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Research Article

RESEARCH ARTICLE

Bacterial community composition in relation to bedrock type and macrobiota in soils from the Sør Rondane Mountains, East Antarctica

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One sentence summary: Lichen- and moss-associated carbon and moisture content, and physicochemical characteristics of bedrock type structure prokaryotic communities in the Sør Rondane Mountains, East Antarctica.

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ABSTRACT

Antarctic soils are known to be oligotrophic and of having low buffering capacities. It is expected that this is particularly the case for inland high-altitude regions. We hypothesized that the bedrock type and the presence of macrobiota in these soils enforce a high selective pressure on their bacterial communities. To test this, we analyzed the bacterial community structure in 52 soil samples from the western Sør Rondane Mountains (Dronning Maud Land, East Antarctica), using the Illumina MiSeq platform in combination with ARISA fingerprinting. The samples were taken along broad environmental gradients in an area covering nearly 1000 km^2. Ordination and variation partitioning analyses revealed that the total organic carbon content was the most significant variable in structuring the bacterial communities, followed by pH, electric conductivity, bedrock type and the moisture content, while spatial distance was of relatively minor importance. Acidobacteria (Chloracidobacteria) and Actinobacteria (Actinomycetales) dominated gneiss derived mineral soil samples, while Proteobacteria (Sphingomonadaceae), Cyanobacteria, Armatimonadetes and candidate division FBP-dominated soil samples with a high total organic carbon content that were mainly situated on granite derived bedrock.

Keywords: Illumina; terrestrial environment; 16S rRNA; inland nunataks; ice free

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INTRODUCTION

The long-term isolation of the Antarctic continent and its history of glacial expansions and retractions since the Late Eocene resulted in highly truncated and simple food webs, comprising a low number of macroscopic taxa (Chown and Convey 2007; Convey *et al.* 2008). Hence, terrestrial life in Antarctica is mainly microbial (Cowan *et al.* 2002, 2014; Vyverman *et al.* 2010) and largely confined to the sparse and discontinuously distributed ice-free regions (Chown and Convey 2007). These regions are restricted to coastal lowlands, the Transantarctic Mountains and parts of the mountain ranges that protrude through the ice sheet across the edges of the continent (i.e. nunataks) (Pugh and Convey 2008), and are believed to have acted as refugia for terrestrial biota during Neogene and Pleistocene ice ages (Convey *et al.* 2008; Pugh and Convey 2008). While it was long believed that soils in the most inland, high-altitude regions were devoid of life, it is now well accepted that even the most arid soils with virtually no organic matter are inhabited by microbial communities (Magalhães *et al.* 2012; Tytgat *et al.* 2014). Because of (i) the low buffering capacities of these organic matter poor and mineral Antarctic soils (Cannone and Guglielmin 2009), and (ii) the close association of microorganisms with their environment (Vrionis, Whyte and Miller 2013), it can be hypothesized that the soil geochemical characteristics enforce a high selective pressure on their bacterial communities, and that different bedrock types support different bacterial communities (Barton *et al.* 2007).

