Geology and elevation shape bacterial assembly in Antarctic endolithic communities

Stefano Larsen [a,*], Claudia Coleine [b], Davide Albanese [a], James C. Stegen [c], Laura Selbmann [b,d], Claudio Donati [a]

[a] Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38098 San Michele all'Adige, Italy
[b] Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy
[c] Pacific Northwest National Laboratory, Richland, WA, USA
[d] Mycological Section, Italian Antarctic National Museum (MNA), Genoa, Italy

HIGHLIGHTS

- Ice-free areas of continental Antarctica support rich bacterial communities.
- Elevation and geology drive the spatial turnover of taxa.
- The influence of variable selection increased with elevation differences.
- Deterministic processes select bacterial assemblages at the limits of life on Earth.

GRAPHICAL ABSTRACT
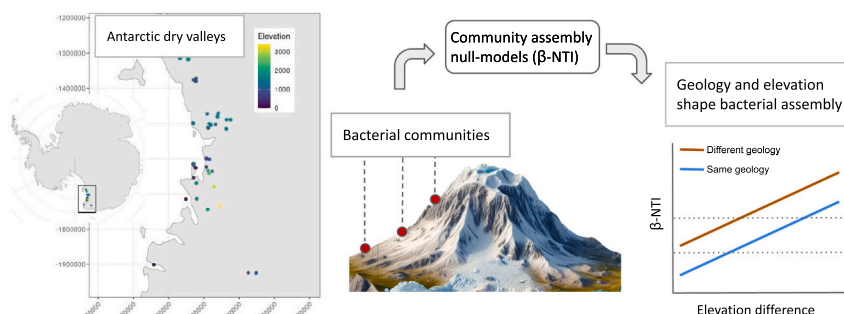
ARTICLE INFO

ABSTRACT

Ice free areas of continental Antarctica are among the coldest and driest environments on Earth, and yet, they support surprisingly diverse and highly adapted microbial communities. Endolithic growth is one of the key adaptations to such extreme environments and often represents the dominant life-form. Despite growing scientific interest, little is known of the mechanisms that influence the assembly of endolithic microbiomes across these harsh environments. Here, we used metagenomics to examine the diversity and assembly of endolithic bacterial communities across Antarctica within different rock types and over a large elevation range. While granite supported richer and more heterogeneous communities than sandstone, elevation had no apparent effect on taxonomic richness, regardless of rock type. Conversely, elevation was clearly associated with turnover in community composition, with the deterministic process of variable selection driving microbial assembly along the elevation gradient. The turnover associated with elevation was modulated by geology, whereby for a given elevation difference, turnover was consistently larger between communities inhabiting different rock types. Overall, selection imposed by elevation and geology appeared stronger than turnover related to other spatially-structured environmental drivers.

Our findings indicate that at the cold-arid limit of life on Earth, geology and elevation are key determinants of endolithic bacterial heterogeneity. This also suggests that warming temperatures may threaten the persistence of such extreme-adapted organisms.

1. Introduction

Continental ice-free areas of Antarctica are among the most inhospitable environments for life on Earth (Convey and Peck, 2019; Leung et al., 2020). The McMurdo Dry Valleys, in particular, are hyper-arid deserts and the only place on Earth where dry permafrost is known to occur (Heldmann et al., 2013). These conditions, coupled with the extremely low nutrient and water bioavailability, render this area a unique Martian analogue on our planet (Cockell et al., 2016; Fairén et al., 2010; Tamppari et al., 2012).

Biological communities inhabiting these regions necessarily reflect these extremely harsh conditions, both in terms of structure and life-history adaptation. Complex life-forms are absent and biomass is almost completely composed of microorganisms. Microbial diversity can be surprisingly high even across arid mineral soils with no apparent source of water or nutrients (Cary et al., 2010); rocks represent the main substratum for life in the scarce ice-free areas supporting the highest permanent biomass of microorganisms, including bacteria, cyanobacteria and fungi (Archer et al., 2017; Cary et al., 2010; Coleine et al., 2021; Friedmann, 1982).

Endolithic growth is one of the most critical adaptations to these harsh environments (Friedmann, 1982), offering relatively buffered conditions that allow organisms to persist across the dry limits for life (Cary et al., 2010). Antarctic endoliths constitute isolated ecosystems and probably represent the simplest, self-sustaining natural communities which do not support higher trophic levels (Archer et al., 2019). The vast majority of microbial species present in rock samples from Antarctica have not been found elsewhere (Coleine et al., 2023), and have diverged from known species long before the glaciation of Antarctica (Albanese et al., 2021). Moreover, endolithic taxa in these polar deserts display unique functional adaptations and tolerance traits to cope with the extreme thermal and moisture stress (Chan et al., 2013). As such, this communities represent an ideal model system for assessing how different ecological processes regulate the assembly of meta-communities likely influenced by a limited number of factors. To date, however, our understanding of the processes that determine the diversity and composition of the endolithic microbiota across Antarctica remains limited, also due to the logistical challenges associated with covering large abiotic gradients in extreme conditions (Koerich et al., 2023).

Traditionally, selection from the abiotic environment has been considered the key driver of microbial assembly, in line with the supposed ubiquity of microbial organisms (De Wit and Bouvier, 2006). However, along with the rapid development and application of metagenomic technologies, the influence of stochastic processes associated with dispersal limitation and historical contingency has clearly emerged in recent years (Aslani et al., 2022; Stegen et al., 2013; Zhou and Ning, 2017). The relative contribution of deterministic and stochastic processes thus appears to vary over a continuum as a function of environmental heterogeneity and the spatial scale of observation (Huber et al., 2020; Larsen et al., 2023; Quiroga et al., 2022; Zhou and Ning, 2017). Yet, an empirical assessment of the mechanisms driving the assembly of endolithic communities at the cold-arid limits of life has not been carried out. In such extreme and isolated conditions, local abiotic factors should drive species composition. For instance, different rock typology may host contrasting microbial assemblages; porous sandstone supports lichen-dominated communities (cryptoendolithic) where fungi are abundant but algae, cyanobacteria, bacteria and archaea are also present (Archer et al., 2017; Coleine et al., 2021; Friedmann, 1982). At lower elevations, granite rocks are more common and typically host microbial communities mainly dominated by cyanobacteria, creeping within cracks and crevices of rocks (chasmoendolithic) (Archer et al., 2017; de la Torre et al., 2003). In addition, because ice-free areas occur from sea-level up to 3000 m altitude, elevation may impose a selective process on community assembly through its effect on temperature regime and water availability (Coleine et al., 2019). However, because endolithic communities of exposed rocks represent islands in a sea of ice separated by hundreds of kilometres (Lee et al., 2022), factors such dispersal limitation and stochastic variation may also influence community assembly. To date, the degree to which deterministic and stochastic processes shape the assembly of the endolithic microbiome remains unknown. Studies in other extreme climates, including alpine grasslands, indicated that elevation can interact with other abiotic drivers to influence the relative importance of dispersal and selection for community assembly (Han et al., 2022; Yu et al., 2019). Here, we test the hypothesis that elevation and geology are able to impose deterministic selection on bacterial communities even under the driest and most isolated conditions for life.

