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RESEARCH ARTICLE

Highly differentiated soil bacterial communities in Victoria Land macro-areas (Antarctica)

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One sentence summary: This study characterizes the diversity and composition of highly adapted soil bacterial communities in three distinct ice-free environments of Victoria Land, in Antarctica, in relation to different physicochemical edaphic parameters.

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ABSTRACT

Ice-free areas of Victoria Land, in Antarctica, are characterized by different terrestrial ecosystems, that are dominated by microorganisms supporting highly adapted communities. Despite the unique conditions of these ecosystems, reports on their bacterial diversity are still fragmentary. From this perspective, 60 samples from 14 localities were analyzed. These localities were distributed in coastal sites with differently developed biological soil crusts, inner sites in the McMurdo Dry Valleys with soils lacking of plant coverage, and a site called Icarus Camp, with a crust developed on a thin locally weathered substrate of the underlying parent granitic-rock. Bacterial diversity was studied through 16S rRNA metabarcoding sequencing. Communities diversity, composition and the abundance and composition of different taxonomic groups were correlated to soil physicochemical characteristics. Firmicutes, Bacteroidetes, Cyanobacteria and Proteobacteria dominated these communities. Most phyla were mainly driven by soil granulometry, an often disregarded parameter and other abiotic parameters. Bacterial composition differed greatly among the three macrohabitats, each having a distinct bacterial profile. Communities within the two main habitats (coastal and inner ones) were well differentiated from each other as well, therefore depending on site-specific physicochemical characteristics. A core community of the whole samples was observed, mainly represented by Firmicutes and Bacteroidetes.

Keywords: Victoria Land; soil communities; metabarcoding; 16S; edaphic parameters; environmental filtering

INTRODUCTION

Antarctica is one of the most extreme environments on Earth, with conditions precluding the survival of most of Earth's life forms, basically allowing microorganism to be the dominant one (Pearce 2012). Only 0.34% (44 000 km^2) of the continent is seasonally or permanently free of ice (Hopkins *et al.* 2006). Ice-free areas

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include the lower-latitude Antarctic Peninsula on the West side, high-altitude mountain peaks of the Ellsworth and Transantarctic Mountains and of the highest mountains in East Antarctica, coastal sites, isolated nunataks, and the McMurdo Dry Valleys.

Victoria Land covers a latitudinal gradient of approximately 8 degrees (from 70°30′ to 78°00′ S), and extends from the Ross Sea to the edge of the Polar Plateau. It consists of two of the 16 biologically distinct, ice-free, Antarctic Conservation Biogeographic Regions (ACBRs), namely North and South Victoria Land, with the Transantarctic Mountains forming a third, encompassing the continent and close-lying islands (Terauds and Lee 2016). This region hosts a wide range of different niches, with minimal human perturbations, suitable for microbial colonization. Many coastal sites of Northern Victoria Land, thanks to milder climatic conditions, have high level of soil moisture contents during the austral summer, leading to the spreading of a vegetation coverage of mosses and lichens. Close to penguin rookeries (e.g. Cape Hallet and Edmonson Point), inputs from penguin guano, feathers, eggs, and corpses result in higher carbon and nitrogen contents in soils. Moving toward the interior of the continent and from sea level to higher elevation sites, the vegetation coverage disappears, soils are often missing and rocks are the main substratum supporting microbial communities (Zucconi *et al.* 2016). South of David Glacier, many isolated nunataks are seasonally free of ice. The McMurdo Dry Valleys in Southern Victoria Land represent the largest ice-free area of the continent, covering about 4500 km^2 (over 15% of the ice-free land on the continent), between the east side of the Transantarctic Mountains and the Ross Sea and Ice Shelf (Bockheim and McLeod 2008). These valleys consist of a pristine mosaic of ice-covered lakes, seasonally glacial-melt-water streams and extremely arid mineral soils (Cary *et al.* 2010). Soils at the margins of lakes and ephemeral ponds and streams, hosting microbial mats (Priscu *et al.* 1999; Laybourn-Parry and Wadham 2014), possibly contribute to the sole primary production of the area and to the diversity, spreading in the surrounding soils organisms, carbon and nitrogen, thanks to wind dispersion (Bottos *et al.* 2014).

Although exposed soils of the ice-free areas only represent a small portion of the continent, they concentrate most of the terrestrial biota and are important oases supporting unique edaphic communities (Bottos *et al.* 2014). Microorganisms are the dominant component of these soils, playing key roles in their ecology and sustainability. Antarctic soil communities appear to be highly specialized and almost entirely structured by abiotic factors, due to extremely limited biotic interactions (Hogg *et al.* 2006; Chong, Pearce and Convey 2015; Van Goethem *et al.* 2016; Lee *et al.* 2019). These communities have to cope with a combination of extreme environmental conditions, such as low temperatures, low water and nutrient availability, high solar and UV radiations and frequent freeze-thaw cycles, and have developed many astonishing adaptations (Onofri *et al.* 2007). Species composing Antarctic communities are often endemic, due to the long-time geographic isolation of Antarctica (Vyverman *et al.* 2010; Durieu *et al.* 2019). Significant advances in our understanding of diversity and functionality of these communities have been gained in the last decades, thanks to the development of new cultivation-independent molecular methods. Despite these advances, Antarctic soils still remain a poorly explored environment and there is still a lack of a clear picture of which forces direct most the composition and function of microbial communities.

The bacterial phyla most frequently observed in Antarctic soils, although with different relative abundances between diverse regions, are Actinobacteria, Proteobacteria, Bacteroidetes, Acidobacteria, Gemmatimonadetes, Deinococcus-Thermus, and Cyanobacteria (Pointing *et al.* 2009; Lee *et al.* 2012; Bottos *et al.* 2014; Ji *et al.* 2015; Wei *et al.* 2015). Many studies also analyzed the diversity of other microbial groups as fungi and algae, both in Victoria Land and in the maritime Antarctica (for example Rosa *et al.* 2020a; Canini *et al.* 2020; 2020b; 2021; Hopkins *et al.* 2021; Newsham *et al.* 2021). All the available studies reported a high proportion of unclassified, unknown and possibly unique taxa, suggesting the need for a deeper microbiological characterization of these environments (Vincent 2000).

Cyanobacteria significantly contribute to the microbial diversity of these soils and increase their stability and nutrient concentration by carbon and nitrogen fixation (Kirby *et al.* 2011). Cyanobacteria, in association with the mineral substrate, other microorganisms (e.g. microfungi, algae, heterotrophic bacteria) and, when present, mosses and lichens, make up the biological soil crusts (BSCs), widely diffused in cold or arid regions (Belnap, Büdel and Lange 2001; Pointing *et al.* 2015).

Reports on bacterial communities in Northern Victoria Land are scattered and only few recent molecular studies are available for this area (e.g.: Niederberger *et al.* 2008; and Kim *et al.* 2015).

The bacterial community of McMurdo Dry Valleys soils has been shown to vary considerably both between and within different sites, as well as from those observed in other regions of the continent (Cary *et al.* 2010). Different drivers have been suggested to explain the bacterial diversity and composition of this area, such as water availability, altitude, salt content, total carbon content and other elements (Aislabie *et al.* 2006; Wood *et al.* 2008; Zegling *et al.* 2011; Lee *et al.* 2012). Clear spatial patterns of bacterial diversity among inland isolated nunataks in Dronning Maud Land were observed too, with bacterial profiles distinct from those found in other Antarctic biogeographic regions (Staebe *et al.* 2019). More recently, new metagenomics approaches revealed diverse microbial communities in East Antarctic soils, but sharing common cold-responsive stress genes necessary for their survival and sustenance in the extreme Antarctic conditions (Koo *et al.* 2018). Despite the great number of existing works, many of them are focused on scattered sampling areas and the parameters determining bacterial communities diversification in these environments are still poorly understood.

Atmospheric and environmental changes are significantly altering local physicochemical conditions of several Antarctic bioregions, with visible changes over a relatively recent time (Flocco, Mac Cormack and Smalla 2019). These changes are expected to alter microbial communities composition and functionality depending on physicochemical soil properties. Possible effects on ecosystems biogeochemistry and functioning have already been suggested as well (Kleinteich *et al.* 2017).

The need to deepen our knowledge of the structure and function of the bacterial community in Antarctic soils is imperative to monitor and predict ecological responses in these changing environments (Bottos *et al.* 2014). The risks of changes in microbial communities structure and the loss of significant biodiversity, due to the disappearance of endemic species and non-native introductions have already been denounced (Cowan *et al.* 2018; Convey and Peck 2019). There is a real risk that this may happen under our eyes without any knowledge, as the real biodiversity of these soils is not yet fully known.