The most intensively studied terrestrial ecosystems in Antarctica are those in the McMurdo Dry Valleys (MDV) in South Victoria Land (see Cary *et al.* 2010 for a review). There, at least 14 phyla of bacteria have been recovered from soils, with Proteobacteria, Actinobacteria, Acidobacteria, Gemmatimonadetes and Bacteroidetes being amongst the most dominant ones (Cary *et al.* 2010). Recently, Kim *et al.* (2015) reported similar observations from Terra Nova Bay, North Victoria Land, where Chloroflexi appeared to dominate the more acidic soils. This agrees well with previous findings that pH, directly or indirectly through affecting the nutrient availability (Gray *et al.* 2014), is one of the most important factors in structuring soil bacterial communities at lower latitudes (Fierer and Jackson 2006). In addition to pH, it appears that electric conductivity (EC) and the organic carbon content or C/N-ratio are the main environmental factors structuring terrestrial communities, while moisture availability has varying effects (Chong *et al.* 2012; Magalhães *et al.* 2012; Geyer *et al.* 2014; Kim *et al.* 2015). Furthermore, the scattered presence of plants provides patches with highly localized nutrient sources and increased protection for microbial communities in the otherwise oligotrophic and harsh Antarctic conditions. For example, it has been shown that plant cover influences the ground thermal regime (Cannone *et al.* 2006; Cannone and Guglielmin 2009), which resulted in a higher bacterial richness compared to bare mineral soils along a latitudinal gradient in the Antarctic (Yergeau *et al.* 2007b). However, the microbial diversity and the factors structuring it in other and particularly more inland locations remain poorly known. This is an important knowledge gap given that findings obtained in one region cannot be extrapolated to others, because of, for example, regional differences in environmental gradients, (micro-)climatic conditions and bacterial community structure. The polar desert soils in the MDV, for example, differ in at least one aspect from those in more inland nunataks, namely their carbon source. In high-altitude regions, contemporary autotrophs (Cyanobacteria, algae, mosses and lichens, and probably also autotrophic bacteria), are solely responsible for the organic carbon supply (Brinkmann *et al.* 2007; Namsaraev *et al.* 2010; Fernández-Carazo *et al.* 2012; Magalhães *et al.* 2012; Ertz *et al.* 2014; Tsujimoto *et al.* 2014; Tahon *et al.* 2016). In contrast, the organic carbon sources in the soils of the MDV are of multiple origins (Cary *et al.* 2010). Apart from recent biological C-fixation, organic matter in some soils of the Dry Valleys is in part derived from old lacustrine sediments, which were deposited in large lakes that existed during the Last Glacial Maximum (Hall *et al.* 2010). In other parts of the region, the carbon stock is influenced by recently deposited cyanobacterial mats derived from the littoral zone of the present-day lakes and rivers or melt-water channels, or by marine inputs (e.g. seal carcasses and sea spray) in the more coastal regions (Cary *et al.* 2010). By contrast, with a few exceptions (Hodgson *et al.* 2010), lakes are absent in high altitude nunataks and their soils were never part of lake or river ecosystems. Moreover, the marine influence is restricted to a small number of marine birds nesting along mountain slopes up to 200 km inland (e.g. Snow Petrel (*Pagodroma nivea*); Ohyama and Hiruta, 1995; Peeters, Ertz and Willems 2011). It follows that the environmental gradients, shaping the microbial communities in these inland nunataks, might be different to those in the MDV.

Here, we aimed to study the effects of bedrock type, the presence of vegetation, the concentration of inorganic and organic carbon, the electric conductivity, the moisture content, and the pH on the bacterial community structure and diversity in inland Antarctic soils. To achieve this, we selected 52 soil samples covering a broad range of environmental gradients from nine nunatak regions situated on either gneiss or granite derived bedrock in the western part of the Sør Rondane Mountains (SRM; Dronning Maud Land, East Antarctica; Fig. 1) and used Illumina sequencing and Automated Ribosomal Intergenic Spacer Analysis (ARISA) fingerprinting (Fisher and Triplett 1999) to assess differences in their bacterial community structure.

MATERIALS AND METHODS

Sampling and site descriptions

The SRM (22° E-28° E, 71°30′ S-72°40′ S) are a 220 km long, east-west oriented mountain range, situated about 200 km inland (Pattyn, Matsuoka and Berte 2010; Osanai *et al.* 2013), with peaks reaching 3300 m a.s.l. (Gorodetskaya *et al.* 2013). The bedrock is mainly metamorphic (e.g. gneiss) with some plutonic (granite) outcrops (e.g. Pingvinane and Utsteinen; Fig. 1). Near the Utsteinen nunatak, in the escarpment zone north of the SRM, the Belgian Princess Elisabeth Station was built, where meteorological data has been collected since 2009 (Pattyn, Matsuoka and Berte 2010; Gorodetskaya *et al.* 2013). Because of the orientation of the mountains, the ice flow from the polar plateau is largely blocked and forced around the range. Katabatic winds are mainly confined to the major outlet glacier to the west (Hansenbreen) or those cutting through the eastern part of the western SRM (Gunnestadtbreen, closest to Utsteinen; and Jenningsbreen, west of the Brattnipane region) (Fig. 1a), leaving the area directly north of the western SRM rather sheltered. As a result, with increasing distance from the plateau more favorable conditions for macrobiotic lifeforms (mosses and lichens) occur. The SRM experience relatively mild winters, with mean air temperatures between –20 and –25°C at Utsteinen (Pattyn, Matsuoka and Berte 2010), during nearly five months (so-called coreless winters), while mean December and January air temperatures are about –8°C. However, large fluctuations in daily air (4°C–5°C) and surface (11°C–14°C) temperatures can occur (Gorodetskaya *et al.* 2013). Precipitation for 2012 was reported to be