We performed shotgun metagenomics to investigate how bacterial composition and diversity varies over a wide elevation range and within the dominant rock types of Antarctica. Specifically, we used phylogenetic null-models to quantify the relative contributions of stochastic and deterministic processes to their assembly. We further used information theory and network analysis of species abundances to quantify the effective number of distinct, non-overlapping communities and identify modules of highly correlated species across locations that could constitute the core of self-sustaining assemblages.

Environmental change is occurring rapidly in Antarctica (Chown et al., 2015; Koerich et al., 2023) and a better understanding of the mechanisms underlying the spatial distribution and assembly of endolithic microbial communities may help elucidate how the resident biota can persist in such extreme environments, and predict ecological impacts of rising temperatures in one of the fastest warming areas of the world.

2. Materials and methods

2.1. Study area

We collected 188 rocks colonised by endolithic communities in thirty-eight sites in Antarctica including McMurdo Dry Valleys, Southern Victoria Land ($n = 80$), and Northern Victoria Land ($n = 108$) during >20 years of Italian Antarctic Expeditions. Different rock typologies (sandstone $n = 138$, granite $n = 42$, quartz $n = 3$, and quartz/dolerite $n = 1$, granite/quartz = 1, mixed = 1) were sampled, but our analysis only includes sandstone and granite. Samples were collected along a latitudinal transect ranging from −62.10008 −58.51664 to −77.874 160.739, and over an altitudinal gradient from sea level to 3400 m elevation, to provide a comprehensive overview of Antarctic endolithic diversity. The presence of endolithic colonisation was assessed by direct observation in situ.

All rocks were excised aseptically by using geological hammers and chisels, posed in sterile plastic bags, preserved at −20 °C immediately upon collection to avoid contamination and transported to University of Tuscia, Italy and stored −20°. Upon arrival in the Tuscia University, each rock sample was crushed and powdered in sterile conditions under the hood to prevent cross-contamination.

2.2. Metagenomes sequencing

Total community DNA was extracted from 1 g of powdered rocks using DNeasy PowerSoil Pro Kit (Qiagen, German), quality checked by electrophoresis using a 1.5 % agarose gel and Nanodrop spectrophotometer (Thermofisher, USA) and quantified using the Qubit dsDNA HS Assay Kit (Life Technologies, USA). Shotgun metagenomic sequencing paired-end libraries were constructed and sequenced as 2×150 bp using the Illumina NovaSeq platform (Illumina Inc., San Diego, CA) at the Edmund Mach Foundation (San Michele all'Adige, Italy) and at the US DOE Joint Genome Institute (JGI).

2.3. Bioinformatic processing

In total, the dataset included 188 metagenomes, of which 18 (JGI dataset) were generated, sequenced, assembled and binned into MAGS as described in Albanese et al. (2021), while 95 metagenomes (FEM dataset) were generated, sequenced, assembled and binned into MAGs as described in Coleine et al. (2023).

For the remaining 75 samples (JGI-FICUS dataset) sequence data was generated at the DOE Joint Genome Institute (JGI) using Illumina technology. An Illumina library was constructed and sequenced 2×151 using the Illumina NovaSeq S4 platform. BBDuk version 38.94 (Bushnell, 2022) was used to remove contaminants, trim reads that contained adapter sequence and homopolymers of G's of size 5 or more at the ends of the reads and right quality trim reads where quality drops to 0. BBDuk was used to remove reads that contained 4 or more 'N' bases, had an average quality score across the read <3 or had a minimum length ≤ 51 bp or 33 % of the full read length. Reads mapped with BBMap (BBTools software package, http://bbtools.jgi.doe.gov) to masked human, cat, dog and mouse references at 93 % identity were separated into a chaff file. Reads aligned to common microbial contaminants were separated into a chaff file. Reads were corrected using bbcms version 38.90 (Bushnell, 2022).

The read set was assembled using metaSPAdes assembler with metaspades version "3.15.2" (Nurk et al., 2017). This was run using the following command line options: spades.py -m 2000 –tmp-dir cromwell_root -o spades3 –only-assembler -k 33,55,77,99,127 –meta -t 16 -1 input.corr.left.fastq.gz -2 input.corr.right.fastq.gz.

Metagenomic contigs were binned into candidate metagenome-assembled genomes (MAGs) using MetaBAT 2 v2.12.1. (Metagenome Binning based on Abundance and Tetranucleotide frequency) (Kang et al., 2019). High-quality reads were mapped on assembled contigs using Bowtie2 v2.3.4.3 (Langmead and Salzberg, 2012). Samtools v1.9 (Li et al., 2009) was used to create and sort the BAM files. The depth of coverage was estimated by applying the MetaBAT2 script "jgi_summarize_bam_contig_depths". Finally, contigs sequences and the depth of coverage estimates were used by MetaBAT2 to recover the 14,503 bins.

The resulting bins were analysed using the metashot/prok-quality v1.2.3 (Albanese and Donati, 2021) (parameters –gunc_filter –gunc_db gunc_db_2.0.4.dmnd) workflow. Completeness, redundant and non-redundant contamination (Orakov et al., 2021) estimates were obtained by CheckM (Parks et al., 2015) v1.1.2 and GUNC. Bins with completeness estimates of <50 %, >10 % contamination and that did not pass the GUNC filter were discarded, resulting in a total of 5769 filtered prokaryotic MAGs. MAGs were classified into "high-quality draft" (HQ) with >90 % completeness and <5 % contamination and "medium-quality draft" (MQ) with completeness estimates of ≥ 50 % and <10 % contamination. Species-level operational taxonomic units (OTUs) were identified by clustering HQ and MQ MAGs at 95 % average nucleotide identity (ANI) using dRep v2.6.2 (Olm et al., 2017), resulting in a total of 2402 species-level OTUs.

The abundance of each representative genome in each sample was estimated using the command *mash screen* (Ondov et al., 2019) from the metashot/containment v1.1.0 workflow (https://github.com/metasho t/containment) using the winner-takes-all strategy to avoid redundancy of the identified genomes.

Approximately-maximum-likelihood phylogenetic tree from the GTDB protein alignments of the 22,402 bacterial OTU representatives was inferred using FastTree (Price et al., 2010) v2.1.11 (default parameters).

2.4. Biodiversity and statistical analyses

2.4.1. Estimating diversity and heterogeneity

To quantify differences in local richness and diversity between sandstone and granite samples and the influence of elevation, we used generalised least squares model (gls) allowing for variance to differ between rock types (function varIdent from the nlme R package). Diversity was expressed as the exponential Shannon index, that is the effective number of species of order q = 1.