With this study, we aimed to characterize bacterial communities associated to unvegetated soils and BSCs along a wide lat-

itudinal gradient encompassing the majority of environments within Victoria Land, through the analysis of 60 samples from 14 localities. The localities have been grouped in three macrohabitats, namely 'Coastal Sites', characterized by the presence of BSCs, inner sites in 'Dry Valley' with soils without evident plant coverage, and a third site close to the 'Mario Zucchelli' Italian Base, named 'Icarus Camp', where samples were collected from a thin layer of weathered material derived from parent granite-rocks slightly sloping towards the sea. We aimed to investigate differences among these macrohabitats and understand the effect of the plant coverage and of associated physicochemical parameters on soil bacteria. This baseline characterization is critically important for monitoring possible future changes in these environments and their inhabitants.

MATERIALS AND METHODS

Sample collection

Samples were collected in sites from 14 localities of Victoria Land, Antarctica, along a latitudinal gradient ranging from 73°31'S (Apostrophe Island) to 77°42'S (Lake Joyce, Taylor Valley). Eight out of 14 localities were located in coastal sites (CS), all in Northern Victoria Land except for Botany Bay, the southernmost one, on the northernmost border of South Victoria Land. All coastal sites were characterized by the presence of soils with BSCs coverage, and, in each locality, two or more sites were selected, underneath crusts of different developmental stages. Botany Bay and Edmonson Point, among the localities, have been designated as ASPAs (Antarctic Specially Protected Areas N. 154 and N. 165, respectively) based on their high richness in mosses and lichens. A further location was Icarus Camp (unofficial name) (IC), located 2 km (1.2 mi) south of the 'Mario Zucchelli' Italian Base (Terra Nova Bay) in the vicinity of a meteorological station. Samples from this locality were analyzed separately, due to the presence of a pristine crust not comparable with the soils from other localities. Samples were collected on a thin layer of weathered material locally derived from parent granite-rocks sloping towards the sea. Finally, five localities were located in the McMurdo Dry Valleys (South Victoria Land) (DV), close to the major lakes of the three main valleys, namely Victoria, Wright and Taylor Valley, but far enough to not be influenced by the water ecosystem (Fig. 1).

In each sampling site, except for Icarus Camp, three replicate samples of about 600g were collected, 100 g for molecular and 500 g for physicochemical analyses, respectively. The replicate soil samples were collected aseptically in an area of about 2 m^2, at 0–5 cm depth after BSCs removal, when present, from the top of the soil. At Icarus Camp, small amounts of the superficial crust were aseptically collected in three different points. Samples were stored at −20°C in sterile bags until they arrived in Italy (about six months) for molecular analyses.

Soil physicochemical parameters

Soil mineral samples were air dried and <2 mm sieved before shipping (about 500 g each sample). Total soil organic C and N were measured by combustion with an elemental analyzer NA 1500 CHNS (Carlo Erba, Milan, Italy). Particle size distribution, cation exchange capacity (CEC) and pH in a 1:2.5 soil: water suspension were determined according to the SISS (Società Italiana della Scienza del Suolo) methods (Colombo and Miano 2015). Soil particle size distribution was characterized 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). Soil exchangeable bases (Na^+, Ca^{2+}, K^+ and Mg^{2+}) were determined by flame atomic absorption spectrometry (AAS 1100B, PerkinElmer, Waltham, MA, USA).

DNA extraction, amplification and sequencing

An aliquot of each sample was homogenized by a pestle and a mortar to help cell lysis by the extraction kit. Total DNA was extracted from 0.2–0.4 g of each homogenized sample, using PowerSoil® DNA Isolation Kit (Mo Bio Inc., Carlsbad, CA, USA), according to the manufacturer's protocol, and stored at −20°C until further processing.

DNA concentration and quality were determined using a NanoDrop ND1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and a TapeStation 2200 (Agilent Technologies, Santa Clara, CA, USA). The V3-V4 hypervariable regions of the 16S ribosomal RNA (rRNA) gene were amplified according to the Illumina 16S Sample Preparation Guide (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf) (Illumina, San Diego, CA, USA). Indexed libraries were prepared by equimolar (4 nmol/L) pooling and sequenced on Illumina MiSeq platform with a 2 × 300 bp run, according to Illumina's instructions.

Bioinformatic analysis of microbiota profiling

The 16S rRNA raw sequences were merged using Pandaseq (Masella *et al.* 2012), then low quality reads (i.e. showing stretches of bases with a Q-score <3 for more than 25% of their length) were discarded. Samples with less than 20000 high-quality reads (19 out of initial 79) were discarded. A subset of 100000 reads for each sample was randomly extracted, in order to obtain a similar number of reads for each sample. Bioinformatic analyses were conducted using the QIIME pipeline (release 1.8.0; Caporaso *et al.* 2010). Filtered reads were de-duplicated and de-noised creating zero-radius Operational Taxonomic Units (zOTUs) by unoise3 algorithm (Edgar 2016) in usearch (v. 11.0.667), discarding those with less than 5 supporting reads. Taxonomic assignment was performed via the RDP classifier (Wang *et al.* 2007) against the Greengenes database (release 13.8; ftp://greengenes.microbio.me/greengenes_release/gg_13_8_otus), with a 0.5 identity threshold. After classification, zOTUs classified as putative chloroplasts or mitochondria were also removed. Dataset was downsampled to the least sequenced sample, in order to have a comparable picture of the taxonomic composition.

Alpha-diversity was measured using Faith's phylogenetic diversity metric (PD_whole_tree), whereas weighted and unweighted UniFrac distances and Principal Coordinates Analysis (PCoA) were used to represent the microbial community structure in beta-diversity analysis.

Statistical analysis

In the analysis of microbial profiles, statistical evaluation among alpha-diversity indices was performed by a non-parametric Monte Carlo-based test, using 9999 random permutations. Permutational multivariate analysis of variance using distance matrices (*adonis* function) in the R package 'vegan' (version 2.0–10; Oksanen *et al.* 2013) was carried out in order to compare the microbial community structure in beta-diversity analysis on unweighted and weighted Unifrac distances (Lozupone *et al.* 2011). For relative abundance analysis, a Kruskal-Wallis test was