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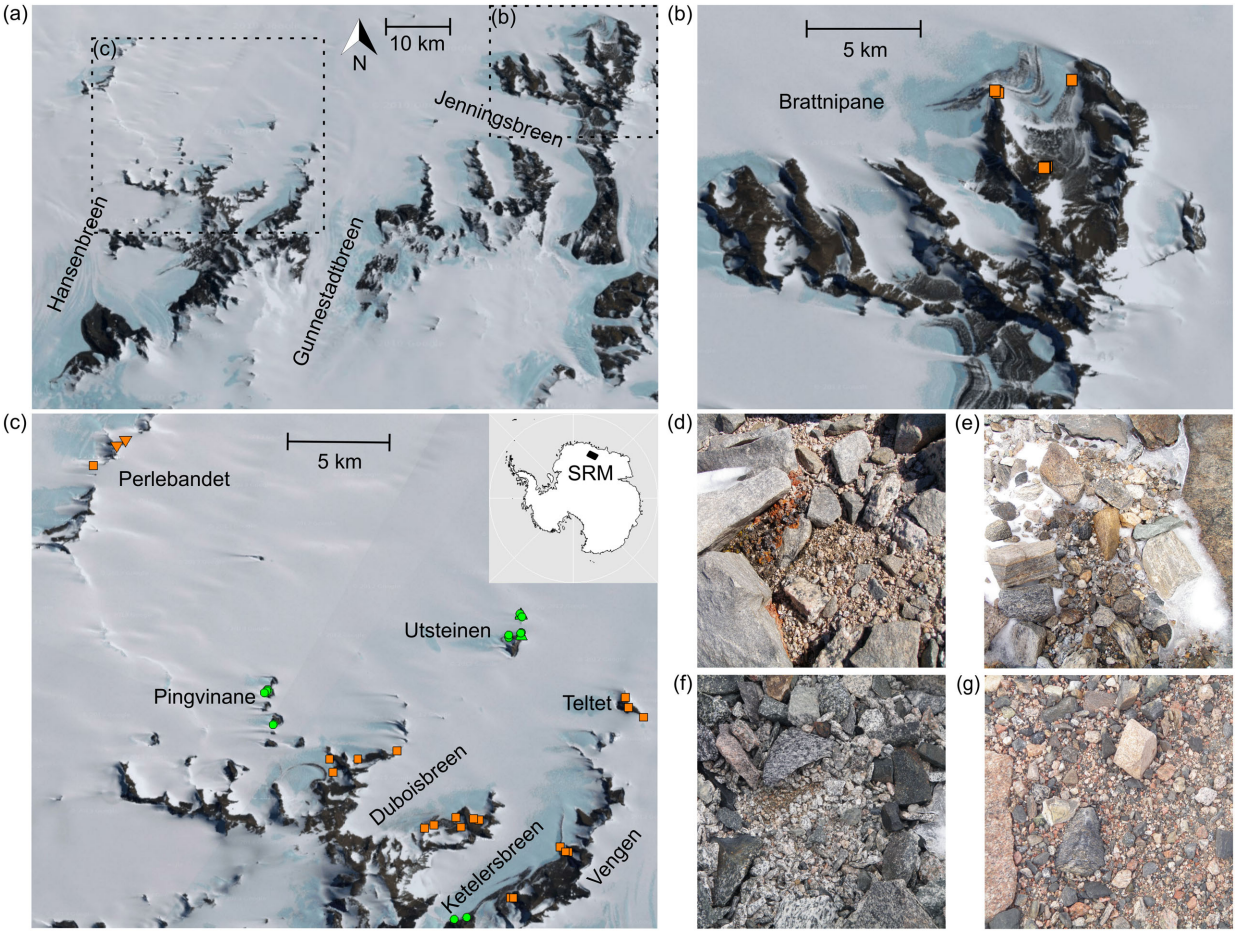

Figure 1. (**a**) Map of the western Sør Rondane Mountains in Dronning Maud Land, East Antarctica, with the major outlet glaciers indicated. (**b**) Detail of the Brattnipane region (BP) with the sampled locations. (**c**) Detail of other sampled regions including the three nunatak sites Utsteinen (UT), Teltet (TE) and Pingvinane (PA), and four areas within the main mountain range, namely the east (DE) and west (DW) sides of the Duboisbreen valley, a granite outcrop south of Ketelersbreen (KB) and Vengen (VE). Granite bedrock is indicated by the green symbols, the gneiss derived samples by the orange symbols. Samples containing macrobiota are denoted with triangles. Images d–g are examples of samples with different bedrock types at different locations. (**d**) Sample UT129 with the presence of orange lichens. (**e**) BP156. (**f**) KB112. (**g**) VE137.

110 ± 20 mm water equivalent year^{-1} (Gorodetskaya *et al.* 2015), although net snow accumulation at Utsteinen is highly variable between years (Gorodetskaya *et al.* 2013, 2015).