To further assess compositional heterogeneity of sandstone and granite metacommunities, and to characterise the exhaustiveness of our sampling, we quantified the *effective number of non-overlapping subcommunities*. This is an approach based on the mutual information between species and samples as proposed in Leinster (2022). In this approach, the exponential of the mutual information of species and samples is interpreted as an effective number of subcommunities. Sampling exhaustiveness for sandstone and granite samples was estimated with a rarefaction approach, whereby we quantified the increase in the effective number of subcommunities on random subsets of increasing sample size. The effective number of subcommunities was computed using the function *norm_meta_beta* from the R package *rdiversity* v2.0.0 (Reeve et al., 2016).

2.4.2. Community assembly null-models

To estimate the relative contributions of different assembly processes we combined taxonomic and phylogenetic null models as originally proposed by Stegen et al. (2012, 2013). In this approach, abundance-based taxonomic and phylogenetic β-diversity between sample pairs are compared to null expectations to quantify the contribution of deterministic and stochastic processes. We opted for this approach over others that similarly seek to estimate the relative importance of niche and dispersal related processes in metacommunities (e.g. Vilmi et al., 2020; Laboucher et al., 2020), because it has been widely tested across different systems (e.g. Aslani et al., 2022), it is supported by theory (Stegen et al., 2013; Zhou and Ning, 2017), and it was specifically developed for microbiological applications.

The beta nearest-taxon index (β-NTI) was used to quantify the degree to which the β-mean-nearest taxon distance (β-MNTD) deviates from null expectations based on 999 random shuffles of taxa across the tip of the phylogenetic tree. As such, the β-NTI accounts for the observed taxonomic and phylogenetic β-diversity and indicates whether stochastic or deterministic processes drive turnover in microbial community composition. Specifically, community pairs with $|\beta\text{-NTI}| > 2$ were considered regulated by deterministic selection, and were further partitioned into *variable* (β-NTI > 2, communities phylogenetically more dissimilar than expected) and *homogenous* (β-NTI < 2; phylogenetically more similar) selection. The remaining pairs with $|\beta\text{-NTI}| < 2$ were considered regulated by stochastic processes. This stochastic fraction was further classified using Bray-Curtis based Raup-Crick (RC$_{bray}$) distances. RC$_{bray}$ values were obtained by shuffling local communities probabilistically, accounting for the relative abundance and occurrence of each taxon. This was repeated 999 times maintaining the observed richness and number of counts in each community, thus obtaining the null distribution of Bray–Curtis distances for each community pair. Then, the distance between the empirically observed Bray–Curtis and null distribution was standardised between -1 and 1. For community pairs with $|\beta\text{-NTI}| < 2$ and values of RC$_{bray}$ < 0.95 we inferred a dominant influence of *homogenising dispersal* (taxonomically more similar than expected), while RC$_{bray}$ > 0.95 (taxonomically less similar

than expected) indicates *dispersal limitation* combined with stochastic ecological drift. When $|\beta\text{-NTI}| < 2$ and $|RC_{bray}| < 0.95$, no particular process dominates community assembly; this scenario is referred to as *undominated*. The null models were run using the algorithms implemented in the iCAMP package (Ning et al., 2020).

Proper inference from the community assembly analysis requires the presence of a phylogenetic signal across taxa, whereby the phylogenetic distance between species reflects their niche differences. We examined the phylogenetic signal with respect to elevation by plotting the Mantel correlogram between species phylogenetic distance and the distance in their elevation niche. The latter was defined as the absolute value in species elevation preference estimated as the abundance-weighted mean of elevation values where the species was observed.

2.4.3. Multiple regression on distance matrices

To further examine the processes imposing selection on bacterial taxa we used multiple regression on distance matrices (MRM). In particular, we regressed $\beta\text{-NTI}$ values against sites' geographic distance and elevation difference. This analysis was used to quantify the extent to which deterministic selection was imposed by (unmeasured) spatially structured environmental variables and variables associated with elevation.

MRM is a multivariate spatial analysis tool that involves the regression of a response distance matrix on multiple explanatory matrices (Lichstein, 2007).

2.4.4. Species association analysis

To identify modules of highly correlated taxa, we used species association analysis. The taxa association network was built using the Semi-Parametric Rank-based approach for INference in Graphical model (SPRING) method (Yoon et al., 2019) implemented in the R package *NetCoMi* v1.1.0 (Peschel et al., 2021). Briefly, the method builds a taxa association network by estimating partial correlations between pairs of taxa using a regularised regression of the abundance of one taxon on all the others. Sparsity of the network is obtained by lasso. Rarefied OTU table was used as input, and the 500 OTUs with highest variance were selected, discarding samples with a total number of individuals <500. To correct for compositionality, which can induce spurious correlations among taxa, data were transformed using the modified centred log ratio transformation (*mclr*) (Yoon et al., 2019). We then partitioned the network into modules of highly connected nodes using the method cluster_fast_greedy that performs an approximate optimization of modularity (Clauset et al., 2004). This modularity defines groups of taxa that are highly correlated across samples.

Finally, to examine non-random phylogenetic structure of taxa within modules, we calculated the standardised effect size of the mean nearest taxon distance (SES. MNTD; using ses.mntd in the picante package; this is also equivalent to -NTI), which represents the number of standard deviations the observed MNTD differs from values expected under the null distribution. Values of SES.MNTD < 2, indicate that taxa within a module are phylogenetically more related than expected by chance. Null values (500) were generated by shuffling taxa abundances across the tip of the phylogenetic tree.

Non-random association of modules with taxonomic composition at the Phylum level, elevation category and rock type was determined using a global chi square test using the R function chisq.test. Associations between modules and specific taxa, elevation categories and rock type were determined using Pearson residuals.

3. Results

After filtering, we identified 5570 high and medium quality bacterial genomes that were dereplicated into 2402 species-level bacterial OTUs Operational Taxonomic Units (OTUs) (see Methods). For each species-level cluster, one representative sequence was selected and taxonomically classified using the GTDB database. All representatives were classified into 16 distinct Phyla, the most common of which was *Actinobacteriota* with 1038 members, followed by *Proteobacteria* with 386 members. On the other side, the least common Phyla were *Myxococcota*, with one member, and *Firmicutes*, with 2 members. The complete taxonomic classification is available via the Zenodo repository (see Data Availability). Out of the 2402 species-level clusters, 48 only were classified at the species level. Abundance of each OTU in each sample was estimated using containment (see Methods). After excluding taxa present with less than ten (10) copies overall and samples with total abundance <2, the dataset included 174 samples (131 in sandstone; 43 in granite) and 2033 species.

3.1. Granite supports richer and more heterogeneous bacterial communities than sandstone

GLS models accounting for different variance between rock types indicated that, on average, granite supported about 55 more bacterial taxa than sandstone (Fig. 1a). When including relative abundances using diversity of order $q = 1$ (Hill-Shannon), granite supported about 20 more 'effective species' than sandstone (Fig. S1).