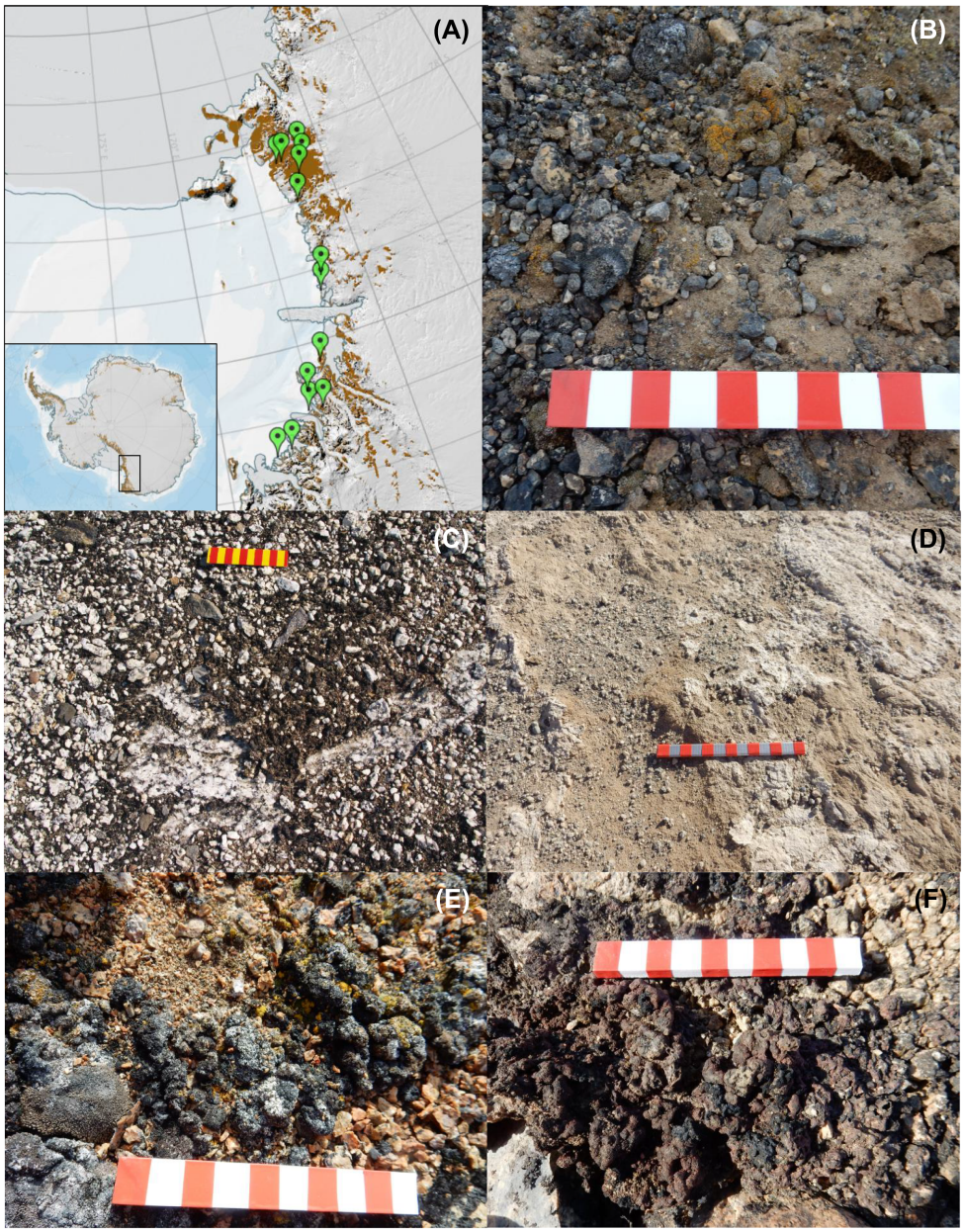

Figure 1. Map of the sampling sites along Victoria Land **(A)** and examples of the different types of samples: **(B)** Apostrophe Island; **(C)** Icarus Camp; **(D)** Lake Joyce; **(E)** Mount McGee; **(F)** Botany Bay (Scale bar 1 cm).

used, followed by multiple pairwise comparisons, choosing a P-value <0.05 as the threshold for statistical significance.

Soil physicochemical parameters were correlated to bacterial composition by calculating Spearman's correlation coefficient; only correlations with a statistically non-zero coefficient (P-value <0.05) were considered.

Permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) was carried out on weighted Unifrac distance matrices of Hellinger-transformed OTU tables with 9999 permutations, with the *adonis* function, in order to determine the effect of each soil parameter on the observed variance of the total community, the communities of CS and DV, and the dominant phyla. To account for correlations among environmental variables, a forward selection of parameters, based on the previous results, was performed, including only significant environmental variables in the final models.

RESULTS

Taxonomic distribution

The final data set was composed of 60 samples out of the initial 79, from 14 localities (Table 1). For the majority of sampling sites, we obtained high quality sequences for at least 2 replicated samples; rarely, for some locations (i.e. Mount McGee and Lake Vanda) we only had 1 sample, due to insufficient DNA quantity during extraction or to low sequencing yield. Eight localities were in coastal sites (CS) (42 samples), characterized by differ-

Table 1. List of sampling sites with coordinates. Altitude, biocrust description and number of samples analysed for each site.

Macro-habitat	Sampling site	Number of samples	Latitude	Longitude	Altitude (m a.s.l.)	Description
Icarus Camp	Icarus Camp site 1	4	74°42'07.1'S	164°0.7'12.6'E	41	Thin crust on a just altered rock substrate
	Icarus Camp site 2	2	74°42'09.0'S	164°0.7'13.3'E	41	"
Coastal sites	Apostrophe Island site 1	2	73°31'09.5'S	167°25'55.3'E	41	Grey crust with few mosses
	Apostrophe Island site 2	3	73°31'10.5'S	167°25'55.3'E	41	Brown thin crust with mosses and lichens
	Apostrophe Island site 3	3	73°31'14.2'S	167°25'56.0'E	37	Low developed brown crust
	Cape King site 1	1	73°35'08.2'S	166°37'19.2'E	144	Black highly developed crust with mosses
	Cape King site 2	1	73°35'08.9'S	166°37'3.5'E	124	Dry thin and black crust with very few mosses
	Cape King site 3	2	73°35'08.5'S	166°37'9.0'E	100	Highly developed crust dominated by mosses. with lichens
	Kay Island site 1	2	74°04'12.6'S	165°18'59.5'E	190	Low developed crust. with some mosses
	Kay Island site 2	3	74°04'11.8'S	165°18'58.7'E	61	Well developed crust. with more mosses
	Mount McGee site 1	1	74°0.0'59.9'S	164°21'28.8'E	185	Medium developed crust. with diffuse moss coverage. Presence of skuas all around
	Edmonson Point site 3	1	74°19'45.2'S	165°07'35.8'E	31	Increasing degrees of crust development. with any apparent colonization in site 3. to a highly developed crust completely covered by mosses and lichens in site 6
	Edmonson Point site 4	4	74°19'45.1'S	165°07'38.7'E	30	"
	Edmonson Point site 5	3	74°19'45.2'S	165°07'49.9'E	30	"
	Edmonson Point site 6	2	74°19'44.8'S	165°07'42.1'E	29	"
	Edmonson Point site 1	2	74°19'37.7'S	165°07'56.5'E	61	Low developed crust. with few mosses
	Edmonson Point site 2	1	74°19'44.9'S	165°07'39.9'E	29	Thin signs of biological colonization
	Prior Island site 1	1	75°40'52.9'S	162°53'38.3'E	102	Grey. thin and low developed crust
	Prior Island site 2	2	75°40'54.5'S	162°53'45.8'E	98	Wet highly developed crust dominated by mosses
	Starr Nunatak site 1	2	75°53'57.9'S	162°35'31.2'E	108	Well developed crust dominated by mosses
	Starr Nunatak site 2	1	75°53'58.7'S	162°35'29.7'E	108	"
	Starr Nunatak site 3	1	75°53'55.9'S	162°35'35.2'E	103	"
	Starr Nunatak site 4	2	75°53'55.3'S	162°35'32.2'E	118	"
	Botany Bay site 1	1	77°00'26.0'S	162°32'39.4'E	115	Black thin crust disconnected from the below soil
	Botany Bay site 2	1	77°00'26.7'S	162°32'40.5'E	94	Black. highly developed crust
Dry Valleys	Lake Fryxell site 1	2	77°36'7.2'S	163°16'5.4'E	28	Well diffuse superficial crust. presence of saline efflorescence
	Lake Fryxell site 2	1	77°36'14.4'S	163°16'13.0'E	21	"
	Lake Hoare site 1	2	77°37'26.2'S	162°53'27.8'E	83	Low developed crust. with saline efflorescence
	Lake Hoare site 2	1	77°37'25.0'S	162°53'27.5'E	91	Medium developed crust
	Lake Joyce site 1	2	77°42'21.1'S	161°34'14.3'E	448	Whitish consistent crusts on a sandy soil
	Lake Vanda site 1	1	77°31'46.0'S	161°34'32.0''E	n. a.	Thin surficial mineral crust
	Lake Vida site 1	2	77°22'28.0'S	161°49'14.6'E	367	Soils with relicts of algal mat
	Lake Vida site 2	1	77°22'20.9'S	161°49'55.3'E	367	"

ent stages of soil crusts development. Five localities were in the Dry Valleys area (DVs) (12 samples), with mostly mineral crusts. Finally, Icarus Camp (6 samples), close to the Italian Base, was characterized by a thin crust coverage on a recently altered rock substrate.

High-throughput sequencing produced a total of 6108017 reads (average of 77 317 ± 88 655 per sample) which, after filtering and downsampling, resulted in 4038617 reads (average of 67 310 ± 25 555 reads per sample). After de-noising and chloroplast/mitochondria and low-abundance zOTU removal (i.e. zOTUs with <5 supporting reads across all samples), a high-confidence dataset of 7201 zOTUs (average of 2014 ± 533 zOTUs per sample) was considered. 78 out of 7201 zOTUs (1.1%) were present in only one sample, and 1032 (14.3%) in at least 50% of the samples, indicating that the composition of each sample was somehow localized. Besides this, we found a core community of 153 zOTUs present in at least 75% of the whole samples. Firmicutes and Bacteroidetes were the two most common phyla within the core community (124 and 25 zOTUs, respectively). Regarding the relative abundance of the different phyla, Firmicutes (average abundance per sample 28.3% ± 21.1% of the total reads) was the dominant group, followed by Bacteroidetes (average rel. ab.: 25.0% ± 10.5%), Cyanobacteria (average rel. ab.: 21.4% ± 17.2%of the total reads) and Proteobacteria (average rel. ab.: 10.7% ± 7.9%) (Fig. S1, Supporting Information). On average, the eight most abundant phyla accounted for more than 97% of the total reads. At family level, 782 zOTUs (10.9% of the total) remained unidentified, and the most abundant families were Ruminococcaceae (14.8% ± 10.9%), Nostocaceae (9.0% ± 10.8%), Bacteroidaceae (7.5% ± 6.1%), Lachnospiraceae (6.2% ± 4.6%), Chitinophagaceae (6.2% ± 7.5%), Phormidiaceae (6.0% ± 8.6%) and Pseudoanabaenaceae (5.2% ± 6.2%). Collected samples showed, in general, a distinct bacterial composition, with a substantial part made by low-abundance families (i.e. <1% rel. ab.), accounting for about 17.5% of total abundance, on average. Finally, among genera, the most abundant ones were *Nostoc* (7.7% ± 9.9%), *Bacteroides* (7.5% ± 6.1%), *Phormidium* (6.0% ± 8.6%), *Faecalibacterium* (5.2% ± 3.8%), *Akkermansia* (3.1% ± 2.5%), *Leptolyngbya* (2.9% ± 4.0%) and *Flavobacterium* (2.6% ± 6.5%); 2743 zOTUs (38.1%) remained unidentified at genus level (Fig. S1, Supporting Information).