During sampling campaigns in the austral summers of 2009 and 2010, 52 terrestrial samples consisting of c. 10–40 g of the upper 3 cm of soil were collected from nine ice-free areas in the western Sør Rondane Mountains, with distances between samples ranging from less than 1 m up to a maximum of ~65 km. Samples were selected according to bedrock type (gneiss or syenite-granite derived), the presence or absence of macrobiota (moss, lichen, algae and/or arthropods) and geographic location (Fig. 1; Table S1, Supporting Information). Thirty-one gneiss samples were collected from the Perlebandet (PB) nunataks in the northwestern most region of the SRM, the east (DE) and west (DW) sides of the Duboisbreen valley, the Vengen (VE) subrange of the Vikinghøgda, the Teltet nunatak (TE) north of Vengen, and the more distant Brattnipane (BP) region to the east. In total, 21 granite-derived samples were collected from three regions, namely two nunatak sites outside the main mountain range (Pingvinane (PA) and Utsteinen (UT)), and a granite outcrop to the south of Ketelersbreen (KB) and Vengen. Mosses and lichens were only recovered from the UT and PB regions, while arthropods were also observed in KB, DE and PA.

All samples were collected aseptically using a spatula, immediately frozen and transported and stored at –20°C until further processing. Detailed information on environmental parameters is presented in supplementary Table S1 (Supporting Information). Coordinates and elevation were recorded using a handheld GPS. Most samples consisted of only gravel and finer weathered rock material (further referred to as abiotic or mineral samples), while 10 samples contained lichens or moss, or were taken directly below lichen or moss biomass. In 10 samples, a darker, soil-like matter (i.e. an accumulation of organic matter) was found, but only six of these coincided with the presence of lichen or moss. Collembolans or mites were observed in five samples, of which four were mineral soils. Macroscopic algae were observed in one sample. In this study, only the samples in which lichens, mosses or algae were observed will be referred to as macrobiota samples.

Prior to analyses, the soil samples were homogenized by shaking the tubes, containing the defrosted sample. Total carbon (TC) was determined by combustion of a subsample, while inorganic carbon (IC) was determined through acidification of a second subsample and subsequent combustion at a lower temperature. In both instances, CO_2 was subsequently measured through infrared spectrometry using a Shimadzu TOC5050A

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analyzer with an SSM5000A module. Total organic carbon (TOC) was calculated by subtracting IC from TC. Prior to pH and EC measurements, homogenized soil subsamples were dried at 40°C for at least 24 h until no further mass reduction was observed, after which the moisture content was calculated. To measure pH and electric conductivity, 15 ml of distilled water was added to 3 g of the sieved soil fraction of <2 mm (ISO 10390 and ISO 11265). EC was measured using an LF96 Microprocessor Conductivity Meter (WTW, Germany) at 20°C, and pH was measured after EC on the same preparation with a P902 Multichannel analyzer (Consort, Belgium). For each sample, the pH and EC were measured in duplicate, and the average of both measurements was used in subsequent statistical analyses.

DNA extraction and sequencing

Of each homogenized sample, 1 g was used for DNA extraction. First, extracellular DNA was removed, following Corinaldesi, Danovaro and Dell'Anno (2005) and intracellular DNA extraction was performed according to Zwart *et al.* (1998). PCRs targeted the V1–V3 hypervariable regions and were performed in duplicate. Primers pA (AGAGTTTGATCCTGGCTCAG, positions 8–27) (Edwards *et al.* 1989) and BKL1 (GTATTACCGCGGCTGCTGGCA, positions 536–516) (Cleenwerck *et al.* 2007) were modified with adapters to complement the Nextera XT index kit (Illumina, USA) and HPLC purified (IDT, Belgium). Each reaction consisted of 2.5 μl 10× buffer (High Fidelity PCR system, Roche, Switzerland), 2.5-μl dNTPs (10 mM) (Life Technologies, Belgium), 0.5 μl of each primer, 0.1 unit of High Fidelity Hot Start polymerase (Roche, Switzerland), 0.5–2 μl of DNA template, and was adjusted to a final volume of 25 μl using sterile HPLC water. An initial denaturation step of 3 min at 95°C was followed by 27 cycles of 30 s at 95°C, 45 s at 55°C, and an extended elongation of 3 min at 72°C in order to reduce the number of chimeric sequences (Engelbrektson *et al.* 2010). Lastly, a final elongation of 10 min at 72°C was executed. Duplicate PCR products were pooled and subsequently purified using a slightly modified Ampure beads XT (Agencourt, Beckman Coulter, USA) protocol, where only 0.8 volumes of beads were used and DNA was resuspended in milliQ water. Tagging was subsequently done with the Nextera XT indices in an eight-cycle version of the amplicon PCR, with the indices replacing the primers. PCR products were purified as described above, with DNA resuspended in a 0.10 M 8.5 pH Tris buffer. DNA integrity was checked using a BioAnalyzer (Agilent Technologies, USA), and concentrations were measured using the High Sensitivity assay for Qubit (Thermo Fisher Scientific, USA), after which the samples were pooled equimolarly. Amplicons were sequenced on an Illumina MiSeq platform giving 2 × 300 bp paired end reads. The run also included a blank (a mixture of several PCRs' negative controls), two mock communities to check overall run quality and benchmark processing variables, and two replicate samples. The replicate samples included one sample, which is included in every Illumina run to check for consistency between different analyses, and one sample that was run twice. Sequences were deposited to the NCBI Sequence Read Archive under accession number SRP067111.