Overall, elevation had no significant effects on taxonomic richness and diversity (Fig. 1B). To quantify the heterogeneity of the granite and sandstone communities, we computed the effective number of distinct subcommunities, as quantified by species-sample mutual information (see Methods), for an increasing number of samples from the two types of rock. Granite samples displayed a consistently higher effective number of subcommunities, along the rarefaction curve, indicating that granite supported a higher bacterial heterogeneity than sandstone (Fig. 1C). Rarefaction of sandstone samples plateaued at about 8.68 distinct subcommunities. The total granite metacommunity was composed of 17.64 distinct subcommunities, but extrapolating the subsampling suggests that a larger number of samples would be needed to reach a possible plateau.

3.2. Elevation was associated with deterministic turnover in composition

The distribution of the taxa over the elevation range (as mean and standard deviation) is shown in Fig. 2. It shows, for instance, how *Actinobacteriota*, *Chloroflexota* and *Proteobacteria* are widely distributed, while *Eremiobacterota* are mostly found at higher elevations. *Cyanobacteria*, on the other hand, appear to form two groups, with taxa found below 400 m a.s.l., and above 1000 m.

Mantel correlograms indicated a significant correlation between taxa phylogenetic distance and their niche distance with respect to elevation preference (Fig. S2). In other words, phylogenetic adjacent taxa tend to prefer habitat with similar elevation, supporting the use of null-model approaches (see Methods) to infer assembly mechanisms. Specifically, we examined how the contribution of different assembly mechanisms varied with increasing pairwise elevation differences across samples, combining both rock types.

Fig. 3 shows the estimated proportion of each assembly mechanism calculated for three groups of pairwise comparisons separated by increasing elevation differences. Each group includes the same numbers of pairwise observations between communities. It is evident how the contribution of 'variable selection' increased with elevation difference (from 21 % to 36 %) while that of 'homogenous selection' declined (from 20 % to 13 %). The increasing influence of 'dispersal limitation' with increasing elevation differences is also evident (from 8 % to 14 %).

We further assessed the specific contribution of elevation-associated variables and geography (reflecting unmeasured spatially-structured environmental drivers) to the selection of microbial communities. To this end, we calculated the $\beta\text{-NTI}$ index for each sample pair, with $|\beta\text{-NTI}| > 2$ providing evidence for deterministic selection (see Methods); then we plotted the $\beta\text{-NTI}$ values as a function of elevation difference between locations as well as geographic distance (Fig. 4). Values of $\beta\text{-NTI}$ increased linearly with elevation difference, but were
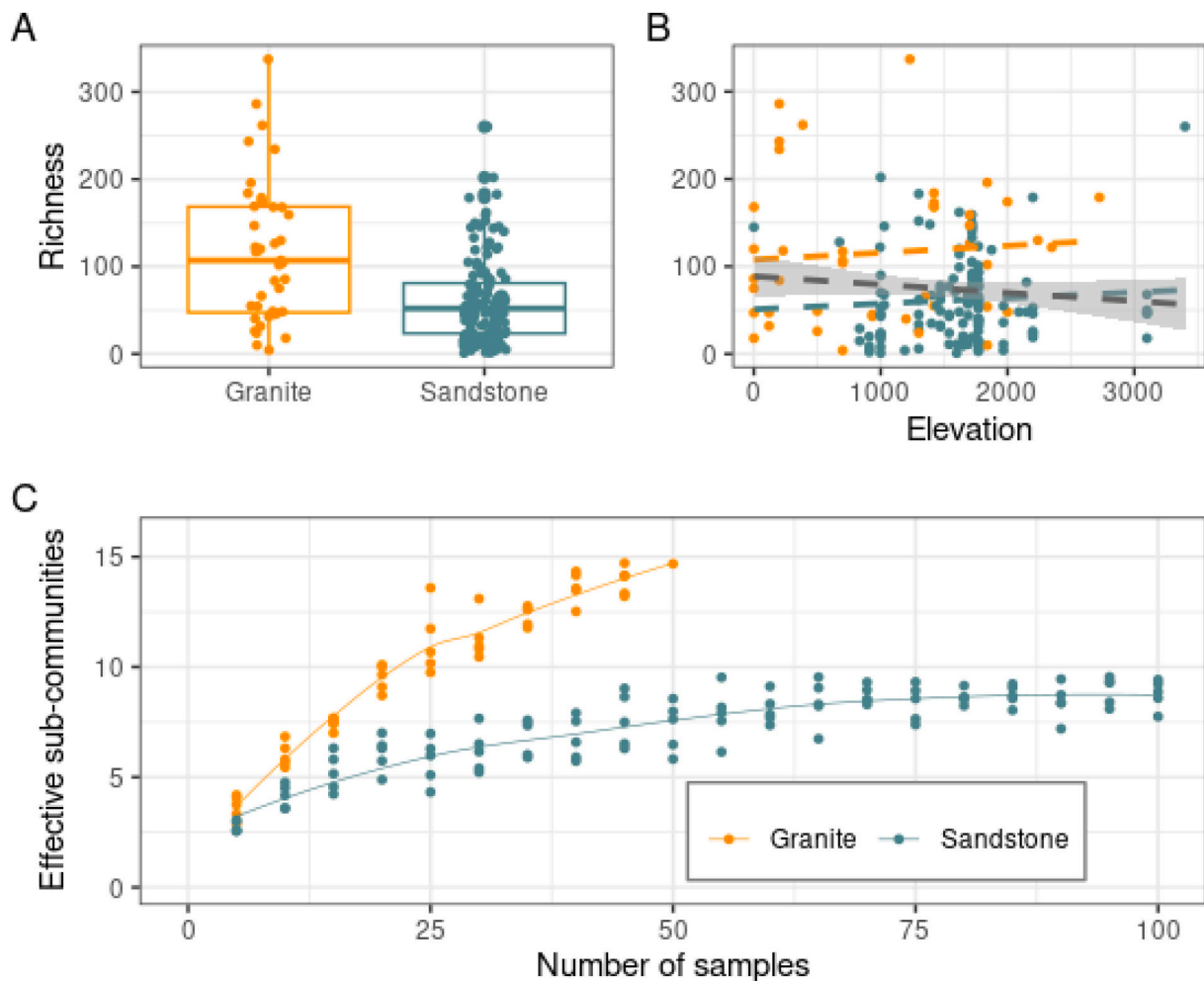
Fig. 1. Bacterial taxonomic richness across (A) rock type and (B) elevation. (C) Effective number of subcommunities as a function of the number of samples for sandstone and granite samples.

contingent on geology. A simple linear model indicates that communities within different rock types were regulated by variable selection (β-NTI > 2) beyond c.1200 m of elevation difference. This elevation threshold increased to c. 2300 m for communities inhabiting the same rock type (Fig. 4). Overall, elevation appeared to exert a stronger influence on community assembly than simple geographic distance, reflecting unmeasured but spatially autocorrelated environmental variables. This inference is based on comparing regressions on distance matrices (MRM). The MRM analyses indicated that elevation difference explained four-times as much variability (about 4 %; $p = 0.001$) in β-NTI compared to geographic distance (1.1 %; $p = 0.003$).

