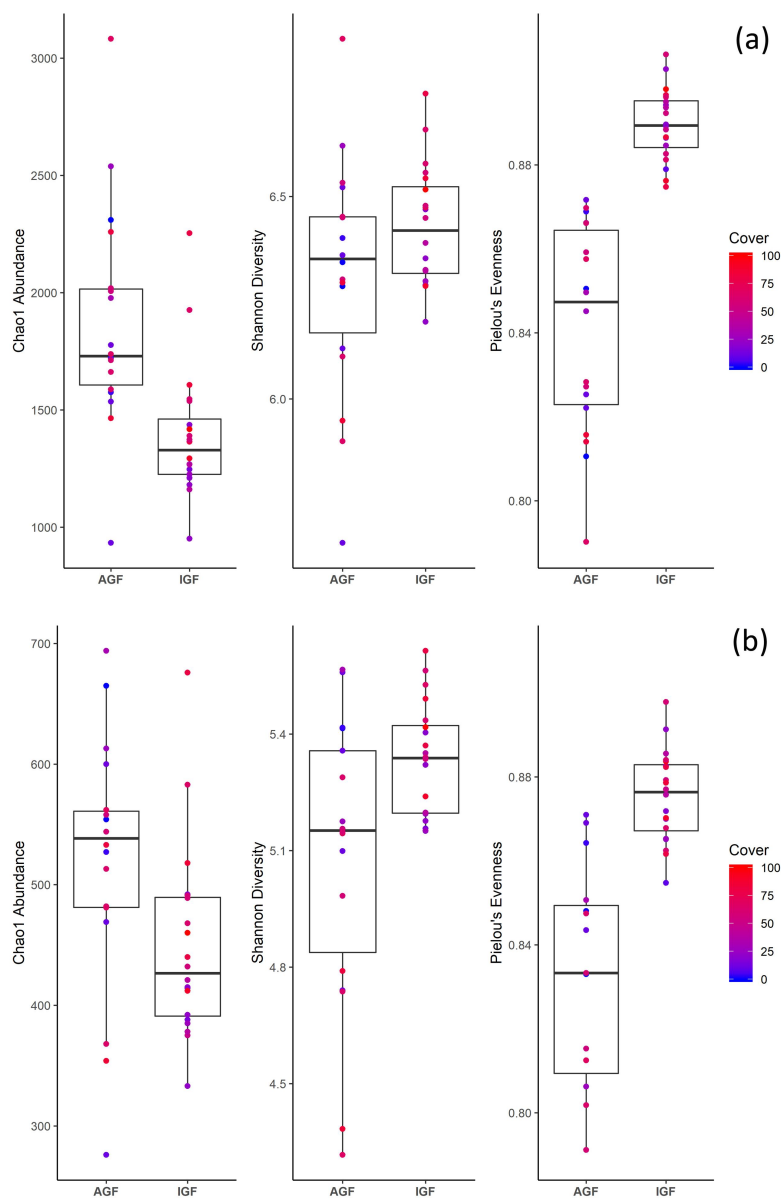


Figure 3. Boxplots showing alpha diversity metrics (Chao1 richness estimator, Shannon diversity index and Pielou's evenness index) variation in both glacier forefields with respect to the total dataset (a) and shared data subset (b). Cryptogamic cover is indicated by dots following the colour scale included in the figure.