ARISA

One out of every five DNA extractions was performed in duplicate, which revealed that the DNA extraction method and ARISA were robust (see Supporting Information for a description of these results). Internal Transcribed Spacer 1 (ITS1) amplification was performed using the universal primers 16S/p2 (CTTGTACACACCGCCCGTC, positions 1390–1407) and 23S/p7 (GGTACTTAGATGTTTCAGTTC, positions 188–208) (Gürtler and Stanisich 1996), the former with a 6-FAM (carboxyfluorescein) label (Sigma-Aldrich, USA). The PCR mixture consisted of 5 μl 10× buffer (Life Technologies, Belgium), 5-μl (2 mM) dNTP (Life Technologies, Belgium), 1 μl (25 mM) $MgCl_2$ (Qiagen, Germany), 0.8-μl (20 μg μl^{-1}) BSA (Roche, Switzerland), 2.5 μl (10 μM) of each primer, 1.25 μl (1 u μl^{-1}) Taq polymerase (Life Technologies, Belgium), 2-μl template DNA and 29.95-μl sterile milliQ water. An initial step of 5 min at 94°C was followed by 35 cycles of 1 min denaturation at 94°C, 1 min of annealing at 56°C and an elongation step at 72°C for 1 min. After a final elongation of 5 min at 72°C, the amplified products were purified, using filter plates (NucleoFast 96 PCR, Machery-Nagel, Germany) and a Tecan Workstation 200 (Switzerland), and the concentration was adjusted to 25 ng μl^{-1} using a NanoDrop 2000 (Thermo Fisher Scientific, USA). Next, a mixture of 0.2 μl GeneScan 1200 LIZ Size Standard (Applied Biosystems, USA), 7.8-μl (1 u μl^{-1}) Hi-Di formamide (Life Technologies, Belgium) and 2 μl of DNA template was made, centrifuged, heated for 4 min at 95°C and subsequently cooled on ice for 2 min. ITS1 fragments were separated on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, USA) for 108 min at 8.5 kV.

Data processing

Forward and reverse reads of the Illumina sequencing were paired using the *fastq_mergepairs* command in USEARCH (Edgar 2010). We used the mock communities to determine the variables giving the best approximation to the original input set of sequences. The minimum length was set to 450 bp, and the maximum length to 570 bp. No ambiguous bases (N) were permitted, and a mismatch of at most 6 bp was allowed for the overlap of paired reads. Further, sequence quality filtering and processing were done using USEARCH, with a minimal Phred score set to Q20 and a maximum expected error of 0.5. OTU clustering was done with UPARSE (Edgar 2013) and *de novo* chimera filtering with UCHIME (Edgar *et al.* 2011). An OTU cut-off of 97% was used to cluster sequences, and the May 2013 GreenGenes training set (DeSantis *et al.* 2006; Schloss 2010; McDonald *et al.* 2012), trimmed to the V1–V3 region, was used with the naïve Bayesian classifier (Wang *et al.* 2007) implemented in Mothur (Schloss *et al.* 2009) to infer a taxonomy for these OTUs. Non-bacterial, chloroplast and mitochondrial OTUs were removed prior to downstream analysis.

