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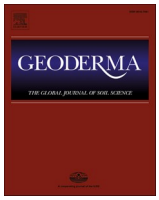

Antarctic rock and soil microbiomes: Shared taxa, selective pressures, and extracellular DNA effects

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ABSTRACT

Highly adapted and often endemic microbial taxa inhabit soils and rocks of extremely cold and dry Antarctic deserts. However, the source populations of these organisms have not yet been clarified. Local hotspots, rather than worldwide wind dispersion, have been described as the primary sources of microbial diversity. In particular, the endolithic niche offers a buffered environment, where layered microbial communities have been described, whose dispersion due to rock fragmentation may influence the diversity and nutrient availability of the surrounding soils. On the other hand, microorganisms thriving in soils could be transported to rock surfaces and colonise them. However, the bacterial taxa that are differentially selected and those exchanged between these two substrata have never been defined before. Additionally, the microorganisms detected in these substrata using DNA-based approaches may not be alive but may correspond to fragments of extracellular DNA originated from dead cells. To identify the taxa that are shared between the two substrata, the selective filters that drive their distributions, and the effects of relic DNA on subsequent interpretations of community structure, colonized rock samples were collected from sandstone outcrops in three localities, as well as soil samples at increasing distances from the outcrops. Homogeneous samples were divided into aliquots, and one of each aliquot was treated for extracellular DNA depletion. Both native and treated samples were screened for their bacterial composition through 16S rRNA gene metabarcoding. The results provide the first demonstration of the strong selection of bacterial communities in rocks and soils, reporting some taxa potentially exchanged between the two substrates. Specifically, genera differentially selected between the two habitats were identified, likely due to their different microenvironmental conditions, such as differences in their thermal regimes. Additionally, extracellular DNA depletion had few effects at the taxonomic level and on the identification of differentially selected genera between the two substrata, but it increased the number of significant correlations of physicochemical variables with the diversity and composition of the soil microbial communities. These findings lead us to the conclusion that, despite the strong selection of the two substrates, there is microbial propagule interchange between soils and rocks in this environment. Extracellular DNA should be carefully considered since it has a significant impact on microbial diversity estimations.

1. Introduction

Microorganisms inhabiting the cold mineral soils of Antarctic deserts are constantly exposed to extreme conditions, including very limited organic nutrients, extremely low moisture, low temperatures, frequent freeze–thaw and wet-dry cycles, fluctuating UV radiation regimes, strong desiccating winds, and locally high salinity (Cowan and Tow, 2004). Despite these environmental constraints, cultivation-independent techniques have highlighted greater prokaryotic diversity within Antarctic soils than previously predicted (Chong et al., 2012; Lee et al., 2012). While the phylum-level composition of these communities does not significantly differ from lower-latitude soils (Bottos et al., 2014), they are highly specialized, with relatively low numbers of dominant species and a high taxonomic and functional diversity of low-abundance taxa (Chong et al., 2015; Delgado-Baquerizo et al., 2018; Fierer et al., 2012). Additionally, due to their geographic isolation,

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harsh physical environments, and relatively simplified trophic relationships, these pristine ecosystems are excellent models for disentangling hypotheses regarding the distribution ecology and evolutionary drivers of microbial life (Barrett et al., 2006; Hogg et al., 2006; Convey et al., 2014; Thompson et al., 2020). Previous studies concluded that abiotic variables strongly influence diversity and community composition in such environments (Feng et al., 2010; Van Goethem et al., 2016; Lee et al., 2019; Severgnini et al., 2021). Furthermore, in many different soil environments, not only *Cyanobacteria* support primary production, but some lineages within the *Proteobacteria*, *Actinobacteria*, *WPS-2*, and *AD3* phyla can fix carbon via the Calvin-Benson-Bassham cycle, scavenging atmospheric traces of H_2 and CO (Bay et al., 2021), which has also been documented in ice-free areas of Antarctica (Ji et al., 2017; Ortiz et al., 2021).

Some studies tried to define the possible contribution of airborne dispersal to Antarctic soil diversity. Recent work revealed strong dispersal limitations and identified stochastic, rather than deterministic processes, as the primary spatiotemporal drivers of soil ecosystem assembly (Lemoine et al., 2023). However, there appears to be a lack of correlation between airborne microorganisms at continental scales and those found in Antarctic soils (Archer et al., 2019). Geographically isolated areas of refugia that were relatively stable throughout glacial advances and retreats can have highly similar community structures (Jackson et al., 2022). In this light, it has been hypothesized that local refugia, such as microbial mats surrounding lakes and ponds or the endolithic communities, could serve as source populations for the diversity observed in the region (Hopkins et al., 2009; Pointing et al., 2009; Cowan et al., 2014).

Cryptoendolithic communities have been described as the predominant life forms in these environments due to the buffered conditions offered by rock porosities (Friedmann, 1982; Zucconi et al., 2016), and their taxonomic diversity, composition, and functionality are gradually becoming clearer (Coleine et al., 2019; Mezzasoma et al., 2022). However, the possible exchange of microbial propagules between the cryptoendolithic communities and the surrounding soils, as well as the possible differential selective pressure of these two substrata, have never been systematically investigated in Antarctica. Indeed, Antarctic soils are formed from rock weathering caused by both physico-chemical processes and biological colonization (Campbell and Claridge, 1987). It has been suggested that the sub-surface growth of cryptoendolithic lichens induces rock surface exfoliation, leading to rock fragments that could serve as vegetative propagules, many of which fall into the soil (Friedmann, 1982). On the other hand, soils can be a source of rock-dwelling microorganisms that can colonize the surrounding rocks. Thus, it is evident that these two substrata experience different microclimatic conditions that can select for different microbial communities, even within adjacent areas.

Additionally, few studies have tried to determine if life forms found through DNA-based methods are metabolically active or present as inactive, dormant, or dead cells in these environments. Studies using different methodological approaches, such as stable-isotope probing (Schwartz et al., 2014), metatranscriptomics (Buelow et al., 2016), and 16S rRNA gene amplicon sequencing (Feeser et al., 2018), yield congruent patterns, with *Proteobacteria* and *Deinococcus-Thermus* generally being active and adapted to Antarctic environmental conditions, while *Acidobacteria* and *Bacteroidetes* appear to be mainly inactive. Furthermore, various studies have shown that extracellular DNA can persist in soils across all biomes in very high proportions, potentially distorting the diversity metric estimations obtained with molecular DNA-based approaches. This effect can be even more pronounced in Antarctic soils, where the limited microbial activity and the combination of dry, cold, and saline conditions can have a long-term stabilizing effect on DNA (Carini et al., 2016).

In this work, we implemented DNA metabarcoding analyses to provide new insights into the prokaryotic diversity and the mechanisms of distribution, ecology, and survival in extremely oligotrophic Antarctic niches. Specifically, our approach was designed to (i) evaluate the possible exchange of microbial propagules between cryptoendolithic communities and the surrounding soils, (ii) elucidate selective pressures on the communities inhabiting these two substrates (in terms of the presence of generalist and specialist taxa for the two substrata), and (iii) evaluate how the extracellular DNA influences the diversity metrics and ecological trends described in this geographic region through DNA-based approaches.