3.3. Species association analysis identifies network modules linked to geology and elevation

Optimal partitioning of the microbial correlation network (see Methods) identified six distinct modules comprising between 18 and 177 taxa. These modules accounted for a variable fraction of the total coverage, with taxa classified in module 1 and 4 being much less abundant than those in modules 2, 3, 5, and 6 (Fig. 5A). According to the standardised MNTD (SES.MNTD; Supplementary Table S1), five modules displayed significant phylogenetic clustering (*p*-value < 0.05), indicating that correlated taxa were phylogenetically more closely related than expected by chance, while phylogenetic clustering was not significant for module 1.

We further identified several phyla that were associated with the different modules. Using a Pearson chi-square test on the distribution of the phyla in the modules (weighted by count data), we found that there was significant deviation from a random distribution (p-value < 2.2e−16). Using standardised Pearson residuals as a measure of the strength of the association (with a threshold of 20) we found that module 2 was associated with excess counts of the phyla *Acidobacteriota*, *Dormibacterota*, and *Proteobacteria*, module 3 of *Cyanobacteria*, *Deinococcota*, and *Actinobacteriota*, module 5 of *Eremiobacteriota*, *Chloroflexiota* and *Armatimonadota*, and module 6 of *Cyanobacteria* and *Proteobacteria* (Fig. 5A).

A Pearson chi-square test on elevation classes showed a non-random distribution of the taxa associated to modules (p-value < 2.2e−16) in samples collected at different elevation. Partitioning the samples in three quantiles according to altitude (Low, Medium and High altitude), Pearson residuals (cut-off value 20) indicated that taxa in modules 3 and 6 were associated with samples collected at low elevation, while taxa in module 2 were more represented in samples at medium elevation, and taxa in module 5 in samples at high elevation. A similar procedure identified a significant correlation between modules and rock type (p-value < 2.2e−16), with Pearson residuals showing a strong association of module 2 and 5 with sandstone and of module 3 and 6 with granite.

4. Discussion

The Antarctic desert is among the most environmentally extreme habitats on Earth. Despite being virtually devoid of complex life-forms
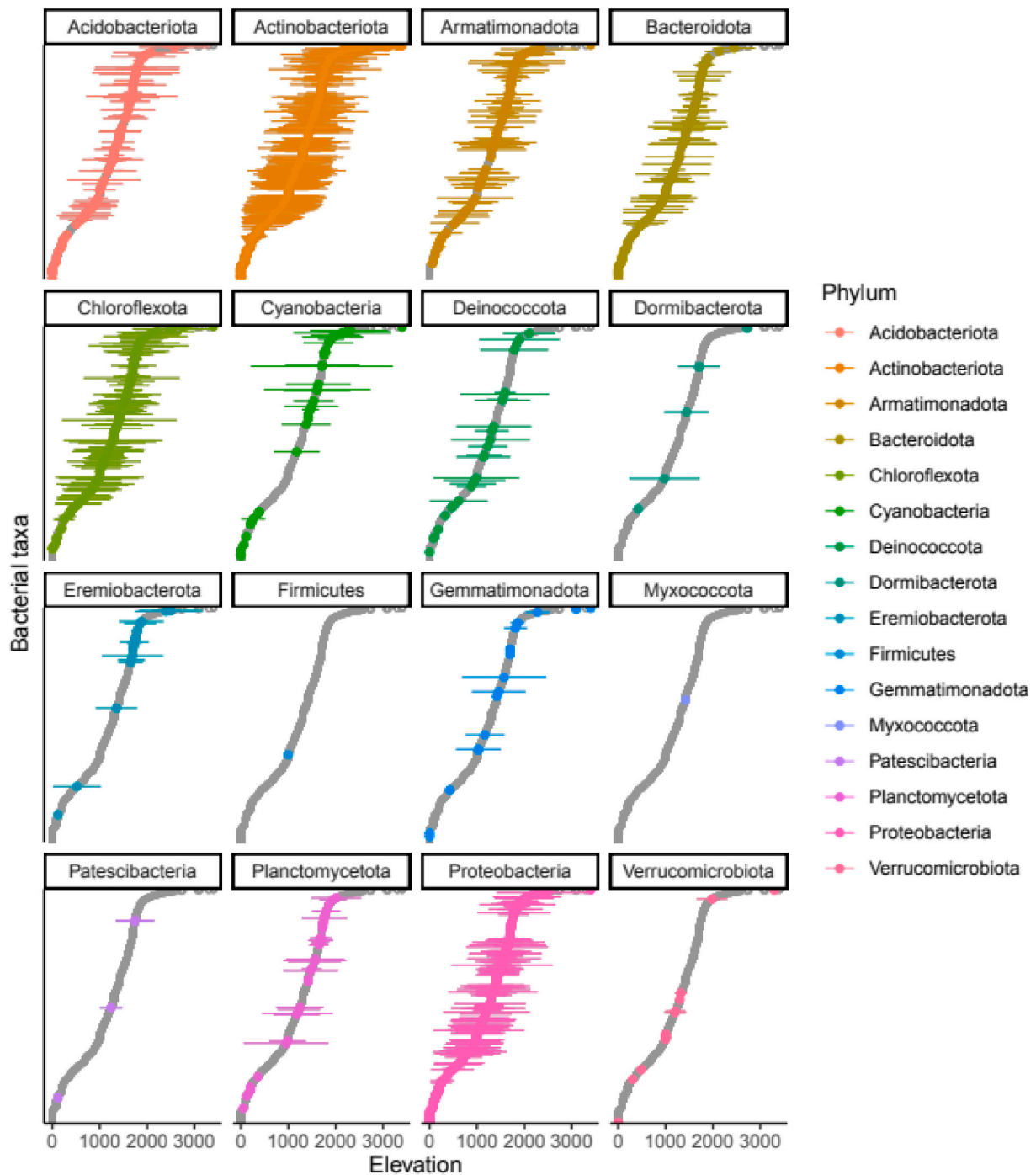
Fig. 2. Distribution of prokaryotic taxa (mean, SD, weighted by relative abundance) over the elevation range (in m). All taxa are sorted along the y-axis based on their mean elevation preference, while each coloured facet highlights the distribution of taxa within a given Phylum.

as vascular plants and animals, mounting evidence indicates that microbial life is surprisingly diverse and spatially heterogeneous, especially within lithic refugia. The discovery of such diversity at the cold-arid limit of life makes the Antarctic deserts a conservation priority (Chown et al., 2015) and understanding the drivers and processes that underpin microbial distributions across this system has both fundamental and applied ecological implications.

