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Research article

Expanding Antarctic biogeography: microbial ecology of Antarctic island soils

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The majority of islands surrounding the Antarctic continent are poorly characterized in terms of microbial macroecology due to their remote locations, geographical isolation and access difficulties. The 2016/2017 Antarctic Circumnavigation Expedition (ACE) provided unprecedented access to a number of these islands. In the present study we use metagenomic methods to investigate the microbial ecology of soil samples recovered from 11 circum-Antarctic islands as part of ACE, and to investigate the functional potential of their soil microbial communities. Comparisons of the prokaryote and lower eukaryote phylogenetic compositions of the soil communities indicated that the various islands harbored spatially distinct microbiomes with limited overlap. In particular, we identified a high prevalence of lichen-associated fungal taxa in the soils, suggesting that terrestrial lichens may be one of the key drivers of soil microbial ecology on these islands. Differential abundance and redundancy analyses suggested that these soil microbial communities are also strongly shaped by multiple abiotic factors, including soil pH and average annual temperatures. Most importantly, we demonstrate that the islands sampled in this study can be clustered into three distinct large-scale biogeographical regions in a conservation context, the sub-, Maritime and Continental Antarctic, which are distinct in both environmental conditions and microbial ecology, but are consistent with the widely-used regionalization applied to multicellular Antarctic terrestrial organisms. Functional profiling of the island soil metagenomes from these three broad biogeographical regions also suggested a degree

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of functional differentiation, reflecting their distinct microbial ecologies. Taken together, these results represent the most extensive characterization of the microbial ecology of Antarctic island soils to date.

Keywords: Antarctic conservation biogeographic regions (ACBRs), Antarctic ecology, functional metagenomics, microbial ecology, sub-Antarctic islands

Introduction

The Antarctic continent hosts some of the most remote environments on Earth where terrestrial life, mostly represented by microorganisms, is permanently exposed to challenging environmental conditions (Cary et al. 2010, Convey et al. 2014, Pearce 2017). The conservation challenges posed by global warming and increasing human impacts on this vast continent have highlighted the importance of establishing an Antarctic regional classification and objectively representative system of area protection based on biological distribution and diversity patterns (Coetzee et al. 2017, Brooks et al. 2019, Leihy et al. 2020). Within this context, and for the region subject to Antarctic Treaty governance (defined as all land and ocean south of the sixty degree latitude parallel), the continental ice-free areas and island groups have been categorised into 16 distinct Antarctic Conservation Biogeographic Regions (ACBRs) based on both environmental and biotic factors (Terauds et al. 2012, Terauds and Lee 2016), providing a useful means to inform Antarctic conservation policies (Chown et al. 2015, Convey and Peck 2019). However, this bioregional classification is not yet comprehensive, baseline biological survey data from some regions of the Antarctic continent are rare or non-existent and, even where data exist for some taxa (including microorganisms), spatial coverage is extremely variable (though see studies reported in Herbold et al. 2014, Archer et al. 2019).

The terrestrial Antarctic realm encompasses the Continental Antarctic, including the islands located close to the continental Antarctic coast (Scott, Siple, Maher and Lauff islands), the Maritime Antarctic (the western Antarctic Peninsula, the Scotia Arc archipelagos (i.e. South Shetland Islands, South Orkney Islands and South Sandwich Islands), plus the outlying Peter 1 Øya and Bouvetøya) and the isolated sub-Antarctic islands located at lower latitudes and generally in proximity to the Antarctic Polar Front (Udvardy 1975, Holdgate 1977, Selkirk 2007, Terauds et al. 2012, Terauds and Lee 2016, Convey 2017). The ACBR classification explicitly applies only to the Continental and majority of the Maritime Antarctic regions (the South Sandwich Islands and Bouvetøya are located north of the area of Antarctic Treaty governance). For convenience, we will henceforth refer to the Maritime, Continental and sub-Antarctic ice-free areas considered in this study collectively as 'Antarctic islands'. Depending on their latitudinal positioning, these islands have widely differing glacial histories (Hodgson et al. 2014), geological origins (Convey 2007, Selkirk 2007, Quilty 2007) and topographies (Hodgson et al. 2014). As such, they are also characterized by different vegetation communities and diversity: from vegetated plateaus and mires with abundant fauna and flora to poorly-vegetated volcanic terrain and montane fellfields, with some Maritime and Continental Antarctic island groups hosting extensive perennial ice-cover (Mosley-Thompson et al. 1990, Hart et al. 1995, Quilty 2007, le Roux and McGeoch 2008). However, all are considered to be extremely remote. The sub- and Maritime Antarctic islands experience high precipitation, with the former characterised by chronically cool temperatures and strongly damped seasonal variation typical of the Southern Oceanic region (Convey 1996, Selkirk 2007, Leihy et al. 2018), while the latter experience much stronger thermal seasonality, similar to that of the Continental Antarctic coastline (Convey 2017). The remoteness of these islands, combined with increasing evidence of limited dispersal due to the extreme inter-island and island-continent distances (McGaughran et al. 2019, Stevens et al. 2021, Short et al. 2022), suggest that strong local homogenizing forces may lead to unique components in the soil microbial communities, as they do for higher plants (Chau et al. 2019).

Researchers have attempted to document the processes that drive island microbial biogeographical patterns across the globe. For instance, island size has been linked with microbial biodiversity (Bell et al. 2005), with larger islands harbouring greater species diversity due to higher colonization rates (Connor and McCoy 1979) and a greater variety of habitats (Williams 1964). Neutral ecological processes such as drift, dispersal limitation and diversification are also thought to have an important impact on the development of island microbial communities due to their isolation from other landmasses (Zhou and Ning 2017). A recent study (Li et al. 2020) suggested that bacterial and fungal island-scale biogeographical patterns are driven by distinct mechanisms. For bacterial diversity, habitat quality and soil chemical properties were suggested to be the main factors affecting species-area relationships, while fungal communities were more influenced by the stochastic process of dispersal limitation within each island.

Despite these efforts to understand global microbial biogeographical patterns across islands, the various peri-Antarctic islands have been the subject of almost no microbial diversity or ecological research. Yet they present excellent opportunities to test general theory and hypotheses about the biogeographic structuring of the region (Bergstrom et al. 2006, Convey et al. 2018 for overviews). Some Maritime Antarctic islands (particularly parts of the South Shetland Islands and Signy Island, South Orkney Islands) have received greater attention from microbial researchers (Chong et al. 2012, Meier et al. 2019, Almela et al. 2021). Where studies do exist, such as in metagenomic analyses of fungal diversity in soils from sub-Antarctic South Georgia (Dennis et al. 2012), these have been analysed

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in the context of wider latitudinal transects (e.g. along the Scotia Arc and Antarctic Peninsula), rather than in comparison with other sub-Antarctic islands. Microbial diversity analyses of the soils of Marion Island, which has a 50-year history of terrestrial research (Smith 1987), have been largely restricted to classical culture-dependent studies (French and Smith 1986) or focussed on specific microbial clades (Sanyika et al. 2012). In one of the few comparative studies of the diversity of a group of microbial eukaryotes across the Antarctic region, Verleyen et al. (2021) concluded that the freshwater diatom communities of different sub-Antarctic islands were largely endemic and shaped by dispersal limitation as well as in situ speciation, resulting in biogeographic separation between those present in different islands and island groups.

The 2016–2017 Antarctic Circumnavigation Expedition (ACE Cruise: Walton and Thomas 2018) provided an ideal opportunity to source biological materials in a coordinated fashion, including soil samples, from a unique constellation of remote and highly inaccessible sub-, Maritime, and Continental Antarctic islands. This sample set provides an unprecedented opportunity to investigate and compare their soil microbial diversity and ecology. The primary aim of this study was therefore to provide a comprehensive comparative assessment of the microbial diversity and functional capacity of soil microbiomes from a number of sub-, Maritime and Continental Antarctic islands, and to investigate the macroclimatic, soil chemistry and spatial factors driving the microbial biogeography of these islands.