ARISA fragment lengths (AFL) were analyzed with GeneMarker v2.2.0 (SoftGenetics, USA). Community fingerprints were checked for quality and were removed when the size standard showed errors or when the ITS1 peaks showed anomalies (e.g. negative values or no peaks). After manually checking baseline noise levels, we set a minimal intensity threshold of 50 RFU (Relative Fluorescence Units). Next, peaks were binned to deal with size-call uncertainty (Brown *et al.* 2005), i.e. 2–4 bp bins for AFLs up to 700 bp, 5 bp bins from 700 to 1000 bp, and bins of 10 bp for larger fragments lengths. Binning is especially necessary for larger fragments (Popa *et al.* 2009). We set a minimal AFL of 350 bp following the observation of Kovacs, Yacoby and Gophna (2010) on minimum fragment lengths in bacterial genomes and in line with the fact that the vast majority of our AFLs were minimally between 459 and 500 bp long. By doing so, we also eliminated the noise observed in some samples associated with smaller sized fragments. We did not eliminate shoulder peaks explicitly, as they were generally contained within a bin.

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Table 1. One-way ANOVA analyses showing the effect of bedrock type or the presence or absence of macrobiota on environmental parameters and the relative abundances of the most abundant phyla. Only significant effects are displayed.

	Bedrock		Macrobiota	
	F	*P*	*F*	*P*
pH	16.570	<0.001	9.861	<0.005
Electric conductivity			4.378	<0.05
TOC content	16.6	<0.001	51.43	<0.001
Moisture content			21.59	<0.001
Actinobacteria	6.688	<0.05	10.39	<0.005
Armatimonadetes	5.977	<0.05	4.367	<0.05
Bacteroidetes			5.339	<0.05
FBP	10.15	<0.005	13.98	<0.001
Planctomycetes			9.534	<0.005
Proteobacteria	5.433	<0.05	16.47	<0.001
Verrucomicrobia			5.7	<0.05

TOC: total organic carbon.

Statistical analyses

The Pearson correlation between abiotic variables was tested. TC showed a high correlation with TOC ($r = 0.99$, $P = 0$) and was removed from the analysis. Values of the remaining abiotic variables were checked for normality (Shapiro–Wilks test) and homoscedasticity (Bartlett's test). Carbon and moisture content were logit transformed, while EC was log transformed. One-way ANOVAs were performed to detect significant differences in the values of abiotic variables for either bedrock or the presence of macrobiota (Table 1). For pH, a Welch t-test was additionally used because this is robust for data with unequal variances. In order to study the effect of bedrock type, independent from that of the presence of macrobiota, on the environmental factors, a one-way ANOVA was used on the mineral samples alone (Table S2, Supporting Information). A similar procedure could not be applied on the macrobiota containing samples, because only two such samples were available from gneiss derived soils.

To investigate the effects of the abiotic factors on the observed richness, the number of OTUs in the NGS data was recalculated based on subsampling to the lowest number of reads (1123) in the dataset. The Pearson correlation between abiotic variables or macrobiota and the Chao1 estimator, and the Shannon and inverse Simpson diversity indices were calculated.

Ordination and cluster analyses were used to assess differences in community structure between the samples and to identify those factors explaining the biotic turnover patterns between the samples. For the multivariate analysis of the ARISA data, one sample was selected randomly out of the replicate samples. The multivariate analyses involved two approaches. First, a SIMPROF analysis (Clarke, Somerfield and Gorley 2008) with 999 iterations, alpha set to 0.05, and average clustering using the Bray–Curtis distance was used for clustering the samples based on their bacterial community structure. SIMPROF is a permutation-based procedure that ranks the pairwise similarities in each group and tests the null hypothesis that samples were all drawn from the same species assemblage. Second, redundancy analyses (RDA) were used to identify the environmental and spatial factors, explaining variation in community structure based on a variation partitioning approach. Apart from the biotic datasets (ARISA and NGS based), this required two other datasets, namely, the one with the environmental variables described above and one with spatial factors. First, the biotic data were Hellinger transformed because this has been shown to be a valid data transformation when analyzing variation between communities at individual sites, using ordination analyses (Legendre, Borcard and Peres-Neto 2005; Peres-Neto *et al.* 2006). The matrix with the spatial variables contained Principal Coordinates of Neighbor Matrices (PCNMs) of the geographic coordinates of the samples (Borcard and Legendre 2002; Dray, Legendre and Peres-Neto 2006). These are positive eigenvalues obtained by principal coordinate analysis (PCoA) of a truncated matrix of Euclidian distances between the sampling sites. Partial RDAs were subsequently run using the forward selection procedure following the protocols described in Peres-Neto *et al.* (2006) in order to identify the factors significantly explaining the variation in bacterial community structure, and to calculate the amount of variation uniquely explained by the environmental factors, the spatial factors and the overlap between both sets of predictors. All downstream analyses and statistics for both datasets were performed in R (R Core Team 2015), with the packages Vegan 2.3-0 (Oksanen *et al.* 2015) and clustsig 1.1 (Whitaker and Christman 2014) for the redundancy analysis (Hellinger transformed data and scaled abiotic variables), the calculation of the PCNM variables (Borcard *et al.* 2004) and the diversity metrics.