Our study helps to fill fundamental knowledge gaps of the processes governing microbial composition across Antarctic deserts indicating that geology and elevation interact to jointly impose deterministic selection over microbiome composition on Antarctic rocks. This is an important discovery as these rocks support the highest permanent

biomass across continental Antarctica ice-free areas (Cary et al., 2010; Coleine et al., 2021). In line with other studies on Antarctic soils and endolithic organisms (Coleine et al., 2019; de la Torre et al., 2003; Dragone et al., 2022), overall composition of endolithic communities was dominated by *Actinobacteriota* and *Proteobacteria*, with relatively high local abundance of *Chloroflexota* and *Eremiobacterota* (mostly in sandstone). The distribution of these taxa was not random, and highly correlated with the type of rock substrate and elevation. The taxa association network analysis showed that different types of communities can be identified. As for the photo-autotrophic component, *Cyanobacteria* were locally abundant especially within granite rocks, although not as dominant as observed elsewhere (Ji et al., 2017; Yung et al., 2014). In
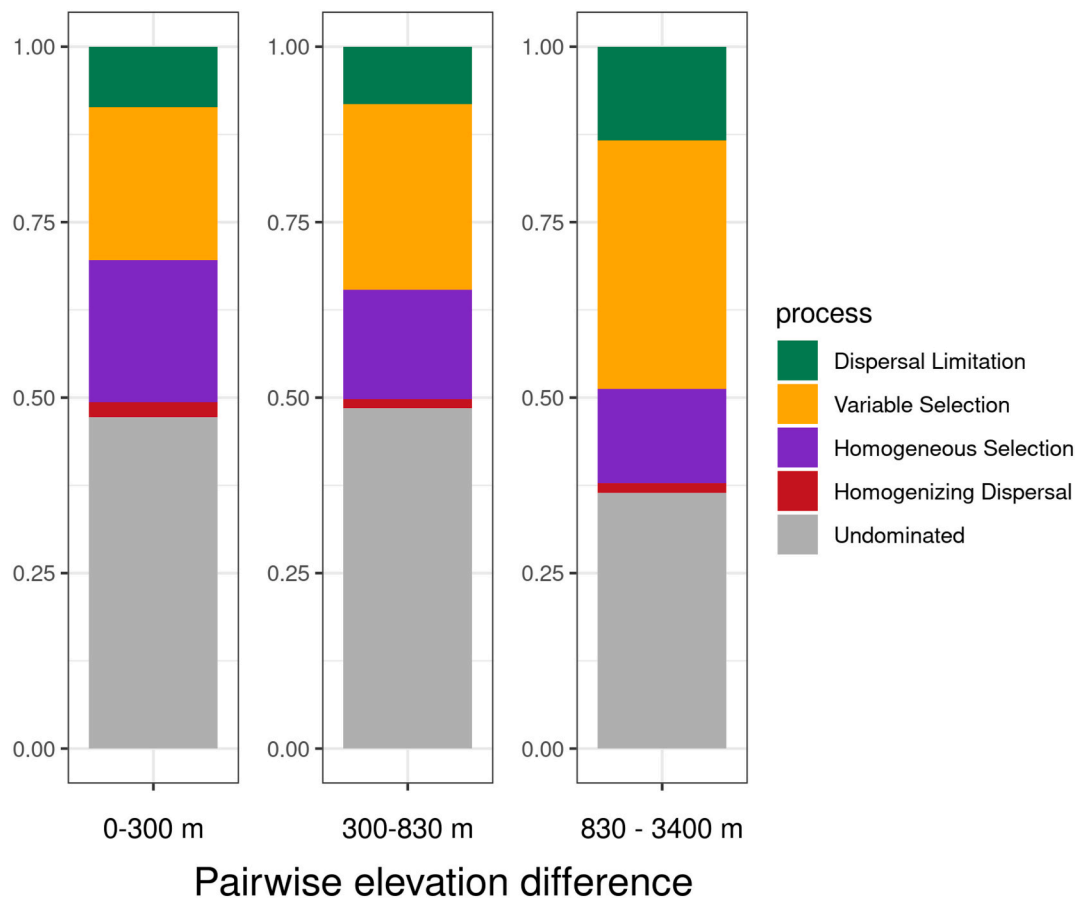
Fig. 3. Contribution of different assembly processes for pairs of communities separated by low (0−300), medium (300–830) and large (830–3400) elevation differences, combining both sandstone and granite samples.

particular, *Cyanobacteria* enriched communities were more frequent in granite samples collected at low altitude; these communities were richer and more heterogeneous than those found in sandstone samples. The surprising abundance and variability of microbial colonisation of granite, and the peculiar abundance of *Cyanobacteria* has been previously noted (Ji et al., 2017). Here it can also be related to the relatively milder climatic conditions of the sites where granites were collected, since elevation for these samples ranged from the sea level up to 700 m asl and exceeded 2000 m for two samples only. Previous studies have shown that water availability and humidity can influence the diversity of endolithic cyanobacteria in the McMurdo Dry Valleys (Rego et al., 2019). Conversely, *Eremiobacterota* (formerly WP-2), which have been previously identified in multiple environments, including Antarctica (Ji et al., 2017), acid mine drainage in North America (Grettenberger and Hamilton, 2021), boreal mosses (Holland-Moritz et al., 2018) and thawing permafrost (Woodcroft et al., 2018) were specifically associated with sandstone samples collected at high altitude. While taxonomic composition was similar to previous work, our results diverged from the expectation that sandstone would harbour higher microbial diversity (Archer et al., 2017; Selbmann et al., 2017). Granite communities were more diverse both in terms of local (alpha) diversity and sample heterogeneity, as quantified by the number of distinct subcommunities. Since our analysis is likely to have underestimated the diversity within granite samples due to their high heterogeneity with respect to the lower number of samples analysed, the divergent diversity between granite and sandstone may be greater than what we observed.

Our results further indicate that spatial patterns in bacterial richness are primarily associated with geology and not elevation and associated abiotic factors. We found only a slight decline in microbial diversity with increasing elevation, but this observation may be affected by the

distribution of granite samples, occurring mostly at lower elevations. Although previous work on Antarctic soils has reported a decline of prokaryotic diversity with elevation (Dragone et al., 2022), our results are consistent with recent works suggesting that elevation has limited effect on microbial richness within endolithic habitat, and that its effect may be mediated by geology (Coleine et al., 2019, 2021).

While elevation has little influence over richness, it was clearly linked to community composition, probably due to an interaction with rock type. Using a framework that combines null-models of taxonomic and phylogenetic β-diversity, we found that spatial turnover in community composition is the result of deterministic assembly processes imposed by variables associated with elevation and rock geology. Null model results indicated that the influence of deterministic variable selection progressively dominated the assembly of communities as elevation differences increased. At the same time, the contribution of homogenising selection declined. Overall, the influence of dispersal limitation on community assembly appeared relatively moderate, despite communities being separated by distances exceeding 600 km.