Methodology

Study sites and sampling

A total of 40 soil samples were collected from 11 Antarctic islands (Fig. 1), under the auspices of the 2016/2017 ACE Cruise (Walton and Thomas 2018) (coordinates in Supporting information). Sampling sites were selected on the

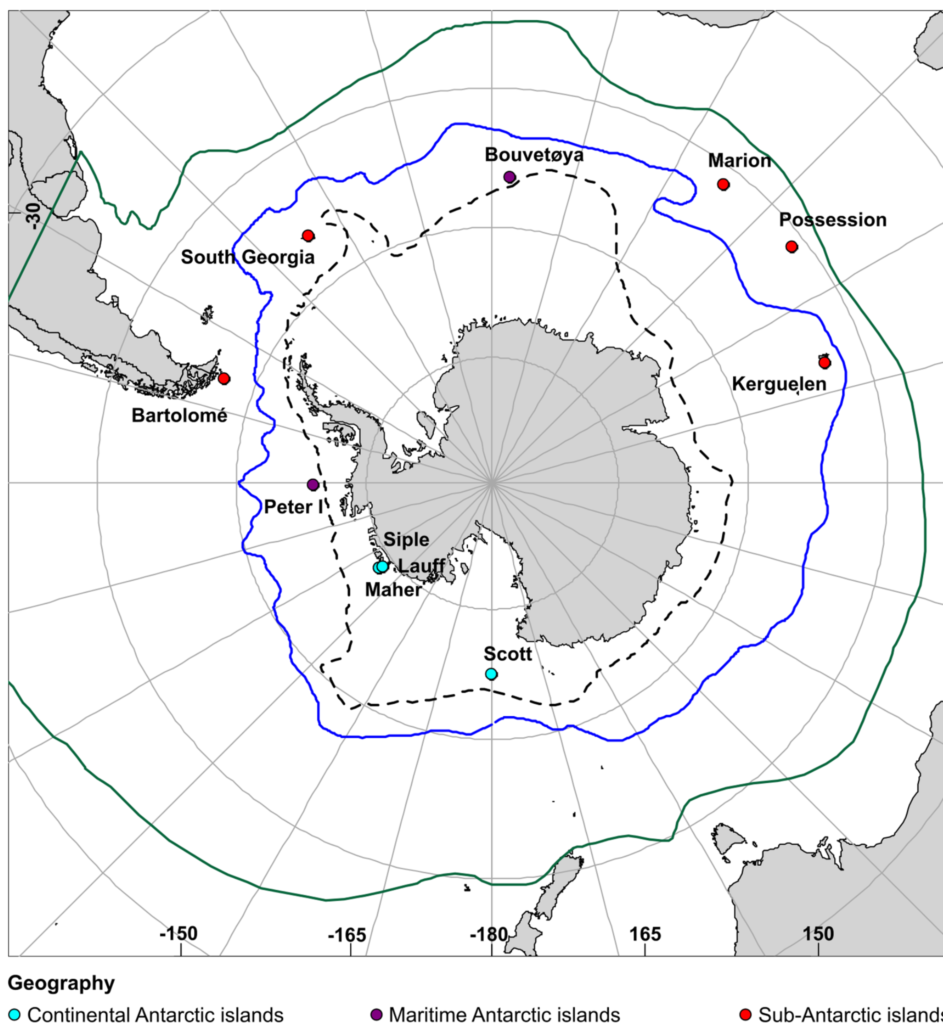

Figure 1. Map showing the Antarctic islands sampled in this study. Islands considered to be part of the Sub-Antarctic, Maritime and Continental Antarctic regions are highlighted in red, purple and light blue, respectively. The main oceanic fronts are represented using the following nomenclature: Sub-Tropical Front (green line); Polar Front (blue line); Antarctic Circumpolar Current southern boundary (dashed line).

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basis of remaining below 300 m a.s.l. and within 4–5 km of the coast. Distances were not standardised because vegetation conditions were sought that mirrored those typical of the sites. This also explains the occasional sampling above 300 m but below 500 m a.s.l. on two sub-Antarctic islands. Sample sites were comparable to the extent possible given the large latitudinal gradient over which they were collected. At each site visited, date, time, GPS coordinates, elevation and environmental conditions were recorded. A trowel, sterilized with ethanol, was used to remove large pebbles from the sampling area and to collect around 700 g of top-soil (0–10 cm depth) into sterile Whirlpak bags (Nasco, Fort Atkinson, US-WI; protocol after Magalhães et al. 2012). New ethanol-sterilized gloves were used at each sample collection site, and sampling equipment was also sterilized between each sampling. On return to the ship, the soil samples for each site were homogenized and immediately separated using the most sterile conditions possible (double nitrile gloves, ethanol-cleaned surfaces and tools) into Whirlpak bags for subsequent analyses. These samples were stored at −20°C until further processing.

Soil physicochemistry and macroclimate analysis

A total of 500 g of soil per sample was used for both DNA extractions and soil physicochemical analysis of 35 of the 40 samples collected during the ACE cruise (Supporting information). Seventeen physicochemical parameters and four climatic variables were measured. The details of how these parameters were measured and analyzed statistically are provided in the supplementary methods (Supporting information).

DNA extraction and sequencing

DNA was successfully extracted using the QIAGEN DNeasy PowerSoil kit and sent for amplicon 16S rRNA and ITS1 sequencing at MRDNA Labs (Clearwater, Texas, USA). In addition, 20 of the extracted DNA samples with the highest DNA concentrations were also sent to Admera Health Biopharma Services (USA) for Illumina Hiseq whole genome shotgun sequencing. Details of the extraction and sequencing protocols can be found in the supplementary methods (Supporting information).

Amplicon community composition analysis

The raw reads from the amplicon sequencing were quality-checked, trimmed (Supporting information), and taxonomically assigned using the QIIME2 pipeline (Bolyen et al. 2019). The details of this process are provided in the supplementary methods (Supporting information). All data analyses of the ASV datasets and statistical analyses were carried out in RStudio (ver. 4.0.3) (www.r-project.org). Before statistical analysis, the prokaryotic and fungal datasets were filtered to remove unassigned reads and reads assigned to mitochondria and chloroplasts, using the *subset_taxa* function from the 'Phyloseq' package (McMurdie and Holmes 2013).

Sample biodiversity was estimated using alpha-diversity metrics, using the Shannon index as a measure of biodiversity. Prior to estimating biodiversity, the prokaryotic and fungal ASV tables were rarefied to the depth of the sample with the lowest number of reads (59 180 reads for prokaryotes, 13 061 reads for fungi, after quality trimming). Biodiversity was subsequently calculated using the *estimate_richness* function from the 'Phyloseq' package. Significant differences in alpha-diversity between groups of islands were calculated using ANOVA (for normally distributed data) (Chambers et al. 1992) or the Kruskal–Wallis test (for non-normally distributed data) (McKnight and Najab 2010). Where significant ($p < 0.05$), pair-wise comparisons between groups were calculated using Tukey's HSD test (Tukey 1977) and the Wilcoxon Rank Sum test (Wilcoxon 1945) for normally and non-normally distributed data, respectively.

Differences in microbial community structure across the datasets were assessed by calculating the beta-diversity dissimilarity between samples. Prior to estimating beta-diversity, the prokaryotic and fungal rarefied ASV tables were $\log(x+1)$ transformed. Beta-diversity of samples was calculated using the Bray–Curtis dissimilarity metric (Lozupone et al. 2011) and visualized in a principal coordinates analysis (PCoA) plot (Jolliffe and Cadima 2016). ANOSIM (Clarke 1993) with 999 permutations was used to test for statistically significant ($p < 0.05$) differences in beta-diversity between island samples. To determine how the number of shared ASVs between samples varied as a function of distance, the zeta diversity decay index was calculated using the 'Zetadiv' package (Hui and McGeoch 2014). For this analysis, decimal GPS coordinates were converted into distance (in meters) from the geographic South Pole using the WGS84 Antarctic Polar Stereographic converter (www.pgc.umn.edu/apps/convert).

Differential abundances of prokaryotic and fungal taxa across samples were detected using the 'ANCOMBC' package (Lin and Peddada 2020) on the relative abundance transformed ASVs table. Samples were grouped into three clusters (sub- /Maritime/Continental Antarctic) following the widely used division of large-scale Antarctic biogeographic regions (Holdgate 1977, Terauds et al. 2012, Convey 2017), and ASVs were clustered at the genus taxonomic level before calculating differential abundance. Differential abundances were calculated using a significance threshold of $p < 0.05$, a zero_cut cutoff of 0.9, and the false discovery rate (FDR) p-adjustment method. Taxa significantly over-represented in a group were subsequently defined as biomarkers for that group.

The 'core' microbial community was defined as taxa at the level of genus that were present in 90% of samples (i.e. 32 or more samples). Abundances of genera belonging to the 'core' microbial community were expressed as relative abundances.

Identification of environmental drivers of soil microbial community structure

Distance-based redundancy analysis (db-RDA) (McArdle and Anderson 2001) was used to estimate the significant ($p < 0.05$)

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environmental variables driving community composition and structure (as expressed by Bray–Curtis distance matrices) across the Antarctic islands. Details of the method used to calculate the explanatory db-RDA model are presented in the supplementary methods (Supporting information). A generalized linear model (GLM) approach was also used to confirm that RDA analysis predictions were valid, via the 'mvabund' package in R (www.r-project.org, Wang et al. 2012). The *manyglm* function was used to fit the multivariate models with a negative binomial distribution. The *anova.manyglm* function was subsequently used to test the hypothesis that microbial communities were influenced by the abiotic drivers identified in the RDA analysis, with PIT-trap resampling of p-values using 1000 iterations. The explanatory power of geographic distances between islands was also assessed using the same approach. GPS coordinates were first converted to distances between samples using the principal component of neighborhood matrix (PCNM) script (Borcard and Legendre 2002) from the 'vegan' package (Oksanen et al. 2020). The resulting PCNM vectors, representing the distance relationships between samples, were tested for significance against the community Bray–Curtis dissimilarity distances using the ordistep method described in the supplementary methods. PCNM vectors with significant associations with community structure were subsequently used in the variation partitioning analysis to calculate the overall explanatory power of geographic distance between islands. Variation partition analysis was performed on the log-transformed ASV datasets and the standardized environmental variable dataset using the *varpart* function in the 'vegan' package. For this analysis, environmental variables were clustered into three groups according to source of the data: soil chemistry, geographic distance and climate. Only environmental variables that were modelled as significant in the db-RDA analysis were included in the groups of variables used for the variation partition analysis.