2. Materials and methods

2.1. Sampling

Samples were collected during the XXXV Italian Antarctic expedition in three different localities of inner Victoria Land: Battleship Promontory (November 27th, 2019), Trio Nunatak (December 2*nd*, 2019), and Richard Nunatak (December 2nd, 2019) (table S1), where stations continuously recording data on air, rock, and soil temperature, humidity, solar radiation, and PAR were installed (Fig. 1). Soil samples were collected in triplicate as close as possible to a sandstone outcrop (within 0–1 m) exhibiting conspicuous cryptoendolithic colonization, and at increasing distances of 50 and 100 m. The gradient was oriented in the direction of the major winds dominating the area, which flow from the polar plateau toward the coast. Rock samples from the colonized sandstone outcrop were also collected in triplicate. Samples were collected using aseptic techniques and sterile collection materials, placed in sterile bags, and stored at −20 °C until processing.

2.2. Soil physico-chemical analyses

Soil samples (about 500 g each) were air-dried and < 2 mm sieved. An aliquot of 10 g before drying was used for measuring the moisture at 105 °C. Physico-chemical properties were analyzed according to standard methods of SISS (Società Italiana della Scienza del Suolo) (Colombo and Miano, 2015). Total soil organic carbon (C) and nitrogen (N) were measured by combustion with an elemental analyzer NA 1500 CHNS (Carlo Erba, Milan, Italy); cation exchange capacity (CEC) was determined by barium chloride ($BaCl_2$) extracts method; soil exchangeable bases (Na^+, Ca^{2+}, K^+, and Mg^{2+}) were analyzed by flame atomic absorption spectrometry (AAS 1100B, PerkinElmer, Waltham, MA, USA); pH was measured in a 1:2.5 soil:water suspension; soil available P was determined by Olsen method and total P by colorimetric method after acid digestion; particle size distribution was obtained by pipette method, particles were sized as sand (0.5–2 mm), coarse silt (0.02–0.05 mm), fine silt (0.002–0.02 mm) and clay (<0.002 mm).

2.3. PMA treatment and DNA extraction

Before the analyses, each rock sample was ground to a fine powder and homogenized under sterile conditions. For both rock and soil samples, one uniform aliquot of 1 g was treated with Propidium MonoAzide (PMA; Biotium, Inc., Fremont, CA, USA), which binds to extracellular DNA or DNA of cells with damaged membranes upon light exposure and inhibits PCR amplification, modifying the method described in Carini et al. (2016). Samples were resuspended in 3.0 ml sterile phosphate-buffered saline solution (PBS) (pH 7.4) in transparent plastic tubes. PMA was added at a final concentration of 40 μM in a dark room. Samples were gently vortexed and incubated in the dark for 30 min under continuous agitation. Samples were then exposed to a 650 W halogen lamp placed 20 cm from the sample tubes, in the ice, for five consecutive 2-minute cycles each followed by vortexing. Then, samples were centrifuged for 30 min at 16,100 xg at 4 °C. The supernatant was removed, and samples were preserved at −20 °C until DNA extraction. DNA was extracted from 1 g of both native samples and those treated with PMA using the DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol.

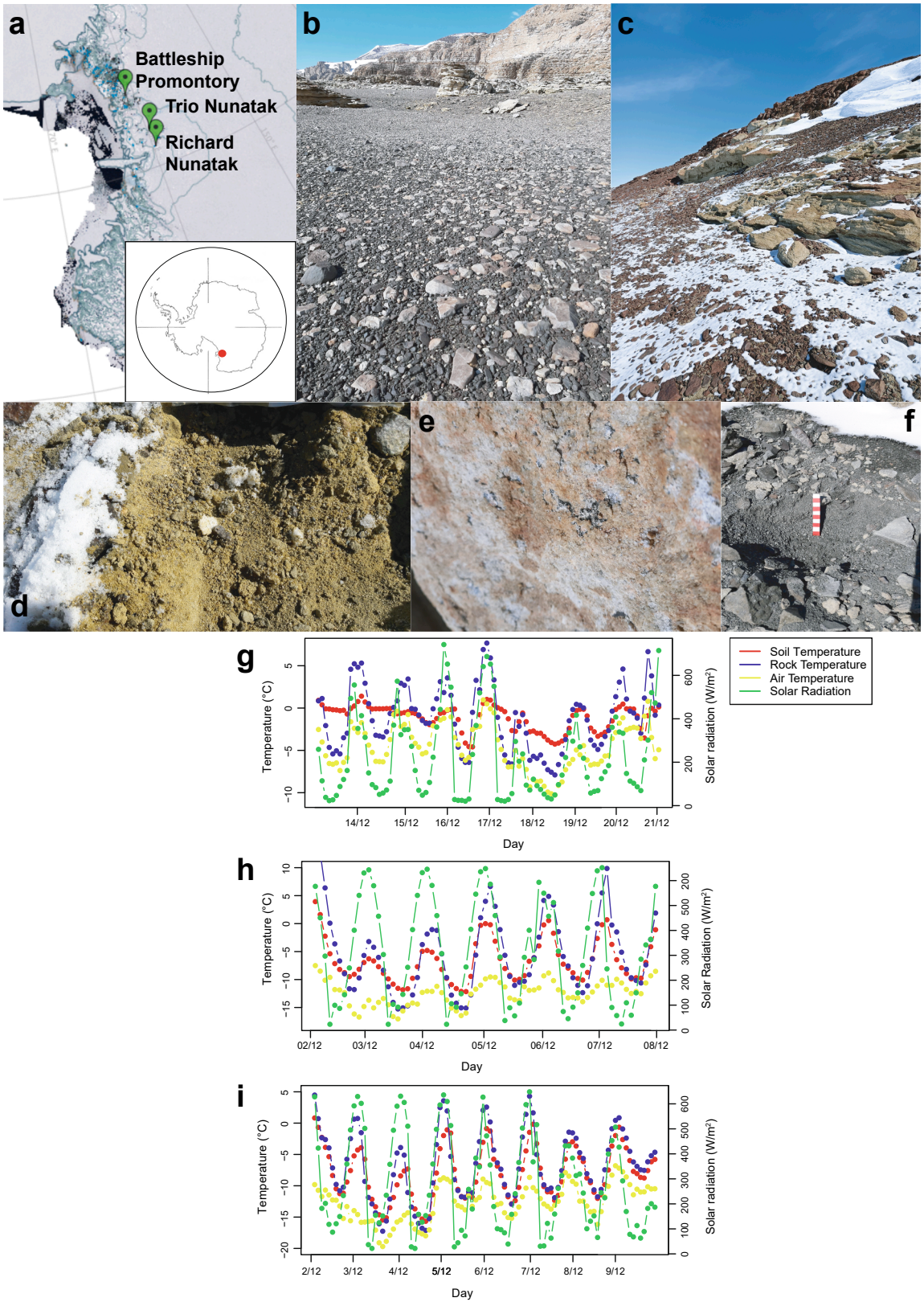

Fig. 1. Sampling sites, temperature and solar radiation measurements. Map of the sampling sites (a); sandstone rock outcrops and adjacent soil at Battleship Promontory (b) and Trio Nunatak (c); details of soil samples at Battleship Promontory (d) and Richard Nunatak (f); detail of weathered colonized sandstone surface at Trio Nunatak (e); temperature trends of rock, soil, air, and solar radiation, monitored by the climatic stations at Battleship Promontory (g), Trio Nunatak (h) and Richard Nunatak (i) (data are relative to the sampling time).