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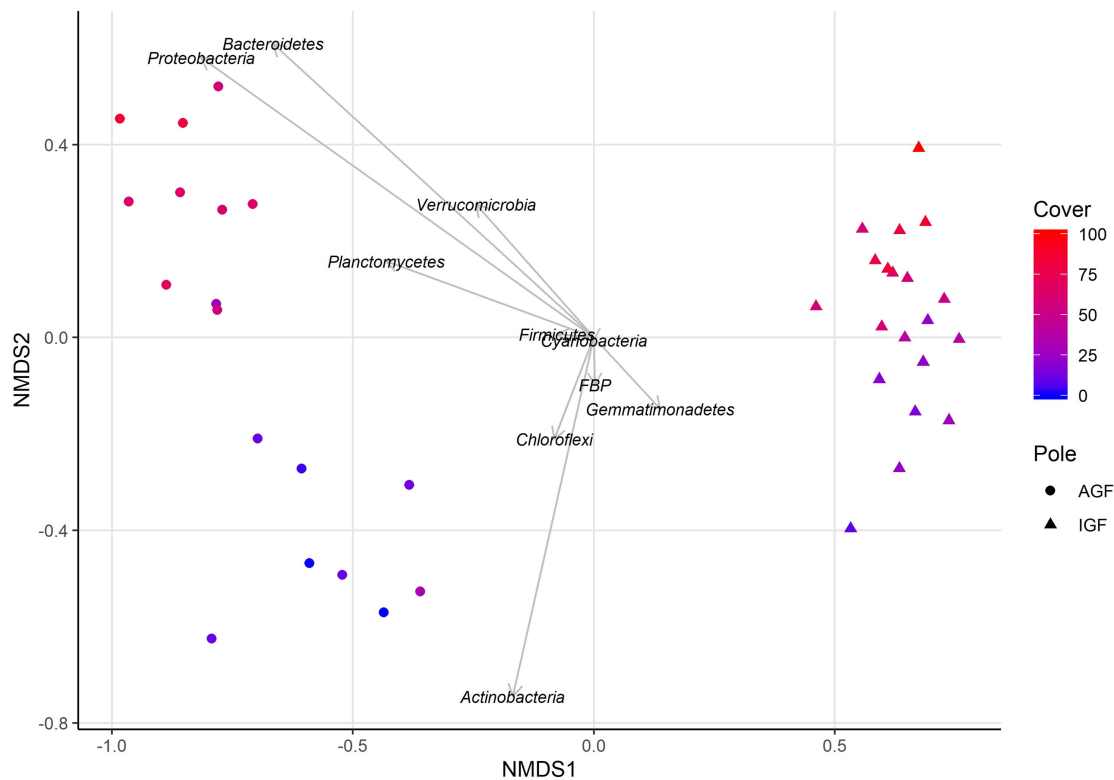


Figure 4. Non-metric multi-dimensional scaling (NMDS) ordination plot of two axis dimensions (stress = 0.079) of Bray-Curtis community dissimilarities based on the phyla assigned to ASVs found in 38 plots. AGF samples are represented by dots and those from IGF by triangles. The NMDS plot shows a high non-metric fit R^2 of 0.999. Cryptogamic cover is represented by dots following the colour scale included in the figure. Vectors are provided indicating the direction and magnitude of influence of the top ten phyla showing a major impact on community structure.

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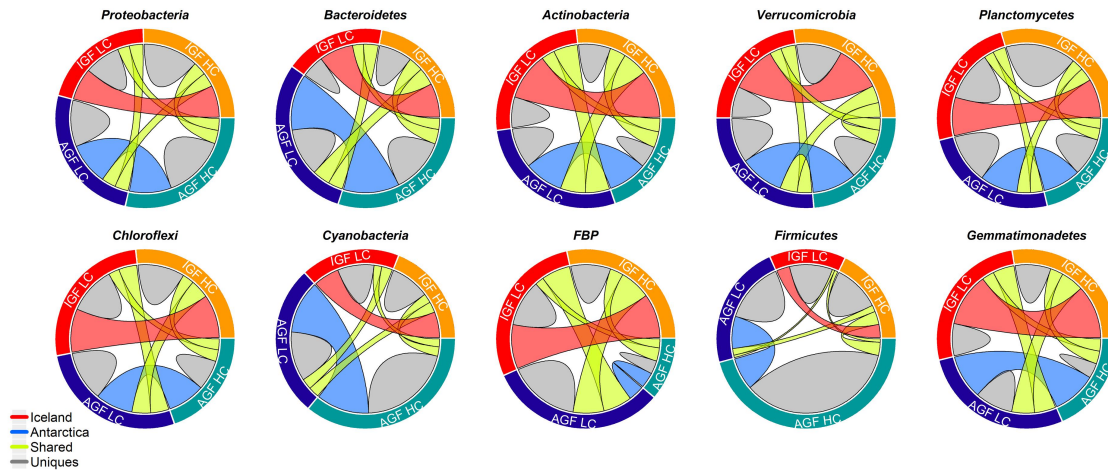


Figure 5. Ribbon maps of shared ASVs for both glacier forefields assigned to the taxonomic groups showing higher contributions to NMDS grouped into the categories of high cover (HC: >50% cryptogamic cover) and low cover (LC: <50% cryptogamic cover). Unique ASVs in each cover category at each glacier forefield are represented as grey areas. Ribbons corresponding to different types of sharing are colour coded: yellow ribbons represent the ASVs shared between both glacier forefields, blue ribbons represent the ASVs shared between both categories of cryptogamic cover for IGF, and red ribbons represent the ASVs shared between both categories of cryptogamic cover for AGF.