Metagenome assembly and functional annotation

The details of the methodology used for the metagenomic sequencing quality control, assembly, and functional annotation are presented in the supplementary methods (Supporting information). Predicted ORFs from the metagenomes were mapped to the KEGG pathway database to obtain lists of complete pathways per metagenome, which were subsequently concatenated to generate a presence/absence matrix of complete biological pathways across the different island soil samples.

The abundances of 155 key marker genes involved in important ecosystem processes such as the nitrogen and sulfur cycles, photoautotrophy and chemoautotrophy (Supporting information) across the metagenomes were calculated by mapping the hidden Markov models (HMM) for these genes against the trimmed reads. The HMM models were either downloaded from the Pfam database (Finn et al. 2014) or generated from gene alignments using the HmmBuild tool (Finn et al. 2011). For the mapping step, trimmed reads were first translated into six possible reading frames, and HMM models were screened against the resulting translated reads with HmmSearch (Finn et al. 2011), using an e-value threshold of 1×10^{-6}. The resulting gene abundances were normalized to reads per kilobase per million (RPKM). Subsequently, the average gene copy number for marker genes was calculated by dividing the normalized abundances of the marker genes by the mean of the normalized read count (in RPKM) of 14 universal single copy ribosomal genes (Supporting information). Linear discriminant analysis (LDA) effect size (LEfSe) (Segata et al. 2011) was performed with marker gene average copy number (with a pre-sample normalization of the sum of values to 1×10^6) to investigate the differential abundance of marker genes across the metagenomes, using the thresholds of $p < 0.05$ and LDA > 2. LEfSe was ran using the 'microbiomeMarker' package (Cao et al. 2022) in Rstudio. For adequate statistical representation, the metagenomes were first grouped according to Bray–Curtis dissimilarity between metagenomes, which were calculated using the normalized marker gene abundances with the 'Phyloseq' R package (www.r-project.org). The significance of the grouping was subsequently assessed using the ANOSIM test, as described for the amplicon datasets, which were summed to 1×10^6, using the thresholds of $p < 0.05$ and LDA > 2. The R package 'pheatmap' (www.r-project.org, Kolde 2019) was subsequently used to generate a heatmap of marker genes that were differently abundant between metagenome groups.

Results and discussion

Antarctic Island soils harbor distinct microbial communities

Considering their geographic isolation and distinct geological history, we hypothesized that the individual island soils would harbor distinct microbial communities, with a very limited degree of microbial interchange. The prokaryotic amplicon sequence data generated from the soil samples supported this hypothesis, with only a small number of 16S rRNA ASVs shared by all islands (two out of a total of 15 773, accounting for 3.9% mean relative abundance across samples). By comparison, 16 ITS ASVs (of 1880, representing 38.8% average relative abundance) were ubiquitous across islands, suggesting a higher degree of fungal dispersal between different island soils. The number of shared ASVs declined with increasing distance between samples for both prokaryotic and fungal ASVs (Supporting information), suggesting that spatial separation plays an important role in determining the extent of soil microbiome homogeneity between islands.

To further explore the differences in soil microbial composition between islands, the beta-diversity dissimilarity scores between samples was compared. The Bray–Curtis beta-diversity dissimilarity scores between samples (Fig. 2A–B) showed that the community structure of prokaryotic and fungal populations could be clearly distinguished ($R = 0.85$ for prokaryotes; $R = 0.69$ for fungi, both $p < 0.01$) according to which island they were sampled from. Using the previously

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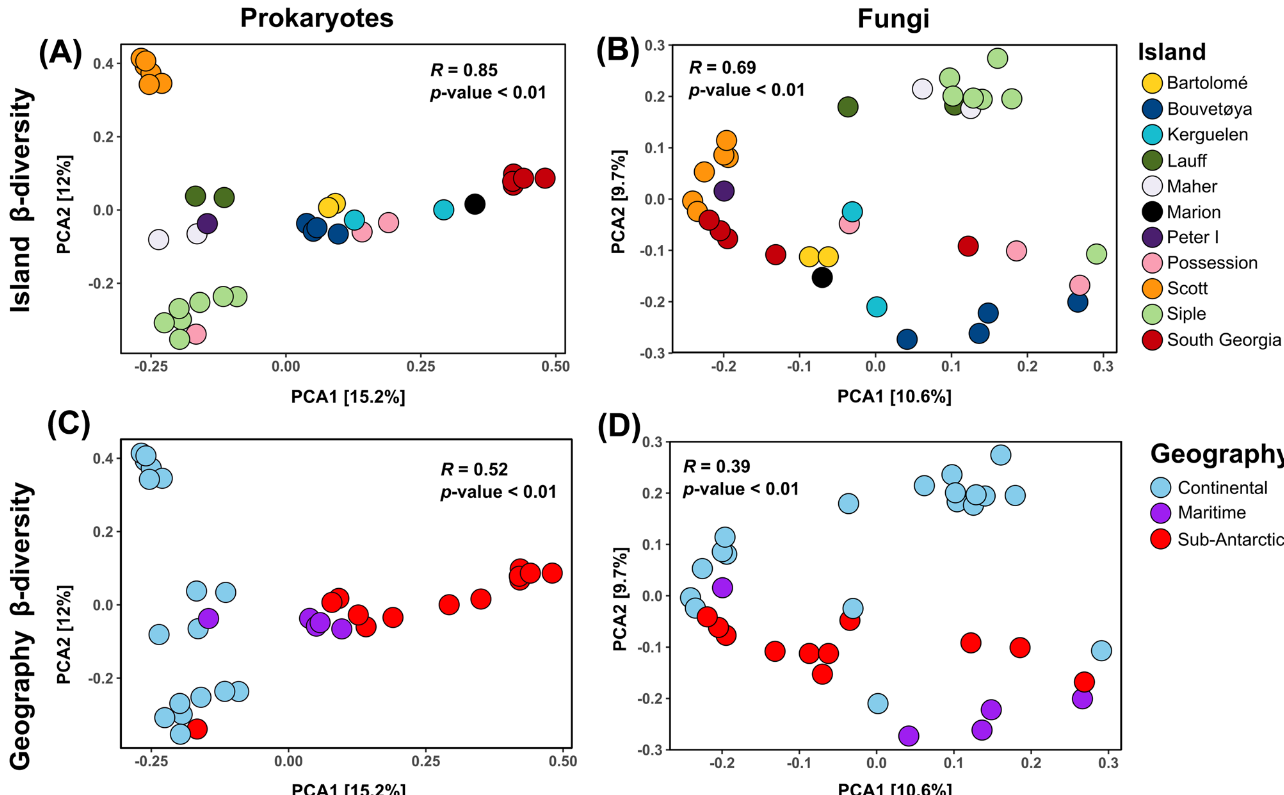

Figure 2. PCoA plot of the Bray–Curtis dissimilarity between samples according to source island, for prokaryotes (A) and fungi (B); and geographic regions (sub-, Maritime, Continental) for prokaryotes (C) and for fungi (D). For each of the sample groupings (Islands and Geographic Region), the coefficient of determination (R^2) and significance level (p) are shown in the plot.

established island groups, based on their geographical location (Continental/Maritime/Sub-Antarctic), microbial community structure was also significantly different ($R=0.52$ for prokaryotes; $R=0.39$ for fungi, both $p < 0.01$) between different island groups, with sub- and Maritime Antarctic islands showing a higher degree of similarity (Fig. 2C–D). These differences were also reflected in a significant decrease (adjusted $p=0.018$) in prokaryotic biodiversity (as measured using the Shannon Index) in Continental Antarctic islands when compared to sub-Antarctic islands (Supporting information). Considering the climatic distinction between these two groups of islands, with the sub-Antarctic being much milder and less seasonally variable (Holdgate 1977), the lower biodiversity in areas closer to the Antarctic continent is not unexpected. By comparison, there were no significant differences in fungal biodiversity across regions, suggesting that the observed climatic gradient across the Antarctic realm has negligible impact on fungal biodiversity. Together, these results suggest that the sub-, Maritime and Continental Antarctic regions, as well as the individual islands, have distinct microbial communities.