2.4. DNA amplification and sequencing

The V4 hypervariable region of the 16S rRNA gene was amplified using 515F (Parada et al., 2016) and 806R (Apprill et al., 2015) primers. The amplification assay consisted of 2 ng DNA, 5x reaction buffer, 1 mM dNTP mix, 500 nM of each primer, and Herculase II fusion DNA polymerase (Agilent Technologies, Santa Clara, CA). The amplification protocol was as follows: 3 min at 95 °C, 25 cycles of 30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C, followed by a 5-minute final extension at 72 °C. Libraries were prepared following the protocol of Minich et al. (Minich et al., 2018). It was not possible to obtain amplicons and/or good-quality libraries to be processed for all available samples (the list of samples for which the amplification or library construction failed is reported in Table S1). The equimolar pool of uniquely barcoded amplicons was paired-end sequenced (2 × 250 bp) on an Illumina MiSeq platform at Macrogen, Inc. (Seoul, South Korea).

2.5. Bioinformatic analyses

Bcl files were converted to Fastq files and demultiplexed using bcl2fastq (v 2.20). Demultiplexed sequences were processed with the Amplicon ToolKit (AMPtk) for NGS data (formally UFITS) v.1.3.0 (Palmer et al., 2018). Starting reads were quality trimmed and PhiX screened, using USEARCH v. 11.0.667 (Edgar, 2010) with default parameters. Reads with less than 100 bp were removed, the others trimmed to the length of 250 bp, and paired-end reads were merged in one step. Obtained reads were clustered in Amplicon Sequence Variants (ASVs), using DADA2 v1.20.0 (Callahan et al., 2016), which includes phiX reads removal and chimera detection. Singleton ASVs were removed from the dataset. Taxonomy was assigned to ASVs with a hybrid approach that calculates the local common ancestor on the results of a Global Alignment (USEARCH/VSEARCH), UTAX, and SINTAX (Edgar, 2010). Alignment was performed on the RDP11 database (Cole et al., 2014). ASVs with no match in the database and those identified as Chloroplasts were removed. Before diversity analyses, the number of reads per sample was rarefied to the lowest library size (72,012 reads) using the *rrarefy* function in the vegan package v. 2.6–4 (Oksanen et al., 2022) in R v. 4.2.1 (R Core Team, 2018).

2.6. Statistical analyses

All statistical analyses were carried out in R v. 4.2.1 (R Core Team, 2018).

The total richness, relative richness, and abundance of dominant bacterial phyla and orders were compared among the different types of samples (rocks and soils), the soils collected at increasing distances from the rocks (0, 50, and 100 m), and the different treatments (both native and PMA treated samples) with a Kruskal-Wallis test (McKight and Najab, 2010) followed by Dunn multiple comparisons (Dunn, 1964), with p-values adjusted with the Benjamini-Hochberg method. Non-metric Multidimensional Scaling (NMDS) analyses of the weighted Bray-Curtis distances of Hellinger transformed matrix of the bacterial community were performed with the following parameters: dimensions = 2, initial configurations = 100, model = global, maximum number of iterations = 200, and convergence ratio for stress = 0.999999. Homogeneity in community variance (Anderson, 2006) was analysed with the *betadisper* function. The significance of the differences in community composition was tested with a permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) performed with the *adonis2* function on Hellinger-transformed Bray-Curtis distance matrices. A CLAM (Multinomial Species Classification Method) statistical approach was used for classifying generalist and specialist ASVs in the two distinct habitats (rock and soil) as described in Chazdon et al. (2011), with the function *clamtest*. Genera differentially associated with rock and soil were obtained with a Linear Discriminant Analysis (LDA) effect size (LEfSe), based on LDA scores of > 2 and p-values of < 0.05 (Segata et al., 2011). The correlations between the richness of the total community and the relative richness and abundance of the dominant phyla and the physico-chemical parameters measured for soil samples were tested with Spearman's rank correlation coefficient and were represented with the *corrplot* package (Wei and Simko, 2021). The effect of soil physico-chemical parameters on community composition was represented via a distance-based Redundancy Analysis (dbRDA) (Legendre and Anderson, 1999), on the Bray-Curtis distances of Hellinger-transformed matrix of bacterial community performed with the *dbrda* function. The effect of soil parameters on the observed variance was determined via PERMANOVA (Anderson, 2001), performed with the *adonis2* function on Hellinger-transformed Bray-Curtis distance matrices. Significant variables were then considered in an additional model, where they were added starting from the most significative one in the previous analysis, to determine the combined effect of soil parameters on the variance of the community.

3. Results

3.1. Dataset

Of the 7,411,303 starting reads, a total of 7,275,324 quality-filtered reads were clustered in 19,090 ASVs. Subsequently, 10,457 chimeras were removed, leaving 8,633 valid ASVs (accounting for 6,636,356 reads mapped to ASVs, 91 % of the total). After singleton removal, 8,631 ASVs were retained. Two samples were excluded because they resulted in a too-low number of total reads (105 and 35 reads respectively; table S1), losing 5 ASVs. 65 ASVs without matches in the database and 47 ASVs identified as chloroplasts were removed. After the rarefaction, the final dataset consisted of a total of 8,375 ASVs obtained from 29 native samples and 27 treated with PMA (see table S1).

1,312 ASVs (15,7 % of the total) were present only in samples not treated with PMA, 1,116 (13,3% of the total) were unique in samples treated with PMA, and 5,947 (71 % of the total) were shared between them. Similar percentages were observed considering rock and soil samples independently (table S2). ASVs unique to samples not treated with PMA often showed high relative abundance for both rock and soil samples. Instead, ASV unique in PMA-treated samples were generally low-abundant (table S2). This effect was particularly evident for samples collected at Richard Nunatak (table S2).