Soil properties

Nitrogen and carbon contents were highly variable. N content was generally lower in DV sites, ranging from 0.005% at Lake Joyce to 0.019% at Lake Fryxell, compared to CS, where values up to 1.093% and 2.031% were recorded at Prior Island and Starr Nunatak, respectively. The same trend was observed for C content, except for Edmonson Point where exceptionally low carbon and nitrogen values were recorded, possibly due to collection sites located far from sites frequented by animals. The pH mostly ranged from slightly acidic to slightly basic values, but with a minimum value recorded at Starr Nunatak (4.82) and a maximum (8.32) at Botany Bay. Soil moisture was lower than 1.0% in DVs and reached values up to 11.7% at Starr Nunatak site 3 and Icarus Camp site 1, and to 14.2% at Kay Island. Regarding soil texture, almost all the sites were dominated by sand (from 49.92% to 98.4%), while coarse and fine silt and clay showed a varying trend (Table 2).

Communities composition of macro-habitats

Bacterial community composition differed greatly according to macrohabitat (Fig. 2), with DV samples displaying a significantly lower biodiversity than CS and IC ($P = 0.003$ and $P = 0.012$, respectively, PD_whole_tree metric). Each macrohabitat showed a distinct bacterial profile ($P = 0.001$, adonis test for all pairwise comparisons, unweighted and weighted Unifrac metrics), with significant differences in phyla composition. DVs samples had a higher abundance of Firmicutes (avg. 52.3% rel. ab.) and were depleted in Cyanobacteria (8.5%) compared to CS ones, that were characterized also by a lower abundance of Bacteroidetes (23.0%) and a higher content of Proteobacteria (13.3%) and Acidobacteria (6.2%). Finally, IC samples showed a low abundance of Firmicutes (5.0%) and a higher proportion of Bacteroidetes (38.6%) and Cyanobacteria (45.4%) (Fig. 2C). Overall, 10 out of the 12 most abundant phyla resulted differentially present in at least one macrohabitat, confirming the macroscopic differences among the bacterial profiles of the three macrohabitats.

Replicated samples from the same site showed similar bacterial profiles, with intra-site distances significantly lower than inter-site distances for CS samples ($P = 0.001$). Replicated DV samples, on the other hand, showed a clear tendency towards a lower distance, but with no significant differences, due to the low number of samples considered (Fig. S2, Supporting Information).

Distinct microbial profiles were highlighted within the same macrohabitat. Beta-diversity analysis of CS samples (unweighted Unifrac distances, Fig. 3A) revealed that Edmonson Point and Kay Island samples were separated from all others (except from Botany Bay), whereas Botany Bay had a composition in between these two former localities. On the other hand, Apostrophe Island, Prior Island and Starr Nunatak samples were undistinguishable one from the other, whereas Cape King resulted significantly different from Edmonson Point, and Kay Island (Table S1, Supporting Information). We could not properly evaluate Mount McGee profiles due to the fact that only 1 sample was available for this locality.

Kay Island and Starr Nunatak were characterized by a significantly higher abundance of Actinobacteria (rel. ab. of 6.5% and 10.6%, respectively), whereas Botany Bay and Edmonson Point were somehow depleted (rel. ab. of 0.8% and 1.1%, respectively). Armatimonadetes were higher in Apostrophe Island and Cape King samples (rel ab. of 1.8% and 3.0%, respectively, compared to an average of 0.3% of the other localities), whereas they were absent in Botany Bay, Edmonson Point and Kay Island. Apostrophe Island was the only locality to show a consistent presence of Eremiobacteraeota (formerly, WPS-2), whereas Abditibacteriota (formerly, FBP) were found in Apostrophe Island, Kay Island, Cape King and Starr Nunatak samples, but missing from all other samples (Fig. 3).

As for CS, a separation based on sampling locality (Fig. 4A) was reported for DV samples (unweighted Unfrac), but with no statistical significances due to the low number of samples per locality. Lakes Hoare, Joyce, and Vida samples had very similar bacterial profiles (average Pearson's correlation of bacterial relative abundance at family level among them: 0.996), composed mainly by members of Ruminococcaceae (31.7%), Bacteroidaceae (18.9%), Lachnospiraceae (13.2%) and by unclassified Clostridiales (10.4%). The composition of bacterial profiles in these sites was evidently different from that of Lake Fryxell, characterized by a greater abundance of Cyanobacteria, such as Pseudoanabenaceae (12.9% *vs*. an average of 0.5% in other localities) and Nostocaceae (8.2% *vs*. an average of <0.1% in other localities), and Lake Vanda, which displayed a high presence of Chitinophagaceae (19.0% *vs*. an average of 0.1% in other localities) and Cytophagaceae (14.7% *vs*. an average of <0.1% in other localities) (Fig. 4B).

Table 2. Soil physicochemical parameters. Bold values have been obtained as mean of the other two values available for the same site.

Sample name	C (%)	N (%)	C/N ratio	pH	Na (cmol/kg)	K (cmol/kg)	Mg (cmol/kg)	Ca (cmol/kg)	CEC (cmol/kg)	Soil Moisture (%)	Sand (%)	Coarsesilt (%)	Fine silt (%)	Clay (%)
Lake Fryxell site 1 sample 1	0.33	0.019	17.4	7.81	2.12	0.45	1.15	2.06	3.53	0.180	87.2	3.2	6.3	3.3
Lake Fryxell site 1 sample 2	0.03	0.010	3.0							0.473				
Lake Fryxell site 2 sample 1	0.05	0.009	5.6							0.181				
Lake Hoare site 1 sample 1	0.16	0.017	9.4	7.98	0.07	0.36	0.88	0.84	2.16	0.091	93.4	2.7	3.0	1.0
Lake Hoare site 1 sample 2	0.11	0.008	13.8							0.088				
Lake Hoare site 1 sample 3	0.14	0.013	10.8							**0.090**				
Lake Joyce site 1 sample 1	0.02	0.019	1.1	6.80	8.32	0.40	1.50	0.75	10.97	0.178	87.9	1.3	5.5	5.4
Lake Joyce site 1 sample 2	0.14	0.005	31.70	6.56	1.59	0.84	2.23	1.39	6.05	0.348	90.2	0.6	4.4	4.8
Lake Vida site 1 sample 1	0.54	0.026	20.8	7.75	0.44	0.20	1.19	1.12	2.96	0.400	94.4	0.2	4.4	1.0
Lake Vida site 1 sample 2														
Lake Vida site 1 sample 2														
Botany Bay site 1 sample 1	2.07	0.085	24.4	8.32	0.07	0.33	1.44	2.43	3.93	0.710	70.4	4.1	18.4	7.2
Botany Bay site 2 sample 1	0.88	0.035	25.1											
Kay Island site 1 sample 1	6.37	0.209	30.5	7.30	0.55	0.42	0.69	0.54	4.91	14.200	75.4	12.2	11.7	0.7
Kay Island site 1 sample 2														
Kay Island site 2 sample 1	10.05	0.343	29.3											
Kay Island site 2 sample 2	6.44	0.227	28.4											
Kay Island site 2 sample 3	**8.25**	**0.285**	**28.9**											
Mount McGee site 1 sample 1	1.89	0.125	15.1	5.91	0.15	0.20	0.58	0.36	2.95	3.300	95.2	1.5	2.9	0.4
Apostrophe Island site 1 sample 1	5.87	0.202	29.1	7.76	0.06	0.14	0.40	0.25	0.85	0.595	74.1	12.3	12.9	0.7
Apostrophe Island site 1 sample 2														
Apostrophe Island site 2 sample 1	6.31	0.243	26.0											
Apostrophe Island site 2 sample 2	5.63	0.221	25.5											
Apostrophe Island site 2 sample 3	**5.97**	**0.232**	**25.7**											
Apostrophe Island site 3 sample 1	5.94	0.222	26.8											
Apostrophe Island site 3 sample 2														
Apostrophe Island site 3 sample 3														
Cape King site 1 sample 1	0.91	0.103	8.8	7.60	0.14	0.34	2.18	1.89	6.42	0.432	93.7	2.5	3.0	0.8