Geographic distance, climate and soil chemistry drive community dissimilarity between Antarctic islands

To further investigate the role of the micro- and macroenvironment in shaping the soil microbial communities of Antarctic islands, soil chemistry data, together with climatic parameters and geographic distance (expressed as PCMN values) were collected and used to model the possible drivers of microbial composition across samples. Similar to the results from beta-diversity analyses, both individual islands and island geographic clusters could be significantly ($p < 0.01$) distinguished according to their soil chemistry (Supporting information). db-RDA identified six variables (pH, CEC, Mg, NH_4, OM and mean annual temperature) that significantly explained 26% ($R^2=0.26$) of the variation in prokaryotic community composition across all samples (Fig. 3A). By comparison, 15% ($R^2=0.15$) of the fungal diversity could be explained by five variables (Ca, K, Density, P and mean annual temperature) (Fig. 3B). Temperature is a known driver of both prokaryotic and fungal community composition and diversity across the globe (Garcia-Pichel et al. 2013, Delgado-Baquerizo et al. 2018, Nottingham et al. 2018), and it is therefore not surprising that this climatic parameter is predicted to drive microbial communities across the Antarctic islands. Similarly, soil pH is an important driver of prokaryotic community structure in terrestrial soils (Lammel et al. 2018, Tripathi et al. 2018), and the results from this study suggest that soil pH (which by itself explained 9.5% ($R^2=0.095$) of the variation in community composition) might also be an important driver in shaping prokaryotic ecology across the different Antarctic islands.

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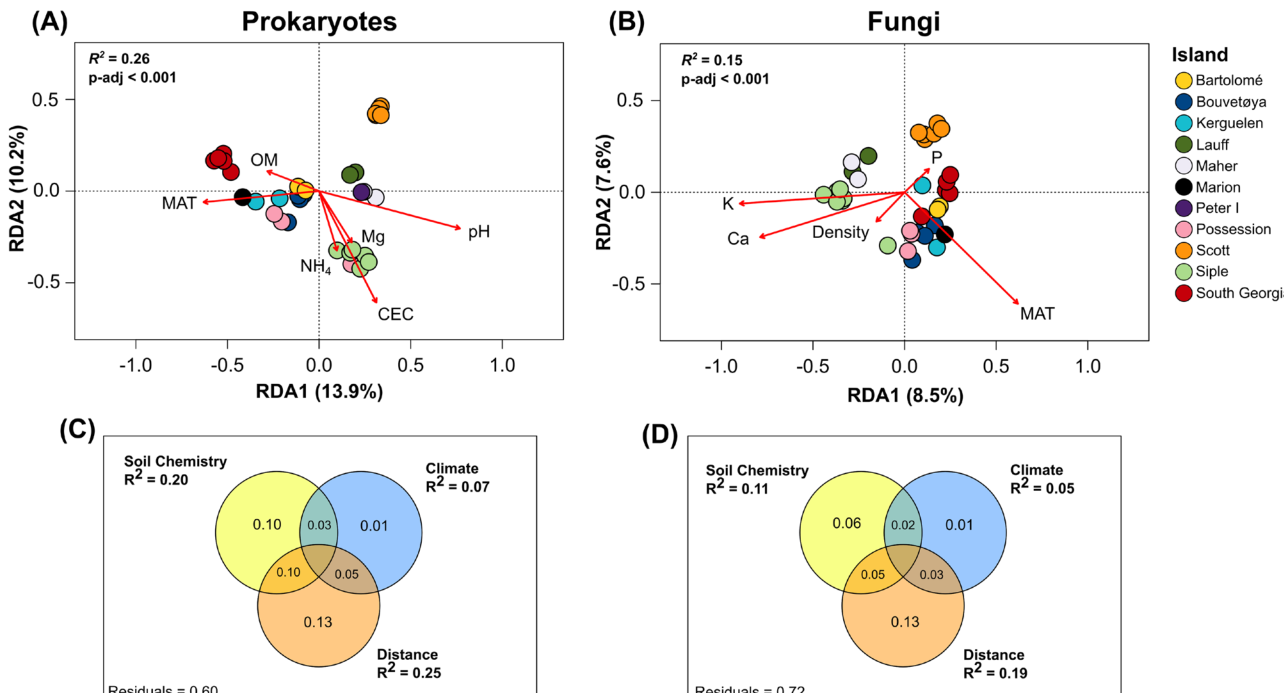

Figure 3. (A–B) Redundancy analysis (RDA) plots showing the effect of explanatory climatic and chemical variables on prokaryotic (A) and fungal communities (B). Samples on the plots are color-coded according to the islands they were sampled from. The degree and direction of effect for each explanatory variable is represented by arrows. Variables are expressed using the following abbreviations: MAT – mean annual temperature; OM – organic matter; K – potassium; Mg – magnesium; Ca – calcium; Density – soil bulk density; P – phosphate; CEC – cation-exchange capacity; NH_4 – ammonia. Both the separate coefficients of determination (R^2) for climatic and chemical variables as well as the cumulative effect are represented in the plots. (C–D) Variation partition Venn diagrams showing the percentage of community dissimilarity explained by soil chemistry (OM, K, Ca, Mg, P, soil bulk density, pH), geographic distance (PCNM) and climate (mean annual temperature) separately, for both prokaryotic (C) and fungal communities (D). Explained percentage is expressed as a fraction, in a scale from 0 to 1.

Surprisingly, pH was not a significant driver of fungal community structure, despite a recent study indicating that both pH and bulk soil density are major drivers of global fungal distribution (Větrovský et al. 2019). Instead, the significance of calcium as a predictor of fungal distribution in Antarctic island soils suggests that components of the fungal populations may be lichen-associated. Calcium is a major driver of lichen diversity (Bačkor et al. 2017) and calcium oxalate crystals are a common byproduct of lichen metabolism (Gadd et al. 2014). In turn, the association of potassium with fungal community structure might be due to the capacity of some fungal taxa to solubilize inorganic potassium, particularly in root-associated systems (Lian et al. 2008, Dominguez-Nuñez et al. 2016). The significance of these variables in driving community composition and structure across island soils was further confirmed via GLM analysis (Supporting information).

Geographic distance between islands (measured as PCNM) was also identified as a significant variable, accounting for 13% (R^2 = 0.13) of variation in fungal and prokaryotic community composition between islands (Fig. 3C–D). This variable explained the highest percentage of soil microbial variability across samples, further giving weight to the hypothesis that geographical distance between Antarctic islands might be a determining factor in differentiation of island communities by limiting microbial dispersal.

It is also necessary to consider the potential roles of other environmental drivers not considered in this study, as well as stochastic processes such as deposition of atmospheric bioaerosols (Barberán et al. 2014, Weil et al. 2017), ecological drift from random growth/death rates within the community (Zhou and Ning 2017), the relative age of terrain (Barrett 2007, Lyons et al. 2016), and other factors such as snow cover and snowmelt cycles (Sorensen et al. 2020).

The core island soil microbiome is dominated by lichen-associated taxa

The phylogenetic analysis of the soil microbial communities revealed a dominance of a relatively small number of phyla across the Antarctic islands. Specifically, a total of 12 prokaryotic phyla (of 48) were present in all samples and accounted for 99% of ASVs across samples (Fig. 4A, Supporting information). The most dominant of these was Proteobacteria (29.0% average relative abundance), followed by Actinobacteriota (13.2%) and Bacteroidota (13.1%). Both Cyanobacteria and Chloroflexi, which are commonly associated with cryptic communities in extreme soil ecosystems

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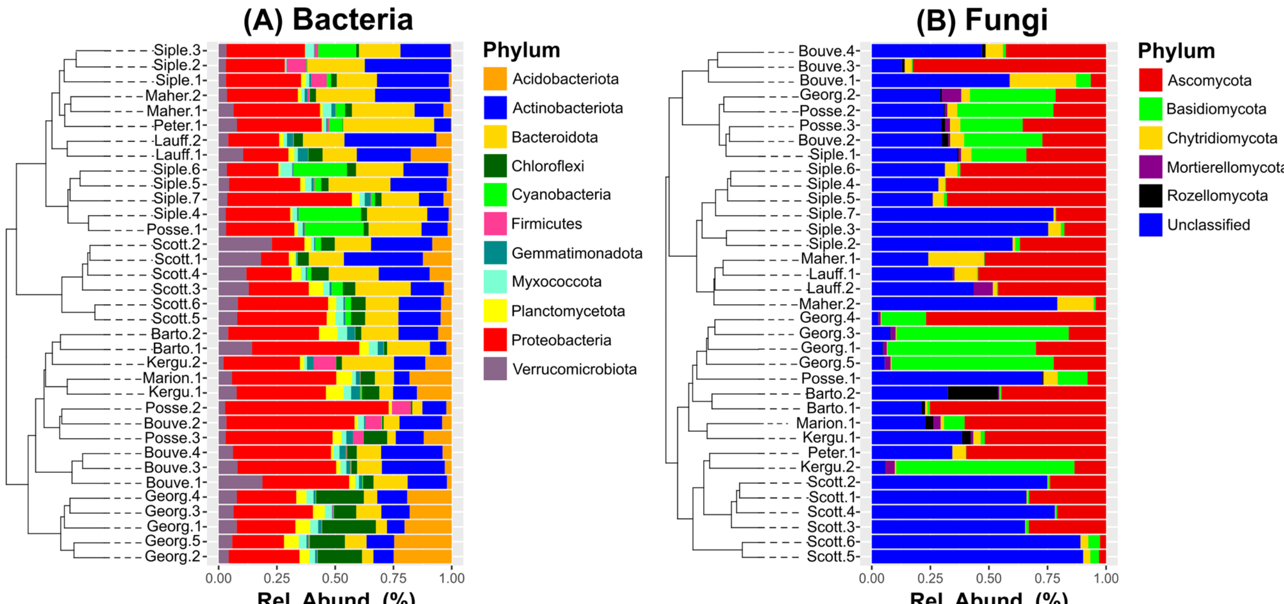