Regarding the distribution in the two substrata, 543 ASVs were unique in rock samples, 5,776 were unique in soil samples, and 940 were shared by both rock and soil for samples not treated with PMA (table S3). For samples treated with PMA, 594 ASVs were unique in rock samples, 5,816 were unique in soil samples and 653 were shared by both (table S3). The proportion of ASVs in common between rocks and soils was highly variable among the different localities and within the different samples in the same locality (table S3). At the taxonomic level, *Actinobacteria*, *Proteobacteria*, *Acidobacteria*, and *Firmicutes* were the phyla mainly represented in ASVs shared by rocks and soils (table S3).

Any difference in the number of ASV shared with rocks was recorded for soil samples collected at increasing distances from the rock outcrops (0, 50, and 100 m). This was also confirmed when considering the different localities independently and both for untreated and PMA-treated samples (data not shown).

3.2. Richness and abundance patterns

Soil ASV richness was significantly higher than rock richness both in native and PMA-treated samples (Fig. 2a). Treatment with PMA did not significantly change the whole richness of both rock and soil samples (Fig. 2a).

Regarding the taxonomic composition, 29 different phyla were identified in the dataset and 1,902 ASVs were only assigned at the kingdom level and could not be assigned to a phylum. PMA treatment did not significantly change the richness of any major phylum except for

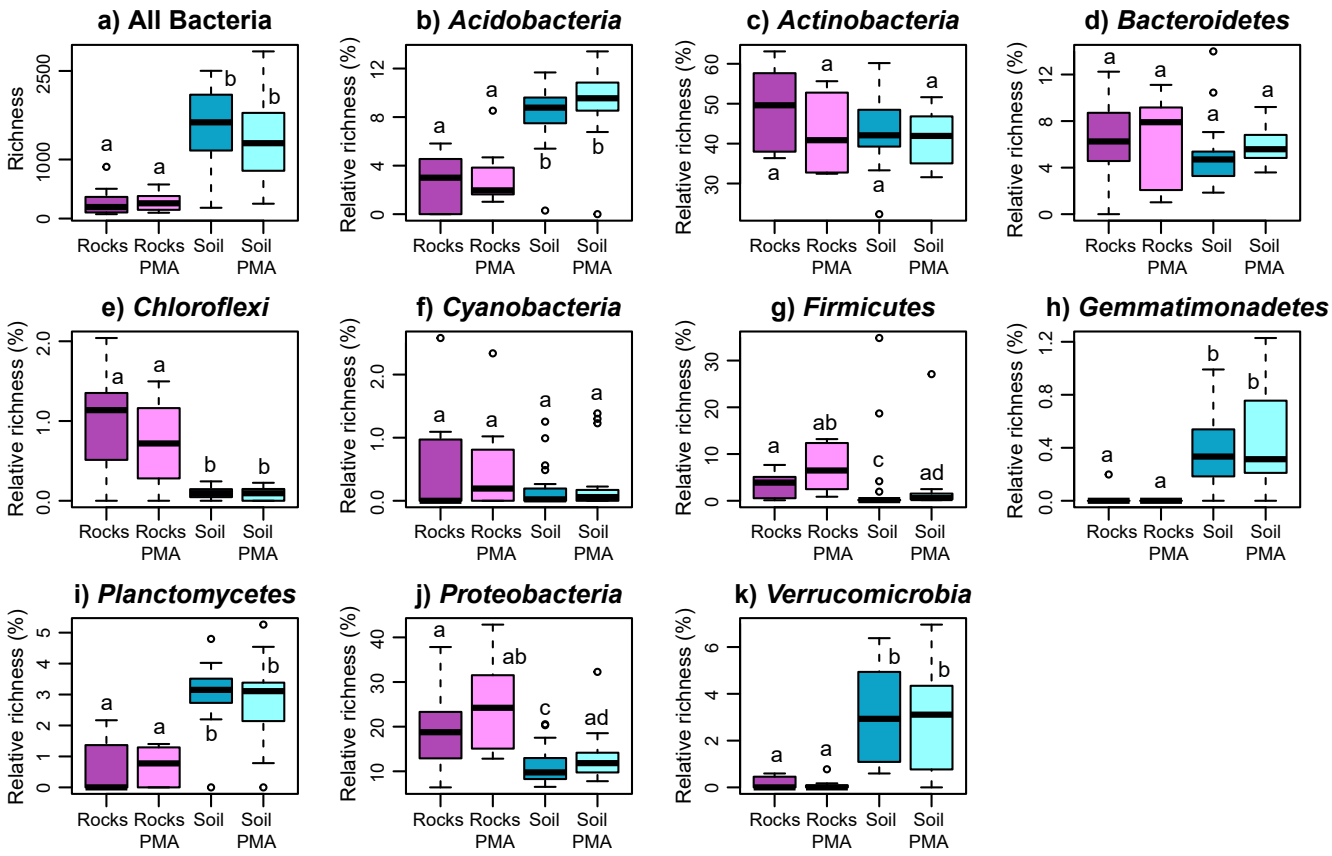

Fig. 2. Richness values within different samples. Differences in the richness of the total community (a) and the relative richness of the most represented phyla (b-k) between rock and soil samples and native and PMA-treated samples. Letters indicate statistically significant differences in a Kruskal-Wallis test followed by Dunn multiple comparisons (p-value < 0.05).

Firmicutes and *Proteobacteria*, which had a slightly higher richness in PMA-treated soil samples (Fig. 2g, j). *Acidobacteria*, *Gemmatimonadetes*, *Planctomycetes*, and *Verrucomicrobia*, had a higher relative richness in soils than in rocks (Fig. 2b, h, i, k), while *Chloroflexi*, *Firmicutes*, and *Proteobacteria* showed the opposite trend (Fig. 2e, g, j). Finally, the type of substrate and the treatment with PMA had no effect on the relative richness of *Actinobacteria* and *Bacteroidetes* (Fig. 2c, d). Similar trends were observed for the relative abundance of the dominant phyla (Fig. S1). No statistically significant differences were recorded among soil samples collected at increasing distances from the rock outcrops in terms of richness and abundance, both in native and PMA-treated samples (figs S2 and S3). Significant differences in the abundance of taxonomic groups between rock and soil samples were identified for several major bacterial orders identified in the communities (Fig. S4), with no statistical differences among soil samples collected at increasing distances from rocks (Fig. S5).

3.3. Differences in community composition

The dispersion of taxa between rock and soil samples and between the samples collected in different localities was not statistically different ($p > 0.05$). NMDS ordinations for native and PMA-treated samples (Fig. 3a, b) resulted in two-dimensional final solutions with stress values of 0.075 and 0.087, respectively. The differences in community composition between rock and soil samples were strongly significant in a Permanova analysis both for native ($R^2 = 0.248$, $p = 0.001$; Fig. 3a) and PMA-treated samples ($R^2 = 0.243$, $p = 0.001$; Fig. 3b). However, excluding rock samples, neither native ($R^2 = 0.126$, $p = 0.178$; Fig. 3c) nor PMA-treated samples ($R^2 = 0.134$, $p = 0.128$; Fig. 3d) showed any statistically significant difference in the composition of soil communities at increasing distances from rock outcrops. Additionally, while rock samples of the three different localities were well differentiated among each other in both native ($R^2 = 0.587$, $p = 0.005$) and PMA-treated samples ($R^2 = 0.576$, $p = 0.003$), a weaker differentiation in community composition was observed for soils of the three different localities, for both native ($R^2 = 0.189$, $p = 0.004$) and PMA-treated samples ($R^2 = 0.213$, $p = 0.001$) (Fig. 3a,b).