Table 2. Continued

Sample name	C (%)	N (%)	C/N ratio	pH	Na (cmol/kg)	K (cmol/kg)	Mg (cmol/kg)	Ca (cmol/kg)	CEC (cmol/kg)	Soil Moisture (%)	Sand (%)	Coarsesilt (%)	Fine silt (%)	Clay (%)
Cape King site 2 sample 1	1.87	0.066	28.3							0.350				
Cape King site 3 sample 1	1.02	0.045	22.7							0.269				
Cape King site 3 sample 2														
Prior Island site 1 sample 1	1.08	0.161	6.7	7.10	0.87	0.22	1.87	2.75	0.17	1.120	49.9	19.0	28.2	2.9
Prior Island site 2 sample 1	9.04	1.093	8.3							1.810				
Prior Island site 2 sample 2	6.62	0.868	7.6							0.990				
Starr Nunatak site 1 sample 1	5.28	0.145	36.4	4.82	0.10	0.08	0.88	1.09	2.15	0.100	69.4	12.6	15.7	2.2
Starr Nunatak site 1 sample 2														
Starr Nunatak site 2 sample 1	4.51	0.603	7.5							6.850				
Starr Nunatak site 3 sample 1	11.17	2.031	5.5							11.700				
Starr Nunatak site 4 sample 1	0.87	0.125	7.0							8.000				
Starr Nunatak site 4 sample 2	0.71	0.111	6.4							7.600				
Edmonson Point site 1 sample 1	0.59	0.027	21.9	6.43	0.09	0.22	0.53	0.22	1.06	0.177	81.0	8.6	9.6	0.9
Edmonson Point site 2 sample 1	0.24	0.018	13.3	6.62	0.42	0.29	0.22	0.37	1.29	0.131				
Edmonson Point site 2 sample 2	0.89	0.037	24.1	6.57						0.221				
Edmonson Point site 2 sample 3	**0.57**	**0.028**	2.5	**6.60**						0.176				
Edmonson Point site 2 sample 4														
Edmonson Point site 3 sample 1	0.05	0.004	12.5	6.61	0.78	0.47	0.20	0.41	1.85	0.180	95.8	3.3	0.8	0.2
Edmonson Point site 3 sample 2														
Edmonson Point site 3 sample 3														
Edmonson Point site 4 sample 1	0.03	0.009	3.3	6.79	0.37	0.45	0.24	0.01	1.07	0.180	97.8	1.8	0.2	
Edmonson Point site 4 sample 2														
Edmonson Point site 5 sample 1	0.06	0.018	3.3	6.47	0.42	0.28	0.24	0.35	1.28	0.190	98.4	1.5	0.1	0.3
Edmonson Point site 5 sample 2														
Edmonson Point site 6 sample 1	1.86	0.204	9.1	6.17	0.63	0.48	0.51	1.30	2.92	0.220	88.1	7.2	3.8	0.1
Campo Icaro site 1 sample 1	0.88	0.107	8.2	6.76	1.30	0.30	1.43	0.56	3.59	8.900	84.4	7.0	7.4	1.2
Campo Icaro site 1 sample 2	1.29	0.047	27.4							11.700				
Campo Icaro site 1 sample 3	**1.09**	**0.077**	**14.1**							**10.300**				
Campo Icaro site 1 sample 4														
Campo Icaro site 2 sample 1														
Campo Icaro site 2 sample 2														

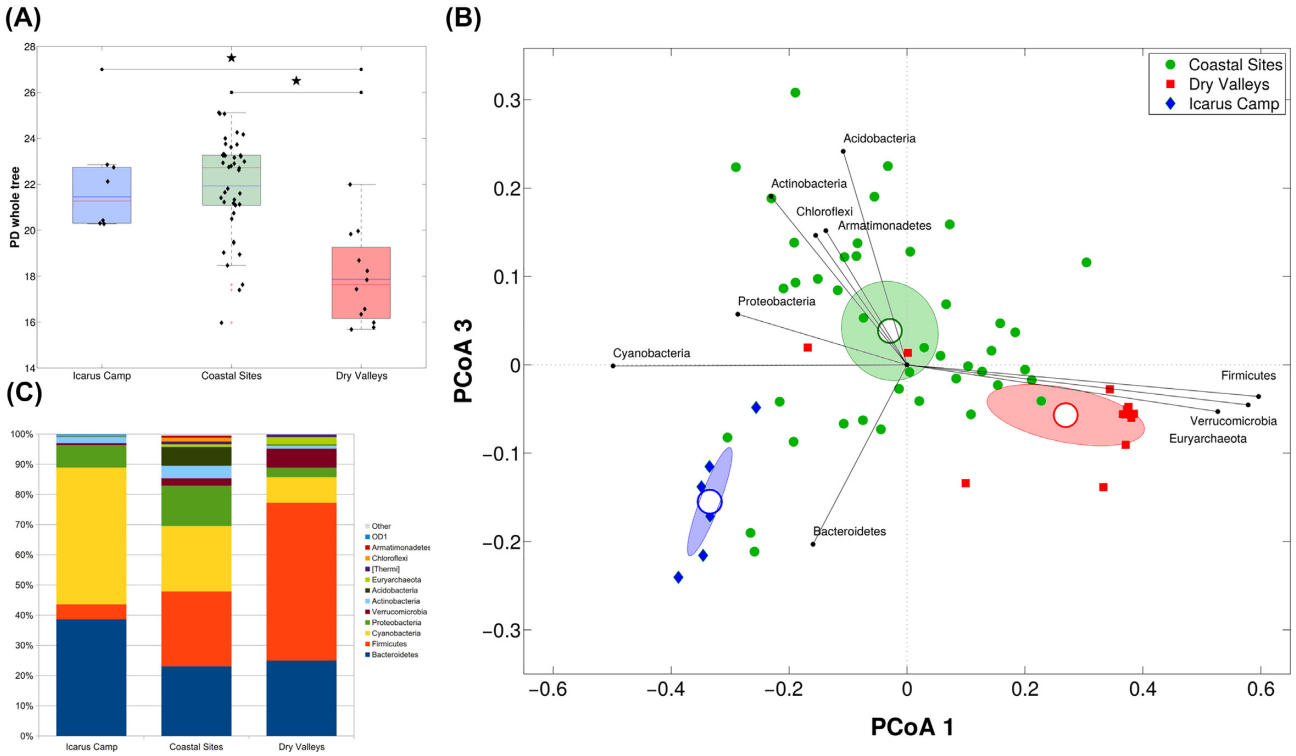

Figure 2. Soil bacterial compositions are deeply different according to macrohabitat. **(A)** Boxplots of samples alpha-diversity (PD_whole_tree metric). Statistically significant differences are marked with an asterisk. **(B)** PCoA of weighted Unifrac distances. Each point represents a sample; data points are colored according to macrohabitat. Centroids represent the average coordinate for the data points in each category. Ellipses represent the 95% SEM-based confidence interval of the data points. The first and third principal coordinates are represented. The biplot of the average bacterial coordinates weighted by the corresponding bacterial abundance per sample was superimposed on the PCoA plot to identify the statistically different bacterial phyla ($P < 0.05$) contributing to the ordination space (black arrows). **(C)** Barplots of average relative abundance of bacterial phyla, according to macrohabitat; only the 12 main phyla were represented; less abundant phyla were grouped in the 'Other category'.