Figure 4. Relative abundance of the dominant (representing 99%) prokaryotic (A) and fungal (B) phyla across samples, which were clustered according to their Bray–Curtis dissimilarity. Relative abundance is expressed as the fraction of total ASVs per samples. Samples are colored according to the islands of origin.

(Lacap et al. 2011, Chan et al. 2012, Magalhães et al. 2012), were found across all samples at mean relative abundances of 5%. Another potentially important phylum present in soils of nine of the islands (with the exception of Bartolomé and Peter I Islands) and ranging between 0.005 and 2.4% relative abundance was WPS-2, which has been recently re-classified as Candidatus Eremiobacterota (Ji et al. 2021). Bacteria belonging to this phylum have been associated with the capability for trace gas scavenging (Ji et al. 2017, Ray et al. 2020), which has been proposed as essential energy production pathway for the survival of microbial communities in extreme biomes (Leung et al. 2020). By comparison, six of the 11 fungal phyla identified in the dataset represented 99% of fungal ASVs (Fig. 4B, Supporting information), with the most abundant known Phylum being Ascomycota (36.5% average relative abundance) followed by Basidiomycota (14.5%) and Chytridiomycota (4.5%). ASVs of unknown taxonomic assignment represented the first and fourth most abundant fractions of fungal and prokaryotic phyla, respectively, highlighting the potential importance of Antarctic Islands as a source of currently undescribed microbial diversity.

Analysis of island soil microbial communities at the genus level also revealed a 'core' microbial population that represented a significant portion of the total microbial communities. For the purposes of this study, the 'core' population was defined as genera that were present in at least 90% of the samples (32 samples or above), and therefore considered to be 'generalist' taxa capable of adapting to the broad range of soil biomes across the sub-, Maritime and Continental Antarctic regions. This 'core' community was composed of 40 bacterial and 18 fungal genera, representing between 9and 68% of the total prokaryote and 6 and 89% of total fungal ASV counts across sampled sites (Fig. 5). In the case of the samples obtained from Scott Island, the low percentage of the overall community represented by the fungal 'core' population can be attributed to the high incidence of unknown fungal taxa (between 60 and 90% of the total fungal community) in these samples, which were not considered in this analysis.

Several of the genera identified in the 'core' community have been previously associated with lichens and mosses. These include the N-fixing cyanobacterial family Phormidiaceae (Bergman et al. 1997), which is associated with moss-covered biocrusts (Maier et al. 2018), as well as the lichen-associated bacterial genera *Chthoniobacter* (Aschenbrenner et al. 2017) and *Nocardioides* (Parrot et al. 2015). Almost half of the 'core' fungal genera were identified as lichenized fungi, including *Cladonia*, *Stereocaulon* (Park et al. 2015, 2018), *Astroplaca* (Arup et al. 2013) and *Mastodia* (Garrido-Benavent et al. 2017). In many of the samples, these constituted the dominant taxa in the 'core' fungal community (Fig. 5). Both the diversity and abundance of lichen-associated taxa in soil 'core' communities of Antarctic islands suggest that lichens may be the common structural and biological platform for the Antarctic island soil microbiome. This would be in accordance with the extensive documentation of lichens across the region (Øvstedal and Smith 2001, Øvstedal and Gremmen 2006, Phillips et al. 2022).

Specialist taxa are differentially abundant across sub-, Maritime and Continental Antarctic islands

Based on our analysis of the microbial composition in the different island soils, the traditionally classified sub-, Maritime and Continental Antarctic islands exhibit distinct

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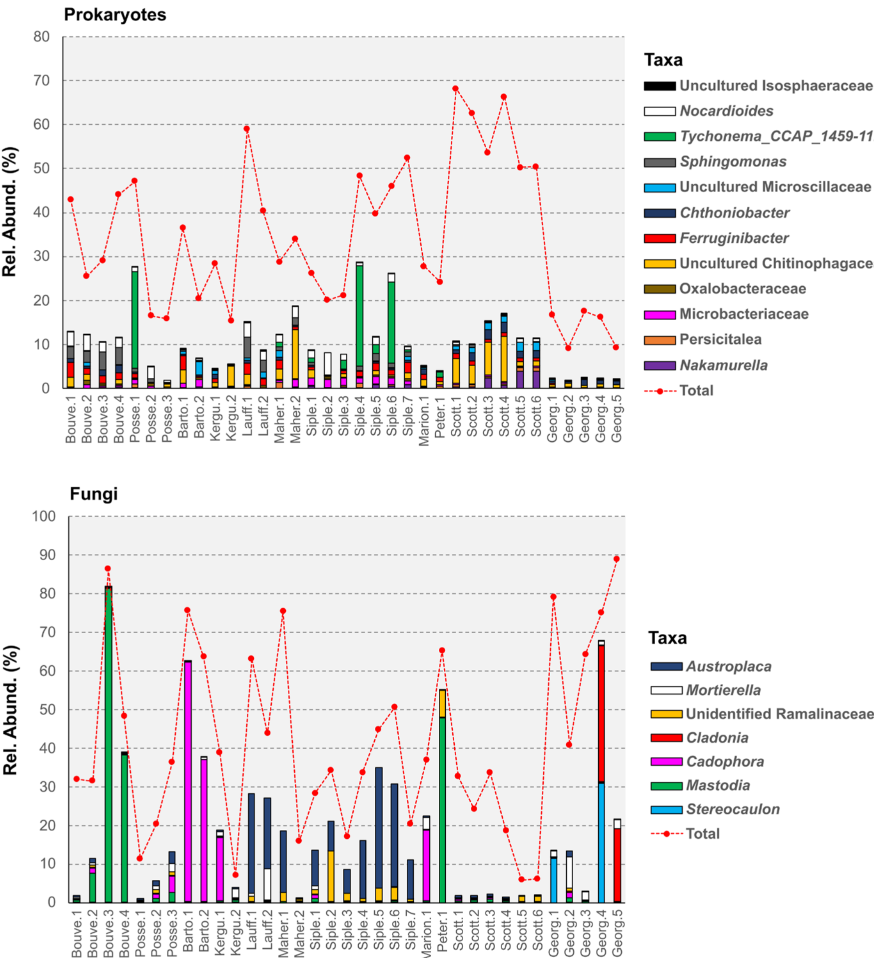

Figure 5. Relative abundance of the 'core' microbial community (red dotted line) across samples, with relative abundances of lichen-associated prokaryotic and fungal genera (barplots) identified in the 'core' community. In both cases, relative abundances are expressed as fractions of the total absolute abundances (read counts) of prokaryotic and fungal ASVs in each sample.

soil microbial communities. To further explore the basis for this division, ANCOMBC discriminatory analysis was performed on the three island groups in order to identify taxa that were differentially represented. This analysis identified 288 prokaryotic and 17 fungal taxa, at the genus level, that were differentially abundant between the three biogeographic regions. For prokaryotes, sub-Antarctic islands exhibited the highest number of over-represented taxa (Supporting information), which is consistent with the sub-Antarctic islands exhibiting significantly higher microbial biodiversity than those of the Maritime and Continental Antarctic. By comparison, both sub- and Continental Antarctic island groups exhibited the same number of over-represented fungal taxa (seven genera).

Several taxa that were differentially abundant between the regions potentially play key roles in the functional capacity of the soil microbial communities (Fig. 6). Bacteria over-represented in Continental Antarctic island soils included the cyanobacterial genus *Phormidesmis*, previously reported in stromatolites (Waterworth et al. 2020), as well as the Chloroflexi genus *Herpetosiphon*, which has been characterized as phototrophic bacteria commonly found in extreme environments (Ward et al. 2018). Similarly, several of the fungal taxa over-represented in the Continental Antarctic islands, including *Glaciozyma* and *Austroplaca*, have been previously characterized as psychrophiles isolated from extreme cold biomes, including Continental Antarctica (Søchting et al. 2014, Firdaus-Raih et al. 2018). As noted above, *Austroplaca* is also a lichenized fungus that constituted a significant proportion of the 'core' fungal community in samples from Siple Island.