Differences in community composition were also confirmed by the distribution of generalist and specialist ASVs in the two distinct habitats from a CLAM test. Generalist ASVs for the two substrata, in native samples, were 172 (2.4 % of the total), mainly assigned to *Actinobacteria* (112 ASVs), while 439 and 2,541 were specialists for rocks and soils, respectively (Fig. 4a). The proportion of generalist ASVs was maintained in PMA-treated samples, with 148 generalist ASVs (2.1 % of the total), and 419 and 2,391 specialists for rocks and soils, respectively (Fig. 4b).

The genera that had significantly higher relative abundance in the two substrata were identified with a lefse analysis, that revealed a higher number of genera enriched in soil than in rock samples. Most of the genera enriched in the two substrata were confirmed also after extracellular DNA depletion. Genera dominant in rocks were *Rhodococcus*, *Rosemonas*, *Ehrlichia*, and *Granulicella*, while genera enriched in soils were *Solirubrobacter*, *Blastocatella*, *Sphingomonas*, *Rubrobacter*, *Gaiella*, *Modestobacter*, and *Marmoricola* (Fig. 4c, d).

3.4. Correlation of soil parameters with bacterial richness, abundance, and composition

All soil samples were coarse textured with more than 90 % sand in 21 of 27 samples. Soils were extremely dry, with water content lower than 4 % in all samples and less than 1 % in more than half of them. Carbon

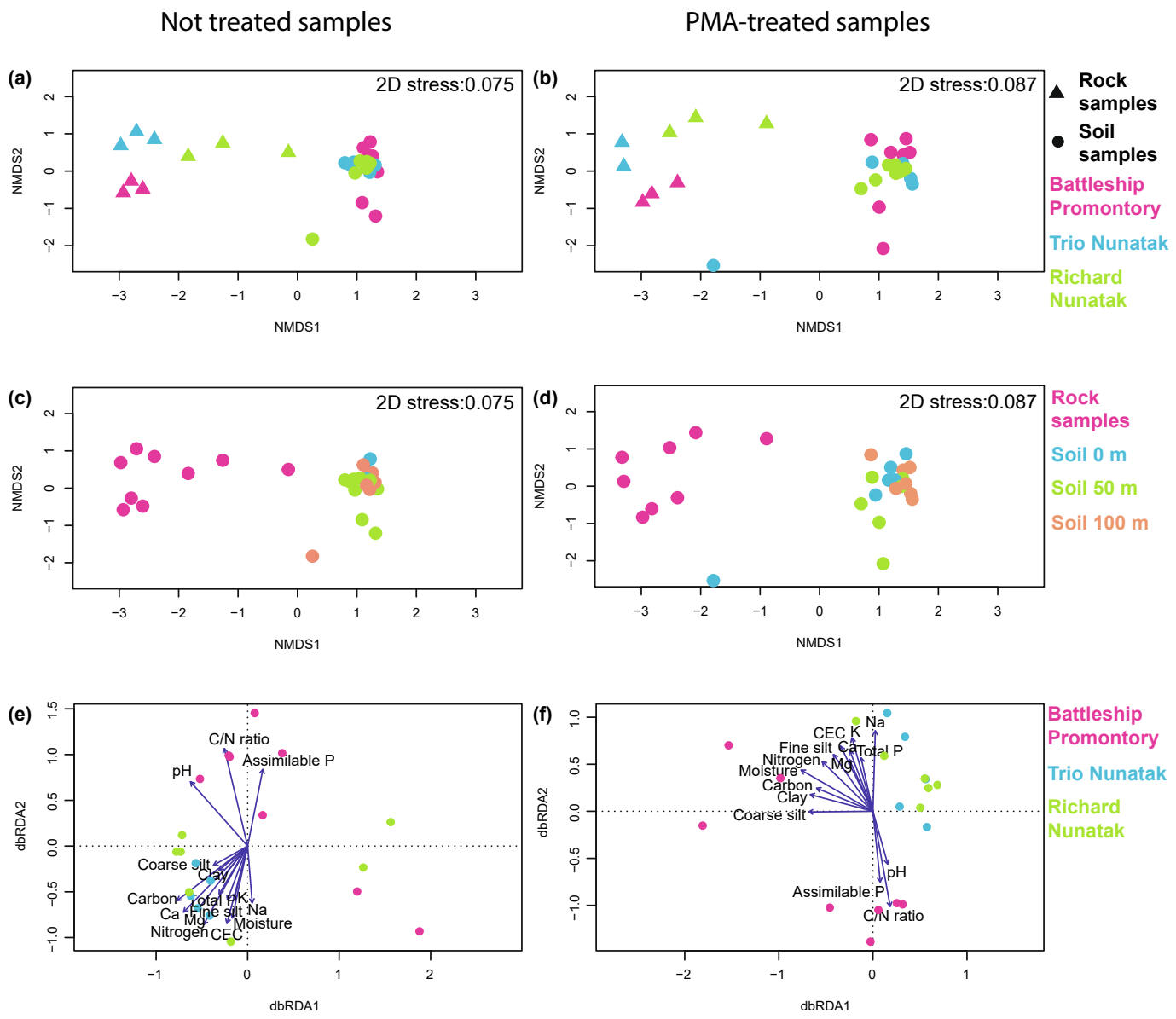

Fig. 3. Communities composition. Nonmetric multidimensional scaling (NMDS) ordinations of the differences (Bray–Curtis distance) in community composition (Hellinger transformed ASVs abundances) for both native (a and c) and PMA samples (b and d). Upper panels (a and b) represent differences among rocks and adjacent soil samples of the three localities. Intermediate panels (c and d) represent differences between the rock and adjacent soil samples at increasing distances. Distance-based redundancy analysis (dbRDA) representing the effect on the variance (Bray–Curtis distance) in soil community composition (Hellinger transformed ASVs abundances) of environmental parameters in native (e) and PMA samples (f).

and nitrogen contents were very low, with the highest values of 0.247 % for carbon and 0.066 % for nitrogen, respectively (table S4).

Few of the correlations tested between physico-chemical parameters and the richness of the total communities and the relative richness of the most representative phyla were significant in native samples, with Mg and Ca being the most significant parameters (Fig. 5a). Instead, when PMA-treated samples were considered, a much higher number of significant correlations was recorded. Among the phyla, *Acidobacteria*, *Bacteroidetes*, *Planctomycetes*, and *Verrucomicrobia* showed the highest number of correlations (Fig. 5b). The most significant parameters were nitrogen, moisture, and exchangeable cations (Na^{+}, K^{+}, Mg^{2+}, and Ca^{2+}), with nitrogen and moisture being always negatively correlated with the relative richness of phyla and the cations being only positively correlated with the relative richness of *Actinobacteria* (Fig. 5b).