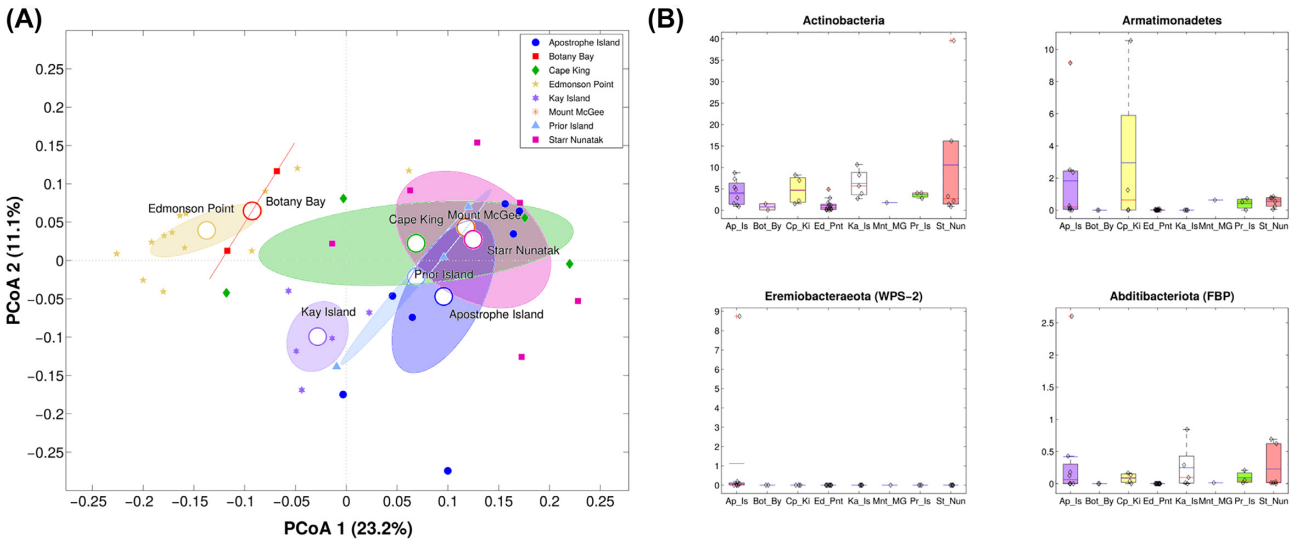

Figure 3. Separation of the taxonomic profiles in CS samples. **(A)** PCoA of unweighted Unifrac distances. Each point represents a sample; data points are coloured according to locality. Centroids represent the average coordinate for the data points in each category. Ellipses represent the 95% SEM-based confidence interval of the data points. The first and second principal coordinates are represented. **(B)** Boxplots of bacterial relative abundances of CS samples divided according to locality. Only the first four differential phyla (P <<0.05i> Kruskal–Wallis test) are represented.

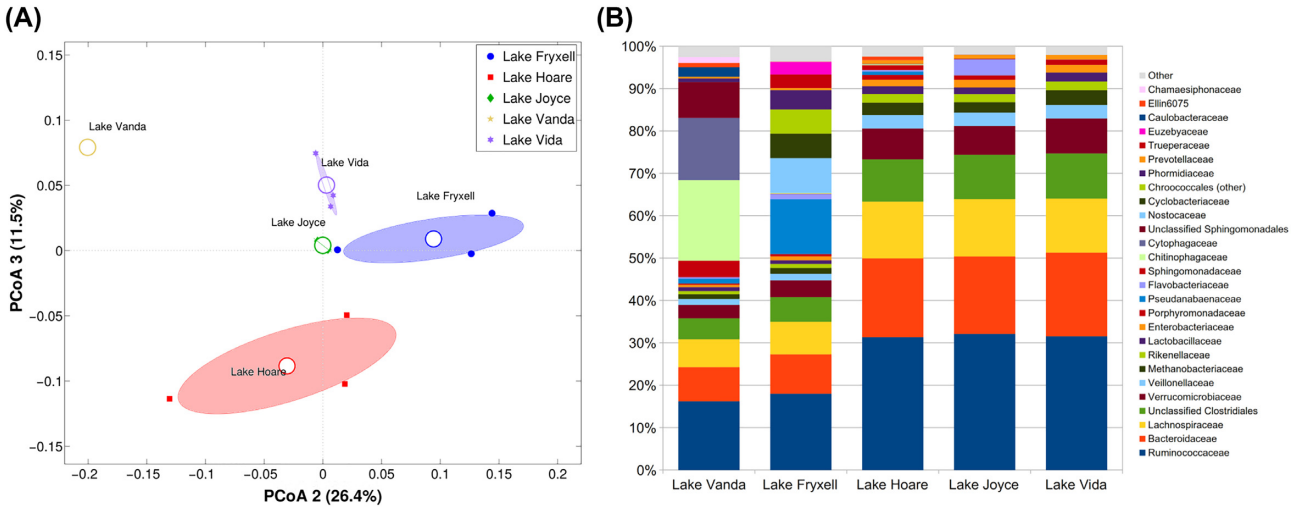

Figure 4. Separation of the taxonomic profiles in DV samples. **(A)** PCoA of unweighted Unifrac distances. Each point represents a sample; data points are coloured according to locality. Centroids represent the average coordinate for the data points in each category. Ellipses represent the 95% SEM-based confidence interval of the data points. The second and third principal coordinates are represented. **(B)** Barplots of average bacterial relative abundances according to locality at family level; only families with rel. ab >1% in at least 1 out of 6 localities were represented; lower abundance families were grouped in the 'Others' category

Among major bacterial phyla, Proteobacteria, Acidobacteria and Actinobacteria showed positive correlations to N, C and coarse silt, whereas Firmicutes, Verrucomicrobia and Euryarcheaota had strong correlations (ρ >0.5) to sand content; notably, Cyanobacteria was the only phylum positively correlated to Na (Table 3).

These results were mostly confirmed by the correlations between some bacterial families and soil parameters (Table S2, Supporting Information). For example, four families of Firmicutes (i.e. Ruminococcaceae, Lachnospiraceae, unclassified Clostridiales and Veillonellaceae) were all positively correlated (ρ >0.5) to sand. Within Proteobacteria, Sphingomonadaceae and Acetobacteraceae were positively correlated to N, C and coarse silt content. Among Cyanobacteria families, Phormidiaceae and Pseudoanabaenaceae were both positively correlated to Na content. Notably, although for phylum Bacteroidetes a significant correlation was observed only with C/N ratio (Table 3), some families classified within it, such as Chitinophagaceae and Sphingobacteriaceae, positively correlated to N and C contents, C/N ratio and coarse silt, whereas Bacteroidaceae was correlated to sand and K contents (Table S2, Supporting Information).

Effect of soil physicochemical parameters on communities composition

To quantify the degree to which edaphic physicochemical parameters could explain the distances in community composition among samples, PerMANOVA analysis was carried out. First, variables were considered individually for the total community, the two CS and DV macrohabitats, and the 8 dominant phyla. Few of the variables tested resulted significant in determining the total bacterial community composition, while none of parameters tested was significant for DV samples and only clay content was significant for CS samples variance (Table S3, Supporting Information). For the total community the soil texture categories were the parameters explaining the highest proportion of variance, followed by C/N ratio, Ca content and pH. Also for the different phyla a great proportion of the parameters tested was not significant for the variance, except for Firmicutes and Proteobacteria, with soil texture and cations content resulting to have a stronger effect (Table S3, Supporting Information). When the edaphic variables were combined to account for correlation among them, a lower number resulted to be independent in explaining the community variance and, in general, the total proportion of variance explained was very low (Table 4). For the total bacteria community, clay was among the independent parameters, with C/N ratio and pH. For the different phyla, a high variability was observed among the parameters, independently affecting the communities variance (Table 4).

DISCUSSION

Antarctic microorganisms have lived isolated from the global gene pool over timescales of evolutionary significance (Vincent 2000) and, therefore, they represent fascinating models for studying survival strategies to stress conditions and defining the biotic and abiotic interactions within communities. A number of studies have been carried out since the second half of the last century to characterize the Antarctic terrestrial communities and their functioning, many of which focused on soil bacterial ecosystems. Following former studies carried out by culture-based approaches, new insights have been provided in the last decades by sequencing and phylogenetic analyses (Lambrechts *et al.* 2019). This study represents a further contribution on this subject. We compared the BSCs communities of three macro-areas (CS, DV and IC), characterized by crusts of different development stage and type, with 60 samples analyzed from 14 localities along a 470 kilometers distance. The bacterial profiles within the same sampling locality, of different sites within each macro-area, among the three macro-areas, and considering the Victoria Land soil community as a whole have been compared.

A common feature of the whole Victoria Land bacterial communities was the dominance of the phyla Firmicutes, Bacteroidetes, Cyanobacteria, and Proteobacteria, followed by Acidobacteria, Actinobacteria, Verrucomicrobia, and Euryarchaeota. These phyla have already been reported as dominant in different terrestrial Antarctic niches as soils (Smith *et al.* 2006; Aislabie *et al.* 2006; Niederbeger *et al.* 2008; Cary *et al.* 2010; Bottos *et al.* 2014; Papale *et al.* 2018), cryptoendolithic

communities (de La Torre *et al.* 2003; Coleine *et al.* 2019) and cryoconite holes (Christner, Kvitko and Reeve 2003; Cameron *et al.* 2012), with no substantial differences even between soils with different nutrient levels and microbial biomasses, as between ornithogenic and mineral soils of the Ross Sea region (Aislabie, Jordan and Barker 2008). Heterogeneous bacterial profiles have been observed among the soil samples here analyzed and most phyla were mainly driven by abiotic parameters and soil texture.