By comparison, many of the biomarkers over-represented in sub-Antarctic islands were identified as acidophilic microorganisms or associated with acidic environments, which is consistent with the lower pH ranges in these island soils. This included the bacterial genus *Acidibacter* and the fungal order Helotiales, members of which have been isolated from acidic biomes (Zijlstra et al. 2005, Falagán and Johnson 2014). A potentially important taxon over-represented in the sub-Antarctic region was an uncultured bacterium from the Chloroflexi family Ktedonobacteraceae, which has been recently shown to have the ability to persist on atmospheric

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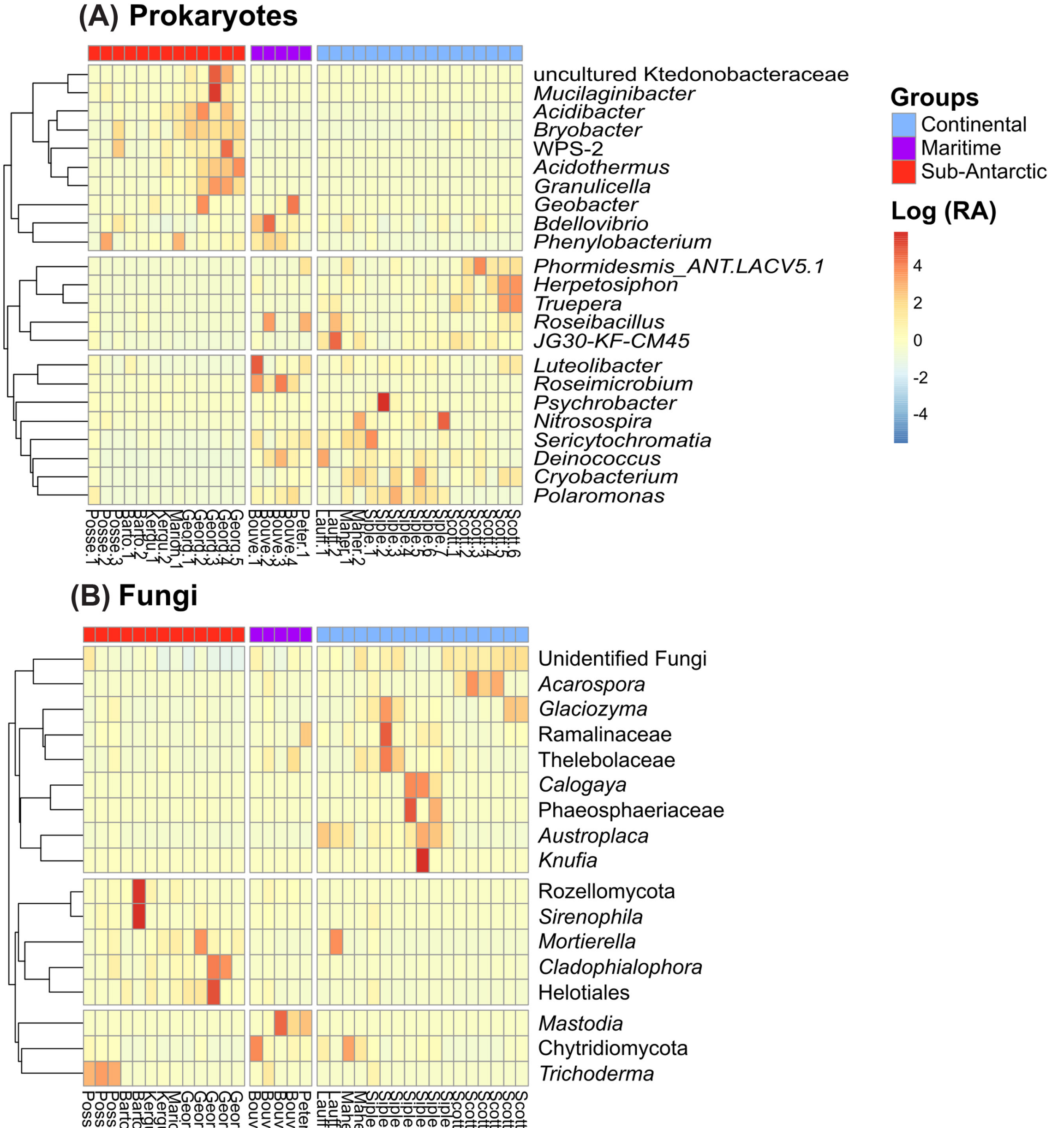

Figure 6. Heatmaps showing the relative abundance of selected prokaryotic (A) and fungal (B) biomarkers across samples, at the genus level, detected from the ANCOMBC discriminatory analysis between sub-, Maritime and Continental Antarctic biogeographical regions. Relative abundances were scaled and centered for each genus-level ASV to account for large differences in relative abundance values between taxa. Rows were clustered according to the calculated Euclidian distance between taxa, based on their abundance across samples. The intensity of the heatmaps corresponds to the log-scaling of each biomarker across sampled sites. Samples were grouped according to the biogeographical region they originated from, as indicated by the bar at the top of each figure showing the sub-Antarctic (in red), Maritime (in purple) and Continental (in blue) Antarctic clusters. Sample names are colored according to their island of origin.

hydrogen and carbon monoxide (Islam et al. 2019), and therefore could play an important role in carbon and energy acquisition in the community by atmospheric chemotrophy (Ji et al. 2017, Montgomery et al. 2021). It is also notable that an unidentified genus from the Phylum WPS-2 was also over-represented in sub-Antarctic islands; this taxon is also a potential candidate for trace gas scavenging autotrophy. Sub-Antarctic islands were also enriched in rhizosphere-associated taxa, including the genus *Bryobacter* (Gao et al. 2019), as well as the family Helotiaceae, many members of which have been characterized as mycorrhizal fungi (Walker et al. 2011).

Over-represented taxa on the Maritime Antarctic islands included psychrotolerant bacterial genera, such as *Polaromonas* (Darcy et al. 2011) and *Deinococcus* (Slade and Radman 2011), as well as genera that are commonly found in more temperate environments, such as the root-associated

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Roseimicrobium (Podar et al. 2020) and *Bdellovibrio*. The latter includes many predatory taxa that have been investigated as possible biocontrol agents (Bratanis et al. 2020). The lichen-forming genus *Mastodia* was over-represented in these island soils, which is consistent with previous observations of this lichenized fungus in Bouvetøya (Engelskjøn 1987) and more widely in the Maritime Antarctic.

The observed differences in abundance of specialist taxa are consistent with the soil chemistries and climatic profiles recorded for the islands, with Continental Antarctic islands representing harsher biomes that might be expected to select for taxa that are more commonly found in extreme environments, while the more acidic soils of sub-Antarctic islands select for acidotolerant, fast-growing taxa. These results therefore strongly suggest that, in addition to geographical distance, the distinct soil chemistry and climate profiles of the geographically-defined Antarctic regions promote within-region endemism and further verify the importance of these filters for structuring terrestrial microbial diversity both in the region and elsewhere (Lee et al. 2012, Bay et al. 2020).

Sub-, Maritime and Continental Antarctic Island soil microbiomes show functional specialization

To investigate the functional potential of the Antarctic island soil microbial communities, 20 soil metagenomes covering all the sampled islands were generated from the whole shotgun metagenomics sequencing dataset (Supporting information) and screened for complete biochemical pathways using the KEGG annotation and pathway finder pipeline (Table 1). The resulting matrix showed a high level of biochemical pathway redundancy in all Antarctic island soils. For example, all soil metagenomes exhibited the functional potential to perform the central carbon metabolic processes, including the TCA cycle (M00009) and the reductive pentose phosphate (RPP) pathway (M00166). Similarly, all soil metagenomes had complete pathways for assimilatory and dissimilatory nitrate reduction (M00346/M00530), denitrification (M00529), as well as the protein complexes required for oxidative phosphorylation. Additionally, genes required for complete methane oxidation (M00174) and nitrification (M00528) were detected in all but one of the sampled islands (Scott Island samples being the exception).

However, the results from the KEGG analysis also suggested that Antarctic island soil metagenomes differed in the abundance of specialized pathways involved in stress response and energy acquisition. To assess these differences more accurately, important biological cycles were profiled across the soil metagenomes by calculating the abundance of 155 key marker genes belonging to these cycles (Supporting information). The most abundant marker genes across the dataset were for catalase and the cytochrome C oxidase subunit 1 (COX1), followed by the gene encoding trehalose synthase. That two of the most abundant gene markers encoded proteins known to be involved in thermal shock and oxidative stress responses (Alfonso-Prieto et al. 2009, De Maayer et al. 2014) is suggestive of common abiotic stress challenges to microbial communities in Antarctic island soils.