Patterns of β-NTI further revealed an interaction between elevation and rock type. In particular, for a given elevation difference, values of β-NTI were consistently higher between communities inhabiting different rock types. This led to a smaller elevation difference (on average) required for communities on different rock types to achieve deterministic divergence in community composition (~1200 m difference), relative to communities within the same rock type (~2300 m difference). In other words, beyond ~1200 m and 2300 m elevation difference between communities within the same or different rock types, respectively, mean β-NTI values increased above the significance threshold, indicating the dominance of deterministic variable selection. In contrast, mean β-NTI values did not increase systematically as a
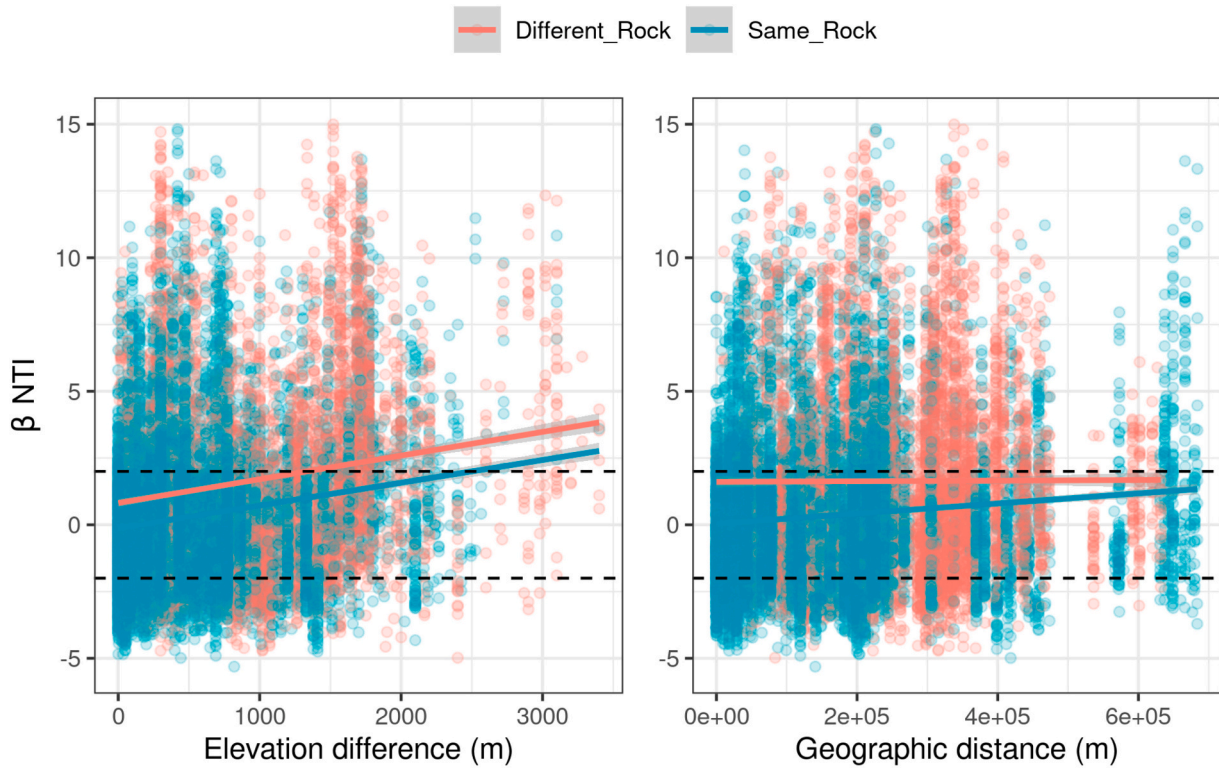
Fig. 4. βNTI values as a function of geology, elevation and geographic distances.
Dashed horizontal lines (−1.96; 1.96) indicate values above or below which pairs of communities are significantly regulated by variable and homogenous selection, respectively. For communities inhabiting the same rock type, variable selection dominated beyond elevation differences of ~1200 m. This elevation threshold increased to ~2300 m for communities within different rock types. When examined as a function of pure geographic distance, mean βNTI values remained within the limits of non-significance.
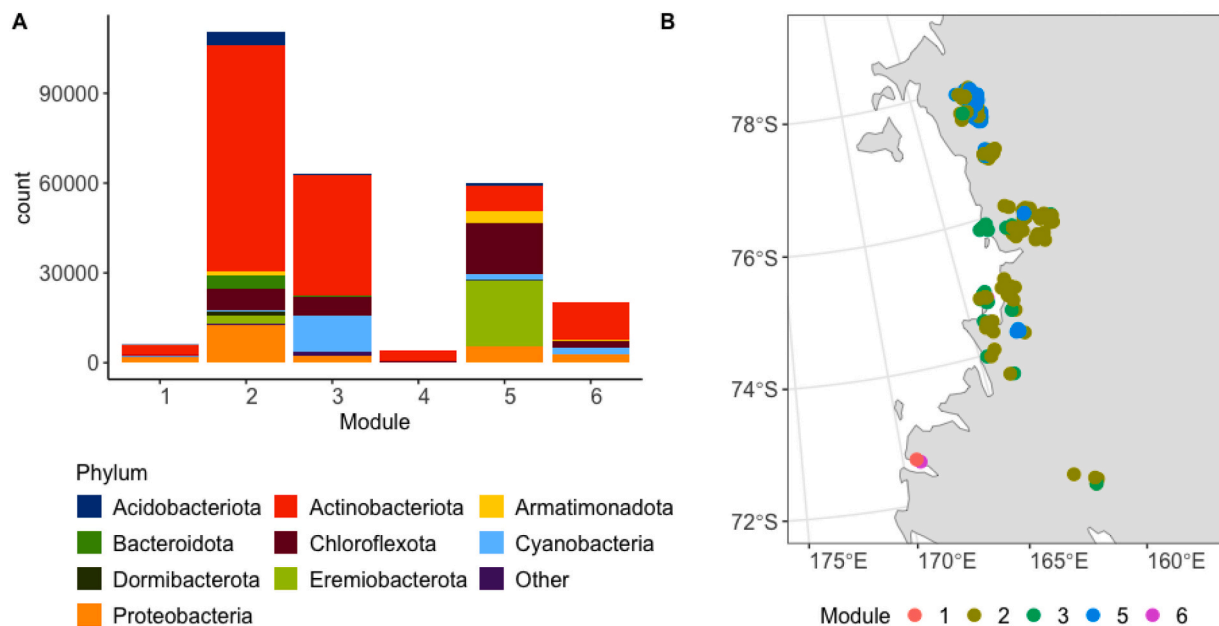

Fig. 5. A) Taxonomic composition of the modules at the phylum level. Each taxon is weighted by abundance; B) geographic distribution of the modules. For each sample, we have computed the fraction of taxa counts associated with each module, and assigned the sample to a module if this fraction exceeded 50 %.

function of pure geographic distance; this suggests that any unmeasured, but spatially structured environment variables had negligible influence on the assembly processes. In a comprehensive meta-analysis, Caruso et al. (2011) identified the signature of both niche-related and stochastic processes in the assembly of microbial communities across the global deserts. Interestingly, they observed deterministic convergence in the distribution of microbial taxa in the Antarctic Dry Valleys, in apparent contrast with the large contribution of variable selection observed in this study. However, only six samples were included in the aforementioned meta-analysis, which did not cover the same elevation gradient

considered here.