A bacterial 'core' community composed of 153 out of the 7201 zOTUs (2.1%), was identified, mainly composed of Firmicutes and Bacteroidetes. This somehow low number of shared zOTUs highlighted a very strong variability among the sites analyzed. A more substantial bacterial 'core' community composed of 48 out of 560 OTUs (8.6%), mostly Actinobacteria and Proteobacteria, was recorded for cryptoendolithic communities inhabiting rocks in Victoria Land, from Stewart Heights (73°30'S) to Battleship Promontory (76°54'S) (Coleine *et al.* 2019). These different percentages might be possibly determined by more stable conditions offered by the endolithic 'rock's habitable architecture' compared to soils, hosting well adapted and more uniform communities (Wierzchos *et al.* 2015). These differences could be linked also to the lack in this endolithic study (Coleine *et al.* 2019) of the southernmost DV sites, characterized by more stressing environmental conditions. The two main phyla composing the core community, Firmicutes and Bacteroidetes, had already been recorded in Antarctic sites, the former from different locations (Yergeau *et al.* 2009; Buelow *et al.* 2016; Ramos *et al.* 2019; Wong *et al.* 2019), the latter as a dominant phylum in Dry Valley soils (Cary *et al.* 2010; Lee *et al.* 2012; Wong *et al.* 2019).

At the lowest level of comparison, replicated samples from the same sampling site showed similar bacterial profiles. Similar bacterial profiles were also observed among different CS samples, suggesting a great influence of the local parameters in determining homogeneous communities. This also occurred when different stages of crust development within the same locality were compared, as for the samples collected at Edmonson Point. The same tendency seemed to occur for DV samples too, but it was not statistically supported due to the low number of samples analyzed. This trend confirmed what already reported for fungal communities characterizing BSCs in North Victoria Land coastal sites and soils along the Taylor Valley, where slightly diversified communities were recorded among samples within the same locality (Canini *et al.* 2020; 2021).

Otherwise, differences were observed among the communities characterizing each macro-area. Differences among CS community structures suggested a strong influence by the local soil physicochemical parameters and crusts development. Besides this, Edmonson Point and Botany Bay, both designed as ASPA based on their rich moss and lichen coverage, and Kay Island were close to each other (non-significant difference between microbial profiles between these sites), suggesting the existence of a sort of common favorable conditions, possibly due to the plant coverage. Similar results were obtained for the fungal component of both coastal and inner sites of Victoria Land, that appeared to be driven by site-specific differences in environmental conditions, particularly edaphic factors, such as exchangeable cations and pH for coastal sites (Canini *et al.* 2020) and soil texture and connected physicochemical properties for inner sites along the Taylor Valley (Canini *et al.* 2021). Unfortunately, no statistically significant differences were recorded among the DV localities community composition, due to the low number of available samples.

A number of different reasons were proposed to explain the biodiversity of soil biota. A soil bacterial diversity frequently exceeding that of lithic communities has been recorded and explained by the aeolian transport of viable cells, increasing the lithobionts contribution to soil biota and reducing the role of soil physicochemistry (van Goethem *et al.* 2016). A high diversity and uniform community structure has already been observed in surface soils from low-carbon sites, explained by the spatial isolation characterizing soils with low water activity and nutrient availability (Zhou *et al.* 2002). This spatial isolation, while enhances the probability of successful colonization by new introduced strains, however makes it difficult for that strains to achieve dominance unless spatial isolation fails (Zhou *et al.* 2002), as for higher water and nutrient availability due to climate change. However, when considering molecular ecology studies based on DNA metabarcoding, it should always be taken into account the possible effect of DNA from spores or dormant propagules and relic DNA from dead cells, the latter having been estimated to represent an high proportion of DNA in soil samples, being preserved for long periods, especially in cold and dry environments (Carini *et al.* 2017; Fierer 2017), and that could inflate the estimations of diversity.

When samples from the three macrohabitats (Icarus Camp, coastal sites, and Dry Valleys) were compared, they resulted to be deeply different from each other, showing distinct bacterial profiles. The most relevant macroscopic difference among them consisted in the crust developmental stages. The plant coverage may reduce the effects of soil physicochemistry in structuring the microbial communities of coastal sites. A plant cover not only provides a greater nutrient supply for heterotrophic bacteria, but also protection from external stressing conditions, in the form of higher and stable temperatures (Yergeau *et al.* 2007). A possible mechanism in which lichenosphere and bryosphere community serve as reservoirs of the underneath soil bacterial diversity may also be hypothesized, but it has to be confirmed with further targeted studies.

Samples from Icarus Camp, homogeneous among each other, were deeply different from CS and DV samples. Here, the thin and young crust, on a recently altered rock substrate, showed a higher abundance of Bacteroidetes and Cyanobacteria compared to the other two macro-habitats. Cyanobacteria are widespread in all polar terrestrial environments, providing organic carbon and nitrogen to these ecosystems (Elser 2002; Pandey *et al.* 2004). They are usually the dominant components of the soil photoautotrophic component in Antarctic BSCs and are the primary colonizers of poor Antarctic soils (Pushkareva *et al.* 2018). Soil studies reported cyanobacteria restricted to wetter, more productive polar locations (de los Rios *et al.* 2004). Accordingly, we here recorded cyanobacteria as abundant components of CS samples, and even more abundant in the thin and young crusts of Icarus Camp. We recorded cyanobacteria on gneiss at Kay Island as well, despite the lower abundance values previously reported for this substrate compared to granite (Pushkareva *et al.* 2018). Among the more commonly recorded genera there were *Phormidium* and *Leptolyngbya*, already found in Antarctic crusts, both in South and North Victoria Land (Aislabie *et al.* 2006; Michaud, Sabacka´ and Priscu 2012; Van Goethem *et al.* 2016; Pushkareva *et al.* 2018). Nostocaceae and some members of this family (*Nostoc* spp.) are photosynthetically active at subzero temperatures and are involved in nitrogen cycling, conferring them higher survival chance under the extreme Antarctic environment (Pandey *et al.* 2004; Pushkareva *et al.* 2018). Cyanobacteria abundance was lower in DV sites. Here, their presence could have resulted from dispersion of aquatic inocula from lacustrine communities or benthic mats, as suggested by Van Goethem *et al.* (2016).

Table 3. Spearman's correlation coefficient between bacterial relative abundances at phylum level in different samples and physicochemical parameters measured in soil. Only statistically significant (P<<0.05i>) coefficients are reported.

	C	N	C/N ratio	pH	CEC	Na	K	Mg	Ca	Moisture	Sand	Coarse silt	Fine silt	Clay
Firmicutes	−0.35	−0.33	–	–	–	–	–	–	–	–	0.56	−0.62	−0.53	–
Bacteroidetes	–	–	0.35	–	–	–	–	–	–	–	–	–	–	–
Cyanobacteria	–	–	–	–	–	0.37	–	–	–	–	–	–	–	–
Proteobacteria	0.38	0.38	–	−0.35	–	–	–	−0.40	−0.36	–	–	0.41	–	−0.48
Actinobacteria	0.57	0.56	0.38	–	–	–	–	–	–	0.39	−0.67	0.81	0.59	–
Verrucomicrobia	−0.32	−0.30	–	–	–	–	–	–	–	–	0.62	−0.70	−0.58	–
Acidobacteria	0.37	0.40	–	–	−0.53	−0.51	–	−0.36	−0.40	–	−0.44	0.68	–	−0.40
Chloroflexi	0.27	0.32	–	−0.56	–	–	–	−0.48	−0.37	–	–	–	–	−0.49
Planctomycetes	–	–	–	−0.38	–	–	–	−0.38	−0.36	–	−0.50	0.60	0.46	–
Armatimonadetes	0.39	0.49	–	−0.49	–	–	–	–	–	–	–	–	–	–
Euryarchaeota	−0.27	–	−0.27	–	–	–	0.37	–	–	–	0.59	−0.70	−0.55	–
[Thermi]	–	–	–	–	–	–	–	–	–	–	−0.55	0.66	0.50	–
Eremiobacteraeota (WPS-2)	0.29	0.31	–	–	–	–	–	–	–	–	–	–	–	–
Abditibacteriota (FBP)	0.55	0.53	0.43	–	–	–	−0.42	–	–	0.40	−0.67	0.73	0.64	–
Fusobacteria	–	–	–	–	–	–	–	–	–	–	–	−0.49	–	–
Parcubacteria (OD1)	–	–	–	–	0.36	0.54	–	0.38	–	–	–	–	–	–

Table 4. Proportion of variation in bacterial community composition at the level of the total community (all bacteria) and the major phyla, explained by soil physicochemical parameters added sequentially (first to the last) in a model, depending on their independent influence in the variance, as reported in Table S3 (Supporting Information). Significant values in bold.