Based on distribution of marker genes across the sequenced samples, soil metagenomes significantly ($p < 0.01$) clustered into the three regional island groups (with the exception of one metagenome from Marion Island and one from Bouvetøya) defined in this study ($R = 0.53$, $p < 0.01$) (Supporting information), which correlated well with the clear biotic and abiotic differentiation between these regions. Of the 155 marker genes, 90 were differentially represented between the three groups (Fig. 7). Soil metagenomes from islands in the sub-Antarctic region were enriched in genes for glycoside hydrolases capable of digesting complex plant polymers, such as endo-xylanases (Glyco_hydro_11) and α-l-arabinofuranosidases (Glyco_hydro_62). This is consistent with the over-representation of rhizosphere-associated and cellulolytic microbial taxa in samples from these islands. Sub-Antarctic islands were also enriched in genes involved in atmospheric gas sequestration and oxidation. These included genes for CoxL and CooS (carbon monoxide dehydrogenases), both of which are involved in carbon monoxide sequestration in aerobic and anaerobic microorganisms (Jeoung et al. 2019), as well as genes encoding several subfamilies of the [Ni-Fe]- and [Fe-Fe]-hydrogenases, which are involved in hydrogen (H_2) oxidation and evolution, respectively (Greening et al. 2016). An exception was observed for sub-family 1h [Ni-Fe] and 1l [Ni-Fe] assimilatory hydrogenase genes, which were over-represented in Lauff and Scott island soils (Continental Antarctic). These two sub-families have been recently characterized as high affinity assimilatory hydrogenases that contribute to microbial energy acquisition in extremely cold oligotrophic soils (Ji et al. 2017, Leung et al. 2020, Ortiz et al. 2021). However, it is also important to note that these two hydrogenase gene families were present in all metagenomes, suggesting that the capability to scavenge H_2 for energy production is a widespread trait, and is not exclusive to soil microbial communities in extreme polar environments (Ji et al. 2017, Bay et al. 2021).

By comparison, soil metagenomes from Maritime and Continental Antarctic islands were enriched in phototrophic gene markers, specifically for several classes of rhodopsins and for photosystems I (PsaA) and II (PsbA), respectively. Many rhodopsins act as light-activated ion-channels that drive energy production (Leung et al. 2020). A total of 40 different rhodopsin classes were found across all metagenomes, 16 of which were over-represented in Maritime Antarctic islands. Over-represented families include the NQ-like rhodopsin cluster, which is associated with microbial communities in environments with high salt contents (Kwon et al. 2013) and has been previously identified in hypolith communities from Antarctic Dry Valley soils (Guerrero et al. 2017).

Genes coding for the orange carotenoid protein, which is involved in photo-protection in phototrophic microorganisms (Kirilovsky and Kerfeld 2013), were also over-represented in Continental Antarctic islands. The enrichment of photo-receptive and photo-protective genes in soil metagenomes from these islands is consistent with the

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Table 1. Presence-absence matrix showing complete biological pathways across the different island soil samples, based on the KEGG pathway module database.

		Lauff	Maher	Siple	Scott	Peter I	Keguerlen	Bartolome	Marion	Possession	Bouvet	South Georgia
Oxidative phosphorylation												
M00144	NADH:quinone oxidoreductase, prokaryotes (15) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00145	NAD(P)H:quinone oxidoreductase, chloroplasts and cyanobacteria (11) (complete)	0	1	1	1	1	0	1	1	0	1	1
M00149	Succinate dehydrogenase, prokaryotes (4) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00148	Succinate dehydrogenase (ubiquinone) (4) (complete)	0	0	1	1	0	1	0	0	0	1	0
M00151	Cytochrome bc1 complex respiratory unit (10) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00152	Cytochrome bc1 complex (9) (complete)	0	0	0	0	1	1	0	0	0	0	0
M00162	Cytochrome b6f complex (7) (complete)	0	0	0	1	1	0	0	0	0	0	0
M00155	Cytochrome c oxidase, prokaryotes (5) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00153	Cytochrome bd ubiquinol oxidase (3) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00417	Cytochrome o ubiquinol oxidase (4) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00156	Cytochrome c oxidase, cbb3-type (5) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00157	F-type ATPase, prokaryotes and chloroplasts (8) (complete)	1	1	1	1	1	1	1	1	1	1	1
Carbon fixation												
M00009	Citrate cycle (TCA cycle, Krebs cycle) (34) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00165	Reductive pentose phosphate cycle (Calvin cycle) (16) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00166	Reductive pentose phosphate cycle, ribulose-5P => glyceraldehyde-3P (7) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00167	Reductive pentose phosphate cycle, glyceraldehyde-3P => ribulose-5P (9) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00170	C4-dicarboxylic acid cycle, phosphoenolpyruvate carboxykinase type (4) (complete)	1	0	1	1	1	0	0	0	0	1	0
M00172	C4-dicarboxylic acid cycle, NADP - malic enzyme type (4) (complete)	0	0	1	0	1	0	0	1	0	0	0
M00171	C4-dicarboxylic acid cycle, NAD - malic enzyme type (9) (complete)	0	0	1	1	1	0	0	0	0	0	0
M00173	Reductive citrate cycle (Arnon-Buchanan cycle) (34) (complete)	0	0	1	0	0	0	0	0	0	1	1
M00377	Reductive acetyl-CoA pathway (Wood-Ljungdahl pathway) (10) (complete)	0	0	0	0	0	0	0	0	0	0	1
M00579	Phosphate acetyltransferase-acetate kinase pathway, acetyl-CoA => acetate (4) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00620	Incomplete reductive citrate cycle, acetyl-CoA => oxoglutarate (17) (complete)	0	0	0	0	0	0	0	1	0	0	0

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Table 1. Continued.

		Lauff	Maher	Siple	Scott	Peter I	Keguerlen	Bartolome	Marion	Possession	Bouvet	South Georgia
Methane metabolism												
M00358	Coenzyme M biosynthesis (5) (complete)	0	0	1	0	0	0	0	1	1	0	0
M00174	Methane oxidation, methanotroph, methane => formaldehyde (4) (complete)	1	1	1	0	1	1	1	1	1	1	1
M00346	Formaldehyde assimilation, serine pathway (10) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00345	Formaldehyde assimilation, ribulose monophosphate pathway (5) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00378	F420 biosynthesis (6) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00935	Methanofuran biosynthesis (6) (complete)	0	0	0	0	0	0	1	0	0	0	0
Nitrogen metabolism												
M00175	Nitrogen fixation, nitrogen => ammonia (4) (complete)	0	0	0	1	1	1	1	1	0	1	1
M00531	Assimilatory nitrate reduction, nitrate => ammonia (4) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00530	Dissimilatory nitrate reduction, nitrate => ammonia (9) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00529	Denitrification, nitrate => nitrogen (10) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00528	Nitrification, ammonia => nitrite (4) (complete)	1	1	1	0	1	1	1	1	1	1	1
M00804	Complete nitrification, comammox, ammonia => nitrite => nitrate (6) (complete)	1	1	1	0	1	1	1	1	1	1	1
Sulfur metabolism												
M00176	Assimilatory sulfate reduction, sulfate => H2S (10) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00596	Dissimilatory sulfate reduction, sulfate => H2S (5) (complete)	0	1	0	0	0	1	1	1	1	1	1
M00595	Thiosulfate oxidation by SOX complex, thiosulfate => sulfate (7) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00616	Sulfate-sulfur assimilation (4) (complete)	1	1	1	1	1	1	1	1	1	1	1
Photosynthesis												
M00163	Photosystem I (5) (complete)	0	1	1	1	1	0	0	0	1	1	0
M00161	Photosystem II (5) (complete)	1	1	1	1	1	0	0	0	1	1	1
M00597	Anoxygenic photosystem II (2) (complete)	1	1	1	1	1	1	1	1	0	1	1
M00611	Oxygenic photosynthesis in plants and cyanobacteria (0) (complete)	1	1	1	1	1	0	0	0	1	1	1
M00612	Anoxygenic photosynthesis in purple bacteria (0) (complete)	1	1	1	1	1	1	1	1	0	1	1
Urea cycle												
M00029	Urea cycle (5) (complete)	1	1	1	0	1	1	1	0	0	1	1
Xenobiotics degradation												
M00551	Benzoate degradation, benzoate => catechol / methylbenzoate => methylcatechol (4) (complete)	0	0	1	1	0	0	1	1	0	0	1
M00568	Catechol ortho-cleavage, catechol => 3-oxoadipate (5) (complete)	1	1	1	1	0	1	1	1	1	1	1
M00569	Catechol meta-cleavage, catechol => acetyl-CoA / 4-methylcatechol => propanoyl-CoA (11) (complete)	1	1	1	1	1	1	1	1	1	1	1

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Table 1. Continued.