RESULTS

Soil properties

A higher abundance of macrobiota was observed in the nunatak sites most distant (i.e. 10–20 km north) from the plateau, with mainly granite derived soils. The granite-derived samples also had a significantly higher average TOC content (One-way ANOVA; $F = 16.6$; $P < 0.001$) than the gneiss derived samples (2.3 ± 6.5% versus 0.2 ± 0.4%) (see also Table 1; Fig. 2a). Sample UT50 had an extremely high TOC content (30.6%), which could be attributed to the fact that it consisted nearly completely of moss. However, the difference in TOC content between both bedrock types remained significant after removing this outlier (One-way ANOVA; $F = 14.7$; $P < 0.001$). The TOC content was also higher in the samples containing macrobiotia (4.7 ± 9.3%), compared to mineral soils (0.1 ± 0.2%; One-way ANOVA; $F = 51.3$; $P < 0.001$). The moisture content showed large differences between samples, ranging from 0.2% to 51.6%, and was on average higher for granite derived samples (9.7 ± 12.3% versus 4.8 ± 6.2%), but this difference appeared to be insignificant. The difference in moisture content between mineral samples and those containing macrobiota, however, was highly significant (One-way ANOVA; $F = 21.6$; $P < 0.001$), with the extreme value of 51.6% again being attributable to sample UT50. On average, samples in which macrobiota were present contained 19.6 ± 14.8% moisture, compared to 3.7 ± 3.9% for the mineral soils. The pH varied from 5.0 to 8.9, with the average pH being significantly lower for granite samples (5.9 ± 0.5) than for gneiss samples (6.9 ± 1.1) (Welch Two Sample t-test; $P < 0.001$); yet, the latter showed a very broad pH range, with sample PB1107 having the overall lowest pH. The pH was also significantly lower when macrobiota were present (Welch Two Sample t-test; $P < 0.005$). The EC ranged from 6 to 890 μS cm^{-1} (on average 100 ± 177 μS cm^{-1}) but was not significantly different between bedrock type and only marginally significantly higher for the macrobiota containing samples compared to the mineral samples (One-way ANOVA; $F = 4.4$; $P < 0.05$; Fig. 2d, Table 1). TOC content showed a weak but significant negative correlation with pH, and a significant moderate positive correlation with both EC and moisture content (Table 2). EC and pH were positively correlated ($r = 0.41$, $P < 0.01$).

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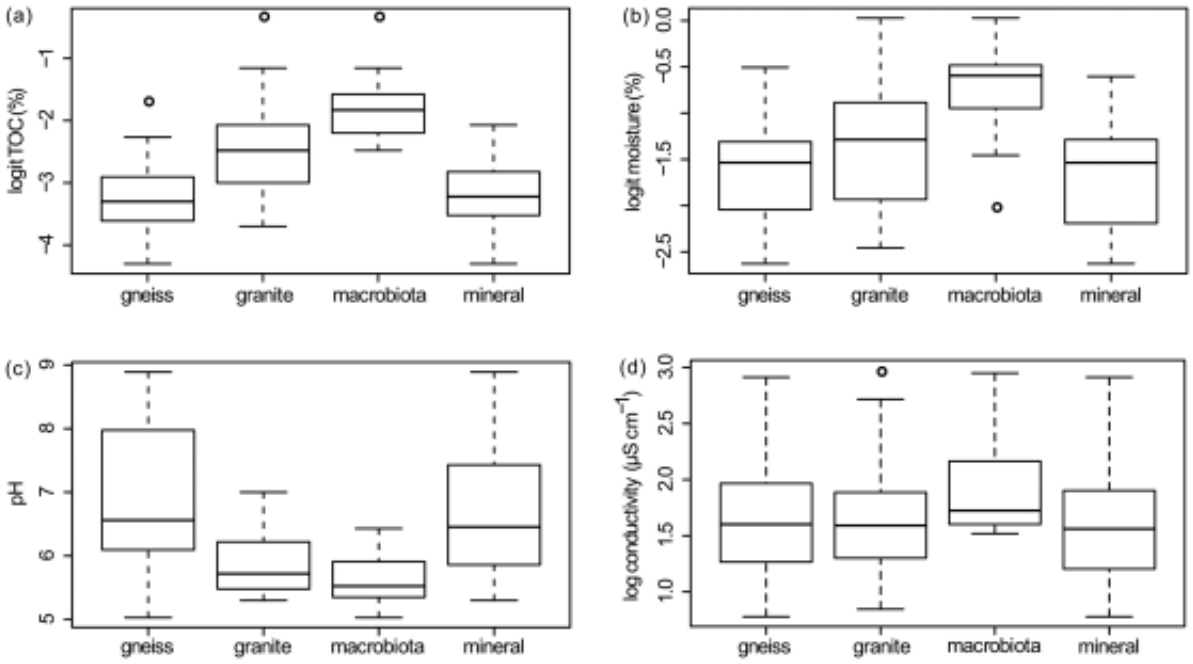