All bacteria			Acidobacteria			Actinobacteria			Bacteroidetes			Cyanobacteria		
Variable	Variance	P	Variable	Variance	P	Variable	Variance	P	Variable	Variance	P	Variable	Variance	P
Clay	**9.712**	**0.0006**	**Moisture**	**7.193**	**0.0069**	**K**	**8.846**	**0.0037**	**Clay**	**9.854**	**0.0023**	**K**	**9.827**	**0.0009**
Fine silt	3.894	0.0505	**Clay**	**6.564**	**0.0202**	Sand	2.829	0.1535	**pH**	**7.963**	**0.0091**	Coarse silt	0.975	0.6842
Sand	0.789	0.6391	Na	3.993	0.0674	Fine silt	2.362	0.2192	**C/N ratio**	**5.383**	**0.0357**	Residuals	89.198	
C/N ratio	**6.183**	**0.0113**	C	1.465	0.4649	Coarse silt	1.084	0.623	Residuals	76.8				
Ca	1.813	0.2525	Residuals	80.785		Na	2.21	0.2472						
pH	**4.286**	**0.0364**				C	0.477	0.8919						
Residuals	73.323					Residuals	82.191							
Euryarchaeota			Firmicutes			Proteobacteria			Verrucomicrobia					
Variable	Variance	P	Variable	Variance	P	Variable	Variance	P	Variable	Variance	P			
C/N ratio	**3.848**	**0.0031**	**Ca**	**5.249**	**0.0027**	**Mg**	**16.652**	**0.0001**	**Clay**	**4.884**	**0.0088**			
Clay	**3.244**	**0.0413**	Clay	2.141	0.1829	**C/N ratio**	**14.482**	**0.0002**	**C/N ratio**	**3.289**	**0.0296**			
Residuals	92.908		Fine silt	2.322	0.1338	**Fine silt**	**6.78**	**0.0056**	Residuals	91.827				
			pH	**3.759**	**0.0166**	Sand	0.418	0.7589						
			C/N ratio	**3.369**	**0.0302**	C	0.771	0.489						
			Sand	2.407	0.1259	Clay	0.473	0.7043						
			C	1.236	0.5657	Ca	2.215	0.1238						
			Mg	2.385	0.1199	**K**	**4.351**	**0.0213**						
			Residuals	77.133		Coarse silt	0.73	0.491						
						CEC	0.332	0.8194						
						Residuals	52.795							

Firmicutes and Euryarchaeota appeared the main drivers of Dry Valley soils, contributing to their ordination space in a PCoA plot. Both were negatively correlated to N, C and coarse silt contents, on average less abundant in DV sites, and positively correlated to sand content, on average more abundant. A presence of Firmicutes can be enabled by their several spores-forming genera, ensuring survival in the Antarctic harsh conditions (Ramos *et al.* 2019). A possible prevalence of Euryarchaeota in oligotrophic soils, being outcompeted by other microorganisms in soils richer in organic matter, was already suggested (Zumsteg *et al.* 2012), and this could explain their driving role in DVs soils, compared to the other sites. Although many studies have reported the absence or a low abundance and diversity of Archaea in Dry Valleys soils possibly linked to the extreme xeric stress (Pointing *et al.* 2009; Van Goethem *et al.* 2016), they seem to be more common than previously reported, possibly previously overlooked due to the limitations of available primers (Lambrechts *et al.* 2019).

Drivers of bacterial communities and taxonomic groups

In this study, tested parameters acted differently on the abundance and composition of different taxa or communities, and, among them, most phyla were correlated with soil granulometry. The sized soil particle fraction, determining distinct microenvironments in terms of water and nutrients availability, and harbouring microbial communities differing in structure, functional potentials and sensitivity to environmental conditions, is an often-overlooked parameter, especially in Antarctica (Hemkemeyer *et al.* 2018). Soil texture, and connected abiotic soil properties, have been accounted as the main parameters affecting the diversity and composition of soil fungal communities along the Taylor Valley (Canini *et al.* 2021) and to a lesser extent in coastal sites of Victoria Land (Canini *et al.* 2020). A significant correlation of same soil texture parameters, namely sand and silt, with bacterial community composition had been reported for soil samples collected at Terra Nova Bay (Northern Victoria Land), with Cyanobacteria, in particular, positively correlated with the silt content (Hemkemeyer *et al.* 2018). Similarly, the predominant effect of abiotic variables has been reviewed by Chong, Pearce and Convey (2015), that reported pH and soil moisture as main drivers of bacterial communities composition and diversity. These parameters are strongly affected by soil texture, indeed soils with coarser granulometry have a lower water and nutrients retention ability, that could be, in turn, influenced by variations in pH. The effect of these parameters, could have been masked by the stronger effect of soil texture reported in our study.

Many studies indicated the high spatial heterogeneity of microbial ecosystems in extreme environments and some of them examined the drivers of the spatial variation observed (Niederberger *et al.* 2008; Bottos *et al.* 2014). Water and organic matter were accounted as the major driving forces for the observed patterns of distribution in Antarctic soil communities (Barret *et al.* 2006; Niederberger *et al.* 2008). A similar heterogeneity was also highlighted for Antarctic soil micro-invertebrates, as nematodes and biotic interactions were accounted as main determinants of this variability (Caruso *et al.* 2019).

Total organic C content was accounted as the most significant variable in structuring the bacterial communities in Dronning Moud Mountains (East Antarctica), followed by pH, electric conductivity, bedrock type and the moisture content, and a reduced role was given to the spatial distance (Tytgat *et al.* 2016). Differently, our results showed only a marginal effect of soil moisture, C and N content on community composition, even if these two latter parameters were strongly correlated with the abundance of many phyla and families. We found that soil texture and pH were among the main drivers of community composition, also analyzing the two main macro areas independently and the different phyla. A complex interaction of salinity, C, moisture, and other variables was observed in soils collected at McKelvey Valley (Dry Valleys) (Pointing *et al.* 2009). These interactions may mask the effect of many parameters in our study, as for C content, resulting not independent when combined with others parameters. The bedrock type and the physicochemical soil properties, namely low water content, high pH and conductivity, as well the geographic separation, were accounted as the ones correlated with the differences in cyanobacterial community composition in four sites from Sor Rondane Mountains (Pushkareva *et al.* 2018). These results strongly differ from ours, where Cyanobacteria were mainly correlated with Na and K content.

However in all the analyses of the variance, we found that despite the wide number of parameters tested a high proportion of observed variance was not explained by these variables. This suggests that further variables should be tested to define their possible role in determining the distribution of such peculiar communities.

CONCLUSIONS

With this work, we characterized the soil bacterial communities of a number of sites spanning a wide latitudinal gradient from North to South Victoria Land, with different edaphic characteristics, representing the main environments in this region. The communities resulted highly diversified among the different sampling localities, as also highlighted by the low number of zOTUs shared among all the samples. The differences in communities diversity and composition found among the three macrohabitats analyzed have been related to local environmental conditions, and soil texture and connected abiotic parameters have been suggested to play a more important role.

A great number of previously unsequenced and putatively new taxa has been recorded, confirming the presence of novel, yet undescribed, and possibly endemic species already evidenced by recent molecular studies for both the bacterial and fungal components of Antarctic terrestrial environments, which are a potential source of novel genes, gene products and compounds (Smith *et al.* 2006; Peeters and Willems 2011; de Pascale *et al.* 2012; Núñez-Montero and Barrientos 2018; Canini *et al.* 2020; Zucconi *et al.* 2020). To deepen the study of microbial communities of extreme environments is, therefore, a priority, particularly in ice-free areas where the range of microbial habitats for colonization is higher (Cowan *et al.* 2018), and the risk of changes as a result of global warming is greater.

DATA AVAILABILITY

Raw sequencing data are available in the NCBI Short-Read Archive (SRA) repository, with accession number PRJNA684555 [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA684555]

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

AUTHORS' CONTRIBUTIONS

LZ, LPDA and SV collected the samples; CM processed soil samples and extracted DNA; TC and CC performed 16S rRNA library preparation and sequencing; LPDA performed soil physicochemical analysis; FC and MS performed statistical analyses; MS, FC, SV and LZ wrote the first draft of the paper. All authors read, commented and approved the final manuscript. LZ and SV supervised and coordinated the study.

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