Similar results were obtained when correlating soil parameters with the relative abundances of dominant phyla, with PMA-treated samples once again showing a higher number of significant correlations. The observed trends in significant correlations were consistent with those observed for relative richness (Fig. S6).

The effect of environmental variables on soil community composition variance was represented in a dbRDA (Fig. 3e, f). None of the parameters tested was significant in explaining the variance of the bacterial communities in samples not treated with PMA via Permanova analysis (Table 1). Instead, many parameters tested were significant in explaining the observed variance of PMA-treated samples when considered independently (Table 1; Fig. 3f). However, when significant parameters were considered in combination, only soil moisture, carbon, and Ca contents were significant, with moisture being the most significant parameter (Table 1). For the variance in the composition of ASVs belonging to the five most representative phyla (*Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, and *Verrucomicrobia*) in PMA-treated samples, moisture, and exchangeable cation contents were

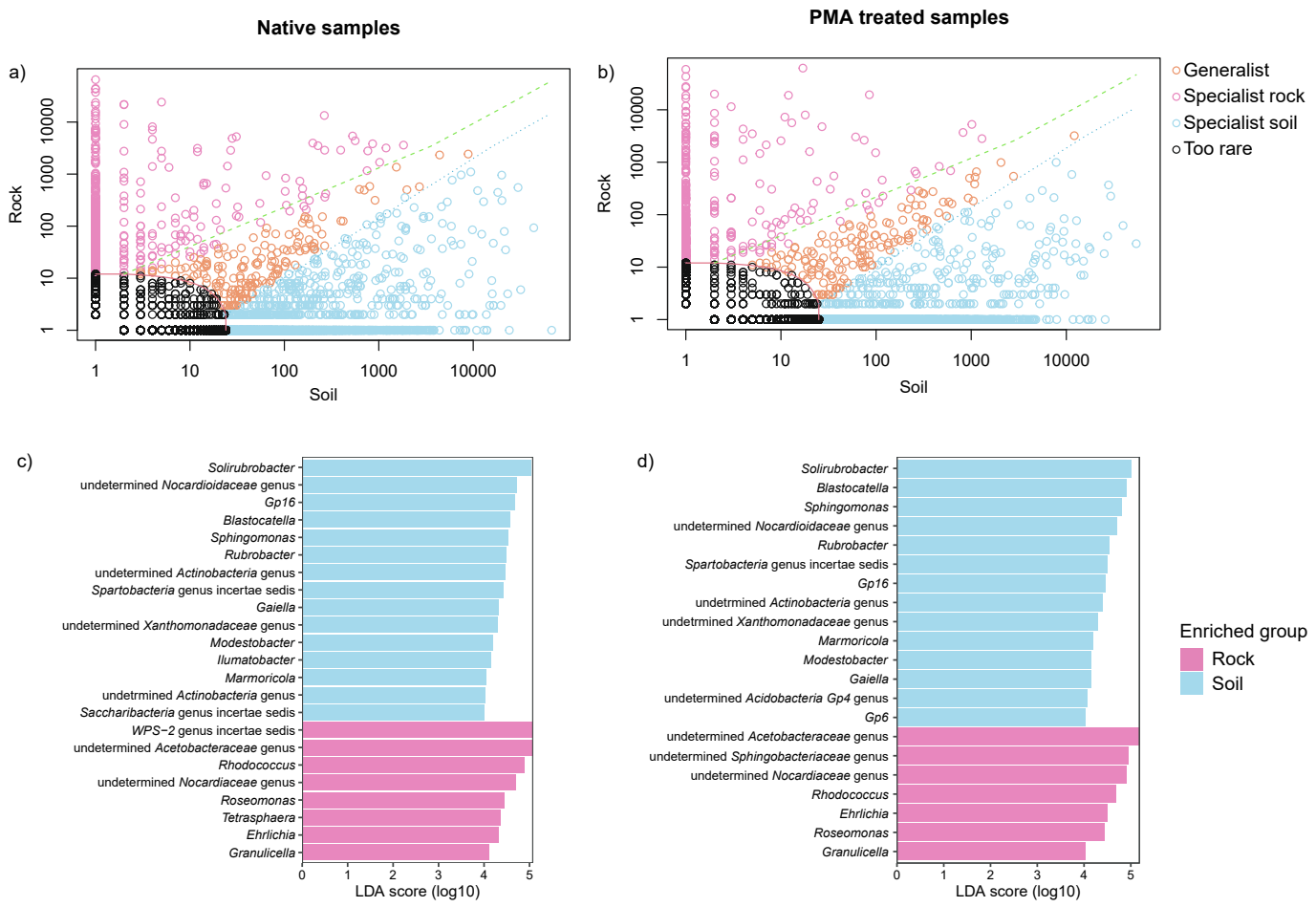

Fig. 4. Taxa shared and differentially selected in rocks and soils. Biplots showing the results of CLAM tests based on pairwise comparisons for native (a) and PMA samples (b). Linear discriminant analysis (LDA) effect size (LEfSe) method for differentially abundant bacterial genera in rocks and soils in native (c) and PMA samples (d).

among the most significant parameters explaining the observed variance in these groups (Tables S5 and S6).

4. Discussion

Molecular studies have advanced our understanding of microbial diversity in Antarctica, but questions remain about dispersal mechanisms and the influence of biological hotspots. In particular, we investigated the potential exchange of bacterial propagules between the lichen-dominated cryptoendolithic communities, which are prevalent in this environment in terms of biomass, and the surrounding soils in three different localities within Victoria Land. Our results show that a large proportion of prokaryotic sequences obtained from the analysis were in common between the rock communities and the adjacent soils, but only a small proportion of ASVs were inferred to be statistically significant generalists of both substrates and are more likely to be exchanged between the two. The proportion of shared ASVs observed in this study (36.6 % and 47.6 % for untreated and PMA-treated samples, respectively) was lower than the 85 % reported by Van Goethem et al. (2016) for endolithic and soil samples from Victoria Valley. However, there was strong variability in this proportion and the mean relative abundance of shared ASVs across the different study sites.