Taken together, these findings support a model in which differences in mineralogy select for divergent assemblages while differences in elevation exert a compounding effect intensifying selective pressures as elevation grows. This would explain why smaller elevation differences are required to deterministically shift community composition across (relative to within) rock types. Surprisingly, given the isolated nature of endolithic habitats, dispersal limitations apparently do not play a major role in differentiating these communities, although it might still influence the microevolution of specific taxa (Gawor et al., 2016).

The joint deterministic influences of elevation and rock type on microbial distributions is further supported by the species association analysis. Six network modules were identified and all included taxa that were closely related phylogenetically. The spatial distributions of these modules were strongly related to elevation and rock type. We infer that closely related taxa that have similar niche requirements are non-randomly associated with particular combinations of elevation and geology.

Our results provide a valuable contribution to understanding patterns and processes shaping the microbiota of Antarctic endolithic habitats, which represent the key refugia for life across the ice-free areas. In line with theory and previous investigations, abiotic factors appeared to be the key driver shaping microbial diversity and composition with effects generally stronger than those simply associated with geographical separation. Moreover the interaction between elevation-associated variables and geology indicates how local physico-chemical variables are driving microbial assembly even where overall conditions are extreme. Understanding how the physico-chemical properties of lithic habitats interact with elevation and climate to regulate microbial biodiversity is among the priorities for research in Antarctica (Chown et al., 2015) where biodiversity studies across large environmental gradients remain rare (Albanese et al., 2021; Chong et al., 2012; Coleine et al., 2019; Dragone et al., 2022).

To conclude, our analyses allow formulating a preliminary conceptual framework underpinning the distribution of bacterial biodiversity in the most extreme environment on Earth (Fig. 6). Our data point to a key role of mineralogy, whose selective pressure increases with elevation, whereby compounding selective forces interact to drive community assembly. The exact mechanisms remain unclear, but variables associated with elevation, such as temperature, water availability and solar radiation are likely involved, as well as the physico-chemical composition of the rocks. As the global climate shifts away from historic conditions, Antarctic organisms will likely experience novel environments and biotic interactions. Changes may occur rapidly; in such extreme ecosystems there is evidence for limited resistance to climate-induced environmental change (Cary et al., 2010; Koerich et al., 2023). Hence, predicting future trends in biodiversity and designing conservation strategies requires a focus on ecological processes and quantitative models that afford long-term forecasts (Koerich et al., 2023). The scarcity of data from the Antarctic continent remains an issue for both conservation and ecological modelling; however, monitoring efforts continue and as more data become available, studies like the present one will provide valuable mechanistic insights for identifying taxa and habitat most susceptible to environmental changes.

CRediT authorship contribution statement

Stefano Larsen: Conceptualisation, Formal analysis, Writing – Original Draft. Claudia Coleine: Conceptualisation, Investigation, Data curation, Funding acquisition, Writing – Original Draft. Davide Albanese: Formal analysis, Data curation. James C. Stegen: Conceptualisation, Writing- Original Draft. Laura Selbmann: Investigation, Supervision, Resources, Funding acquisition, Writing – Original Draft. Claudio Donati: Resources, Formal Analysis, Writing- Original Draft.
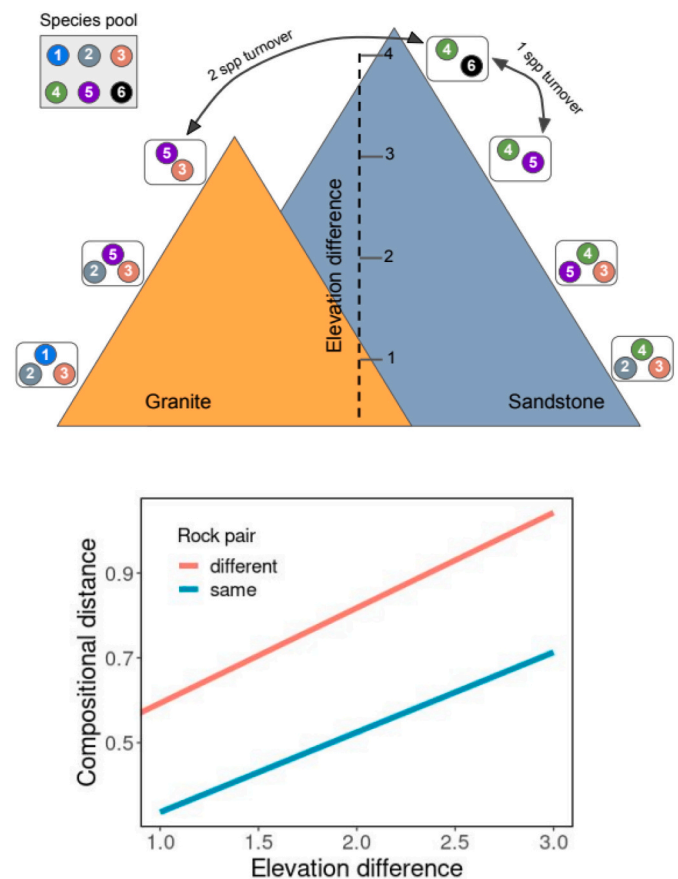

Fig. 6. Conceptual diagram of the combined effect of geology and elevation on the assembly of endolithic bacterial communities as emerged from this study. The upper panel shows the distribution of six species (coloured circles with numbers) within seven communities over the elevation gradient. In this example, each community consists of 2–3 species and is located in either granite or sandstone substrate. Within the same rock type, pairs of communities experience turnover of one species for each step over the elevation gradient. However, across different rock types, turnover increases to two species for each elevation step, reflecting the underlying differences between geology. The lower panel shows the relationship between elevation distance and compositional distance. In this conceptual example, compositional distance simply represents the turnover of species, whereas in Fig. 4 the null-model derived β-NTI index is used.

Declaration of competing interest

Data availability

All data generated and used in this study are available via the Zonodo repository with DOI: doi:https://doi.org/10.5281/zenodo.8297605.

Codes for running the analyses are available at https://github.com/stefanolarsen/AntarcticMicro.

Acknowledgements

of PNRA projects. The Italian Antarctic National Museum (MNA) is kindly acknowledged for financial support to the Mycological Section of the MNA and for providing rock samples used in this study stored in the Culture Collection of Antarctic fungi (MNA-CCFEE), University of Tuscia, Italy. H.P Lovecraft is acknowledged for his work on Antarctic mountains.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.168050.

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