		Lauff	Maher	Siple	Scott	Peter I	Keguerlen	Bartolome	Marion	Possession	Bouvet	South Georgia
M00638	Salicylate degradation, salicylate => gentisate (4) (complete)	1	0	1	0	0	1	1	1	1	0	1
M00623	Phthalate degradation, phthalate => protocatechuate (4) (complete)	1	0	1	1	1	1	1	1	1	1	1
M00878	Phenylacetate degradation, phenylaxetate => acetyl-CoA/succinyl-CoA (11) (complete)	0	0	0	0	0	0	0	0	0	0	1
M00545	Trans-cinnamate degradation, trans-cinnamate => acetyl-CoA (9) (complete)	1	1	1	1	0	1	1	1	1	1	1
Drug resistance												
M00627	beta-Lactam resistance, Bla system (3) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00642	Multidrug resistance, efflux pump MexJK-OprM (4) (complete)	1	1	1	1	1	1	1	1	1	1	1
M00698	Multidrug resistance, efflux pump BpeEF-OprC (4) (complete)	1	0	1	1	0	1	1	1	0	1	1
M00641	Multidrug resistance, efflux pump MexEF-OprN (4) (complete)	0	1	1	1	0	0	0	1	0	0	0
M00649	Multidrug resistance, efflux pump AdeABC (4) (complete)	0	0	1	0	0	0	0	0	0	0	0

observed over-representation of phototrophic organisms such as Cyanobacteria and Chloroflexi in this sample set and supports the consensus that phototrophy is an important strategy for energy production in soil microbiomes from these islands.

Genes encoding ribulose-1,5-bisphosphate carboxylases/oxygenases (RuBisCo), the key enzymes in the fixation of carbon dioxide in the Calvin–Benson–Basham cycle of phototrophic and chemoautotrophic microorganisms (Ellis 1979, Raven 2013), were also enriched in soil metagenomes from Continental Antarctic islands. This observation, particularly the over-representation of genes for type IE RuBisCo, further highlights the importance of photoautotrophy in these soil microbiomes. The type 1E high affinity carboxylase has previously been identified in microbial communities from Antarctic soils (Ji et al. 2017), and has been shown to fix carbon dioxide using hydrogenase-driven H_2 assimilation as an energy source (Grostern and Alvarez-Cohen 2013). More recently, Ortiz et al. (2021) have suggested that RuBisCo type IE proteins are used in conjunction with [Ni-Fe]-hydrogenases to couple H_2 and carbon monoxide (CO) fixation, and their over-representation in metagenomes from Continental Antarctic islands suggests that these soil microbial communities may be heavily dependent on RuBisCo-mediated rare gas scavenging for carbon acquisition.

Concluding remarks

Taken together, the results from this study suggest that Antarctic island soil microbial communities are distinct both taxonomically and functionally, shaped by the spatial isolation of the islands and their local environments. We consistently identified evidence for the importance of the environment as a driver of community specialization, both in terms of adaptation to abiotic stresses and adaptation to the trophic relationships in the environment.

The findings from this study provide further support for the distinct biodiversity characteristics of the sub-, Maritime and Continental Antarctic regions. In particular, our data support individual sub-Antarctic islands forming distinct biogeographical regions in a conservation context (cf. the area formulation developed by Terauds et al. (2012) and Terauds and Lee (2016) for the area of Antarctic Treaty governance). We also argue that the differences in climate and biomes between these regions form natural barriers against the dispersal of microbial communities between islands, barriers that are being progressively eroded through anthropogenic activity (Pertierra et al. 2017, Hughes et al. 2019, Baird et al. 2020).

We acknowledge that the sampling regimes of the Antarctic islands are far from comprehensive. For some of the islands, constraints imposed by local climatic conditions and complex logistics allowed very limited shore-time in the current study. While the samples acquired are clearly not fully representative of the islands' edaphic diversity, the full sample set nevertheless provides a unique basis for comparative

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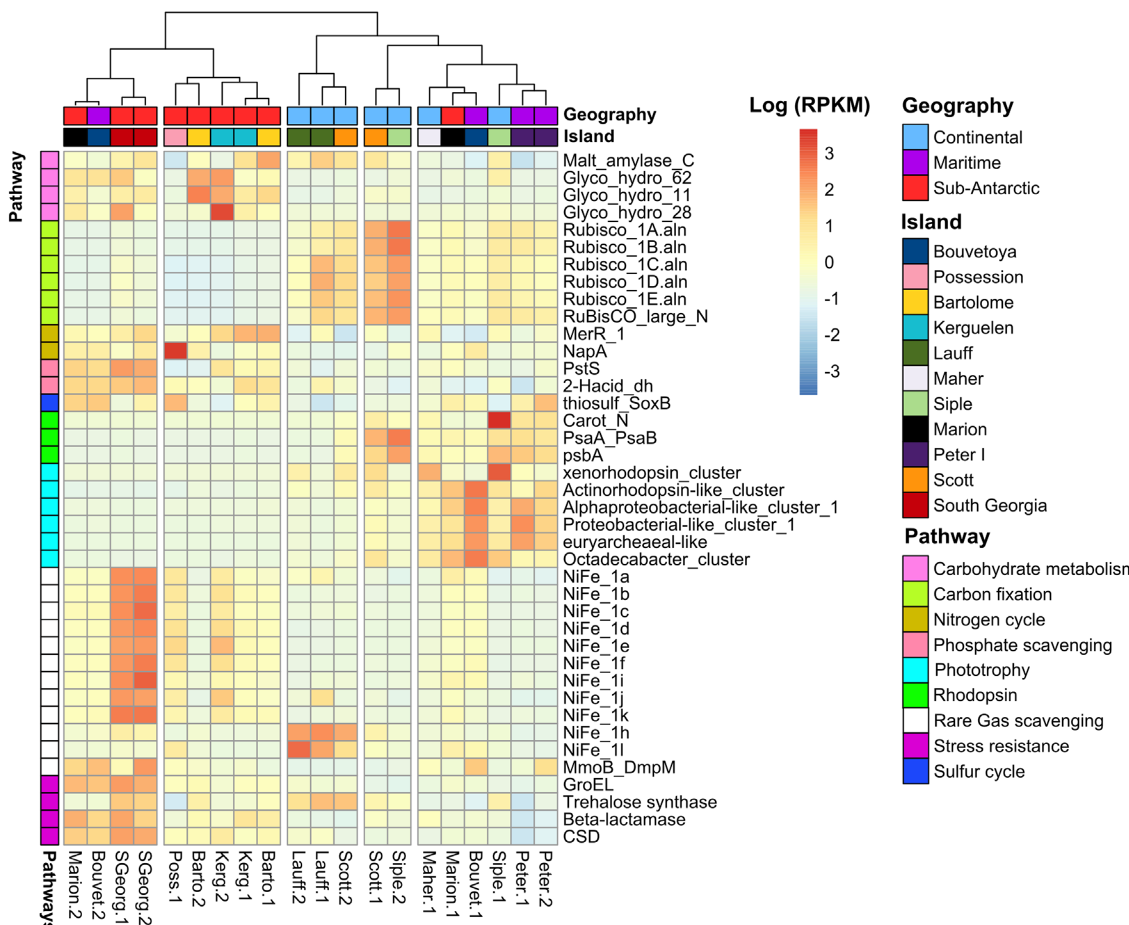

Figure 7. Heatmap of core gene markers that were differentially represented between island soil metagenomes. Gene markers are grouped into clusters according to the biological pathways they take part in or the class of genes they belong to. Metagenomes were clustered according to the Bray–Curtis dissimilarity distance between functional profiles of the 155 marker genes used in this study. Metagenomes are labelled according to sampled island from which they were sequenced, as well as the biogeographical region to which these belong. The intensity of the heatmaps is represented as the log scale of average gene copy number of each gene marker across the metagenomes.

analyses of microbial diversity and functional capacity. As the first extensive survey into the microbial ecology of Antarctic islands, this study provides an important milestone in our understanding of these environments. Additionally, this study provides a compelling justification for the expansion of the rationale of the existing ACBRs to regions north of 60° latitude, to include the sub-Antarctic islands surrounding the Antarctic continent and the Maritime Antarctic South Sandwich Islands and Bouvetøya, and will form a knowledge base for future conservation efforts in these regions.

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Author contributions

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Data availability statement

All sequence data used in this study (16S and ITS amplicon and short whole-shotgun sequences) are available on the NCBI sequence repository database under the Bioproject PRJNA868884 (www.ncbi.nlm.nih.gov/bioproject/PRJNA868884). All scripts used in this study are available from the Zenodo Digital Repository: https://doi.org/10.5281/zenodo.7997130 (Lebre 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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