We found abundant ASVs identified as taxa of oligotrophic *Actinobacteria* in both substrata, which were also the most represented group among the generalist ASVs detected, with orders *Actinomycetales* and *Solirubrobacterales*, previously described as dominant in both endolithic and soil niches in Antarctic environments (Lee et al., 2012; Chong et al., 2015; Wei et al., 2016). Strains belonging to these taxa have been characterized as belonging to clades distinct from genomes found outside Antarctica, with locally adapted populations that thrive in Antarctic environments (Ortiz et al., 2021). Additionally, certain species of *Actinobacteria* have been reported as the main players in chemolithoautotrophic carbon fixation (Ji et al., 2017; Bay et al., 2018). Due to their ability to obtain energy sources from atmospheric gases, these species may remain metabolically active in both substrates even in the absence of photosynthetic organisms, such as lichens, which are extremely abundant in endolithic communities but scarce in soils (Canini et al., 2023). Members of *Acidobacteria*, encompassing many oligotrophic species and described as very frequent in Antarctic niches (Ji et al., 2017; Van Goethem et al., 2018), including many endemic species (Ortiz et al., 2021), were shared between rocks and soils. However, many studies suggest that members of this phylum might be mainly metabolically inactive in Antarctic soil environments (Schwartz et al., 2014; Feeser et al., 2018), surviving in different dormant forms (Schwartz et al., 2014; Buelow et al., 2016) and only becoming active for short periods due to their ability to tolerate large fluctuations in soil water content (Ward et al., 2009). Despite its broad ecological amplitude, we found this taxon to be more abundant and diverse in soils compared to rocks, even after the depletion of extracellular DNA by PMA treatment. Instead, *Proteobacteria* were found highly abundant in rock samples. This observation is consistent with several studies conducted on Antarctic endolithic communities (Mezzasoma et al., 2022, Coleine et al., 2020). ASVs assigned to this phylum, in particular those belonging to the orders *Rhodospirillales* and *Sphingomonadales*, were among the

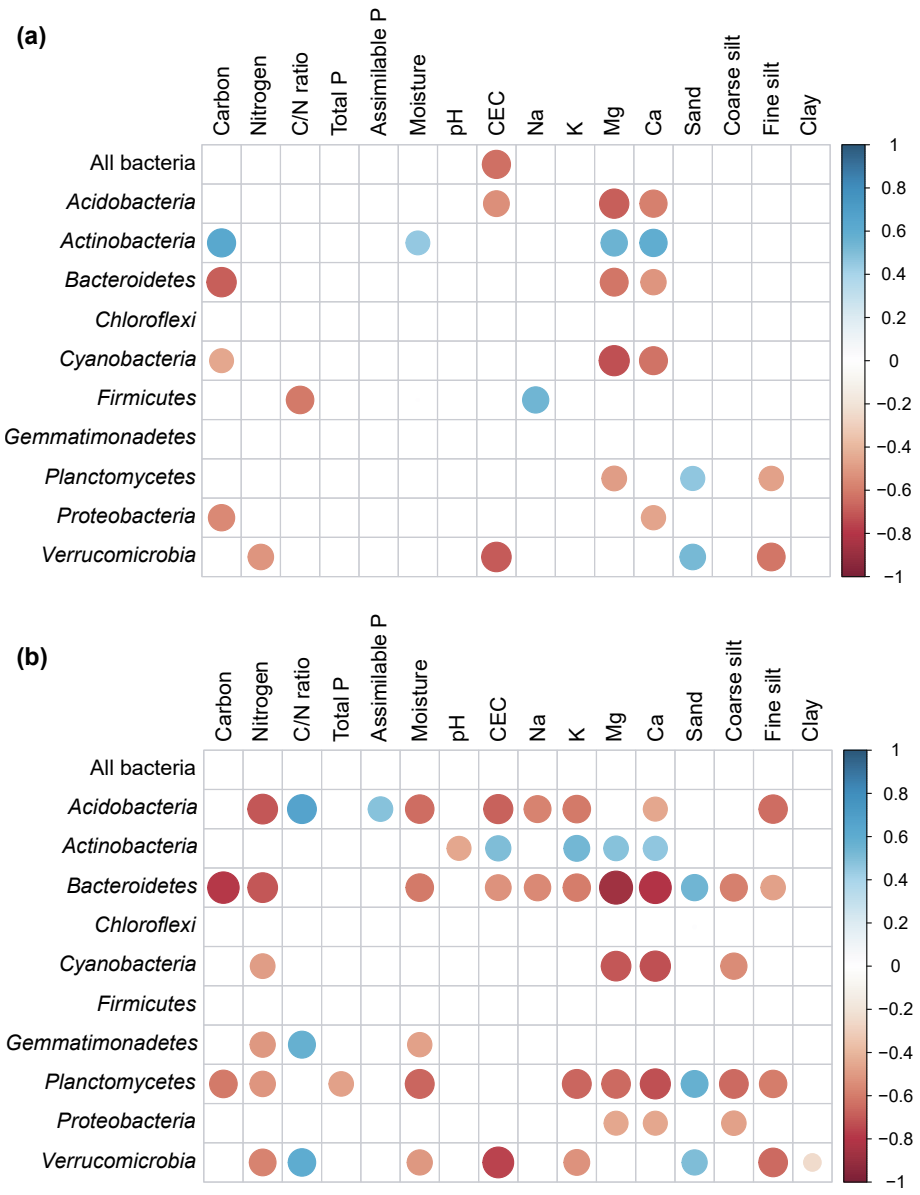

Fig. 5. Correlations with environmental parameters. Spearman's rank correlation of the richness of the total community (all bacteria) and of the relative richness of most represented phyla with the soil physicochemical parameters, measured for both native samples (a) and PMA treated samples (b). Rho values are represented in the correlogram corresponding to the colours and size of the circles. Only significant correlations are shown (p-value < 0.05).

main shared taxa between rocks and soils.

Instead, some genera showed significantly different abundance between the two substrata. Interestingly, the specifically selected genera were more numerous for soils. We found that the four genera dominant in rocks were already reported as widely abundant in Antarctic lichen-dominated cryptoendolithic communities (Mezzasoma et al., 2022). Among them, *Rhodococcus* and *Ehrlichia* were also found in Antarctic soils (Sun et al., 2023; Pearce et al., 2012) and *Granulicella* in soils from cold environments (Männistö et al., 2012). Additionally, *Rhodococcus* was also found in hydrocarbon-polluted Antarctic soils with strong bioremediation potential (Bej et al., 2000). As for the rocks, genera reported as dominant in soils have been documented in many different Antarctic soil studies and only marginally in rock samples (Wang et al., 2015; Huang et al., 2017; Lambrechts et al., 2019), with only *Blastocatella* and *Rubrobacter* having been documented as widely diffused in endolithic communities (de la Torre et al., 2003; Mezzasoma et al., 2022). Additionally, *Rubrobacter* and *Modestobacter*, reported from Dry Valleys water tracks (Niederberger et al., 2015; George et al., 2021), both are known for their resistance to environmental constraints, with *Rubrobacter* having astonishing resistance to desiccation and ionizing radiation (Ferreira et al., 1999) and *Modestobacter* producing melanized cell walls and having been reported from many different desert environments (Mevs et al., 2000; Reddy et al., 2007; Golinska et al., 2020). This confirms that these genera are representative of the groups that are differentially selected between rocks and soils.

Although the endolithic environment hosts the highest standing biomass in this area (Cowan and Tow, 2004; Cary et al., 2010; Cowan et al., 2010; Archer et al., 2017), comparisons with soils collected from the same areas demonstrated astonishingly higher diversity in the soil environment, as also highlighted by other similar studies (Van Goethem et al., 2016; Rego et al., 2019). This makes it clear that, despite the release of biomass from rocks and a potential exchange between soils and rocks, there are still undetermined sources of microbial propagules playing that may be transported through winds blowing in the area, such as microbial mats surrounding lakes and ponds, which may play a crucial role in the soil composition of this region. Additionally, the

Table 1
Effect of environmental parameters on communities composition. Proportion of variation in community composition explained by soil physicochemical parameters considered independently for both native and PMA-treated samples, and considered in a combined model for PMA-treated samples, depending on their independent influence in the variance

Independent parameters					Combined parameters PMA samples		
	Native samples		PMA samples				
Variable	Variance	p-value	Variance	p-value	Variable	Variance	p-value
Carbon	8,17	0.073	**11,73**	**0.039**	**Moisture**	**13,16**	**0.0070**
Nitrogen	7,89	0.111	**11,31**	**0.029**	**Carbon**	**8,24**	**0.0437**
C/N ratio	8,32	0.159	10,09	0.076	Clay	4,14	0.5440
Total P	5,76	0.326	7,79	0.099	Sand	7,86	0.0508
Assimilable P	5,99	0.243	7,52	0.156	Nitrogen	6,18	0.1659
Moisture	6,70	0.201	**13,16**	**0.008**	**Ca**	**9,55**	**0.0093**
pH	6,96	0.188	6,94	0.181	Mg	2,22	0.9557
CEC	6,91	0.179	**9,99**	**0.039**	Fine silt	4,27	0.5082
Na	4,50	0.514	**9,60**	**0.045**	CEC	5,56	0.2182
K	4,97	0.511	9,30	0.070	Na	3,22	0.7922
Mg	8,50	0.063	**10,32**	**0.040**	Residual	35,60	
Ca	8,18	0.099	**10,68**	**0.023**			
Sand	5,82	0.280	**11,41**	**0.034**			
Coarse silt	3,98	0.751	12,80	0.060			
Fine silt	5,92	0.278	**9,89**	**0.036**			
Clay	5,26	0.398	**11,71**	**0.040**			

biomass released from rocks could be an important source of organic matter for life within soils. The temperatures recorded for rocks and soils in the three stations were always higher than ambient air temperatures, with soils providing a niche characterized by lower mean temperatures and less daily variation compared to rocks (Fig. 1), which could be one of the reasons for the higher diversity observed in soils, however, it is evident that also other factors could play a significant role in the differentiation of the communities between the two substrates.

The absence of diversity and composition gradients in soils at increasing distances from the rocks, despite accounting for the predominant wind direction, may be attributed to a general homogenization caused by the wind patterns in this region (Doran et al., 2002). Consequently, these winds likely affect the transport of abiotic materials and the dispersal of microbial propagules throughout the entire area considered (Šabacká et al., 2012). Aeolian transport could potentially mitigate the deterministic effects of rock dispersal in contributing to the edaphic microbial communities (Lemoine et al., 2023).

In terms of the effect of extracellular DNA or DNA from damaged cells on the observed diversity, a significant proportion of ASVs were found to be absent after PMA treatment. At the taxonomic level, PMA treatment resulted in a significant increase in the relative richness and abundance of the phyla *Firmicutes* and *Proteobacteria*, particularly the order *Cytophagales*. Similar studies also showed increased representation of specific taxa after PMA treatment. For instance, Carini et al. (2016) found a significant increase in the abundance of *Actinobacteria* and *Alphaproteobacteria* and a decrease in *Verrucomicrobia* after relic DNA removal. In a comparison of DNA extraction methods that include or exclude extracellular DNA removal in Antarctic soil samples, Tahon et al. (2018) reported an increase in the relative abundance of *Acidobacteria* and a decrease in *Actinobacteria*, *Cyanobacteria*, *Chloroflexi*, and *Bacteroidetes*. This indicates that the effect of extracellular DNA at the taxonomic level may be strongly variable and needs to be assessed in each study to accurately estimate the diversity of microorganisms potentially present in a viable state within the examined communities. However, despite this variability, the impact of extracellular DNA was less pronounced when examining shared or differentially selected ASVs and taxa between rocks and soils.

When using DNA-based approaches to infer taxonomic abundance and diversity, the assessment of relic DNA becomes pivotal in understanding the key drivers of soil diversity and functionality. In our study, the importance of evaluating extracellular DNA was particularly evident when considering the effect of physico-chemical parameters on bacterial richness and variance, which was mostly hidden in samples not treated with PMA. Indeed, the strong effect of local environmental filtering on Antarctic soil microbial communities is well documented (Cary et al., 2010; Tytgat et al., 2016; Lee et al., 2019). In our case, the limited effect of soil physico-chemical variables, as described for samples not treated with PMA, was not consistent with the abovementioned works. Conversely, the depletion of extracellular DNA allowed us to highlight a strong effect of several abiotic variables, in particular soil texture and exchangeable cations, which have already been described as main predictors of microbial diversity in similar Antarctic environments (Canini et al., 2021; Severgnini et al., 2021).

5. Conclusions

The simplified structure of terrestrial Antarctic communities permitted us to identify the patterns of microbial colonization in the two substrata, rock and soil, in a straightforward way. Although the two substrata shared numerous ASVs, their different selective pressures influenced the distribution of several taxa, resulting in the uniqueness of each substratum. This effect was also highlighted after extracellular DNA depletion. This leads us to speculate that several microbial propagules may be actively thriving in both substrata, especially since the shared taxa between them are known for their astonishing metabolic adaptations to this environment. These findings provide new important information into microbial propagule sources and survival mechanisms within extreme environments. Nevertheless, the greater diversity found in soils indicates that other sources contribute to the overall diversity of this environment. Our results also indicate that the presence of extracellular DNA can inflate estimates of microbial community abundance and composition, even if the full extent of relic DNA influence on current Antarctic soil surveys has yet to be fully evaluated. To the best of our knowledge, while some authors have already reported significant effects of soil extracellular DNA on describing spatial and temporal variability in communities (Fierer et al., 2017), this study represents the first demonstration of how it can limit our ability to detect the extent to which environmental parameters structure soil communities. Finally, this study provides a baseline characterization of microbial diversity in rock and soil in relation to soil physico-chemical characteristics in sites where a long-term monitoring system has been established to measure biologically relevant environmental parameters in order to track the response to climate change.

CRediT authorship contribution statement

Fabiana Canini: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Byron J. Adams: Writing – review & editing. **Luigi P. D'Acqui:** Writing – review & editing, Investigation. **Federica D'Alò:** Writing – review & editing. **Laura Zucconi:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets generated and analysed during the current study are available in the NCBI SRA repository, BioProject ID: PRJNA922885.

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Appendix A. Supplementary data

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