Figure 2. Comparison of abiotic variables between both bedrock types (gneiss and granite) and purely mineral or macrobiota containing samples. (a) Total organic carbon content; (b) moisture content; (c) pH and (d) electric conductivity.

Table 2. Pearson correlation (*r*) and significance levels (*P*) among abiotic variables and between abiotic variables and diversity indices and estimators. Only significant (bold) or nearly significant ($P < 0.1$) effects are displayed.

	TOC		Electric conductivity		pH		Moisture	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Electric conductivity	0.46	<0.001	–	–	0.41	<0.005		
pH	−0.28	<0.05	0.41	<0.005	–	–		
Moisture	0.47	<0.001					–	–
OTUs							0.28	<0.05
Shannon							0.36	<0.01
Inverse Simpson	0.26	0.07					0.3	<0.05
Chao1							0.29	<0.05

Taxonomic composition of the bacterial communities

The Illumina sequencing resulted in nearly four million reads, of which 395 512 high-quality sequences remained for the 52 samples after processing. A total of 3360 OTUs were obtained, based on a 97% cut-off level, which could be assigned to 24 phyla and 189 genera. At the phylum level and genus level, 214 OTUs (985 sequences) and 2534 OTUs (314 419 sequences), respectively, remained unclassified. The average number of sequences per sample equaled 7606 ± 4574 with on average 260 ± 87 OTUs.

In total, 32% of the reads were identified as belonging to the Acidobacteria (Fig. S1, Supporting Information), of which 95% were closely related to isolate *Ellin6075* (Joseph *et al.* 2003) and assigned to class Chloracidobacteria (subgroup 4). Proteobacteria, Cyanobacteria and Actinobacteria were the other most abundant phyla (21%– 15%). Chloroflexi, the candidate division FBP, Bacteroidetes and Armatimonadetes, represented between 3.3% and 2.6% of the sequences, while the remaining phyla all represented less than 1% of the sequences (or less than 3% of the total number of sequences combined). Actinobacteria had the highest number of OTUs (689), followed by the Proteobacteria (683), Bacteroidetes (397) and Chloroflexi (355). However, when the number of OTUs in relation to the number of sequences was considered, Firmicutes had the highest relative number of OTUs (44 for 126 sequences) for those phyla represented by more than 12 sequences (i.e. excluding (candidate) phyla BRC1, Elusimicrobia, GN02, AD3, Nitrospira), followed by candidate phylum OD1 (26 OTUs, 209 sequences) and Planctomycetes (176 OTUs, 2149 sequences). Interestingly, Acidobacteria were represented by only 145 OTUs.

Drivers of microbial community structure

At the phylum level, Proteobacteria and Acidobacteria, respectively, showed a significant positive and negative correlation with TOC, EC and moisture content (Table 3). Armatimonadetes, candidate phylum FBP, and Planctomycetes showed a positive and Actinobacteria a negative correlation with TOC and moisture content, while Armatimonadetes additionally showed a negative correlation with pH. Deinococcus-Thermus, showed a positive correlation with electric conductivity, and inversely, a negative correlation with moisture content. Lastly,

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Table 3. Pearson correlation (*r*) and significance levels (*P*) between abiotic variables and the relative abundances of the 13 most occurring phyla. Only significant correlations are displayed.

	TOC		Electric conductivity		pH		Moisture	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Acidobacteria	–0.35	<0.05	–0.38	<0.05			–0.32	<0.05
Actinobacteria	–0.41	<0.005					–0.49	<0.001
Armatimonadetes	0.52	<0.001			–0.3	<0.05	0.41	<0.005
Cyanobacteria							0.32	<0.05
FBP	0.43	<0.005					0.31	<0.05
Planctomycetes	0.3	<0.05					0.29	<0.05
Proteobacteria	0.58	<0.001	0.37	<0.01			0.56	<0.001
Deinococcus-Thermus			0.31	<0.05			–0.31	<0.05

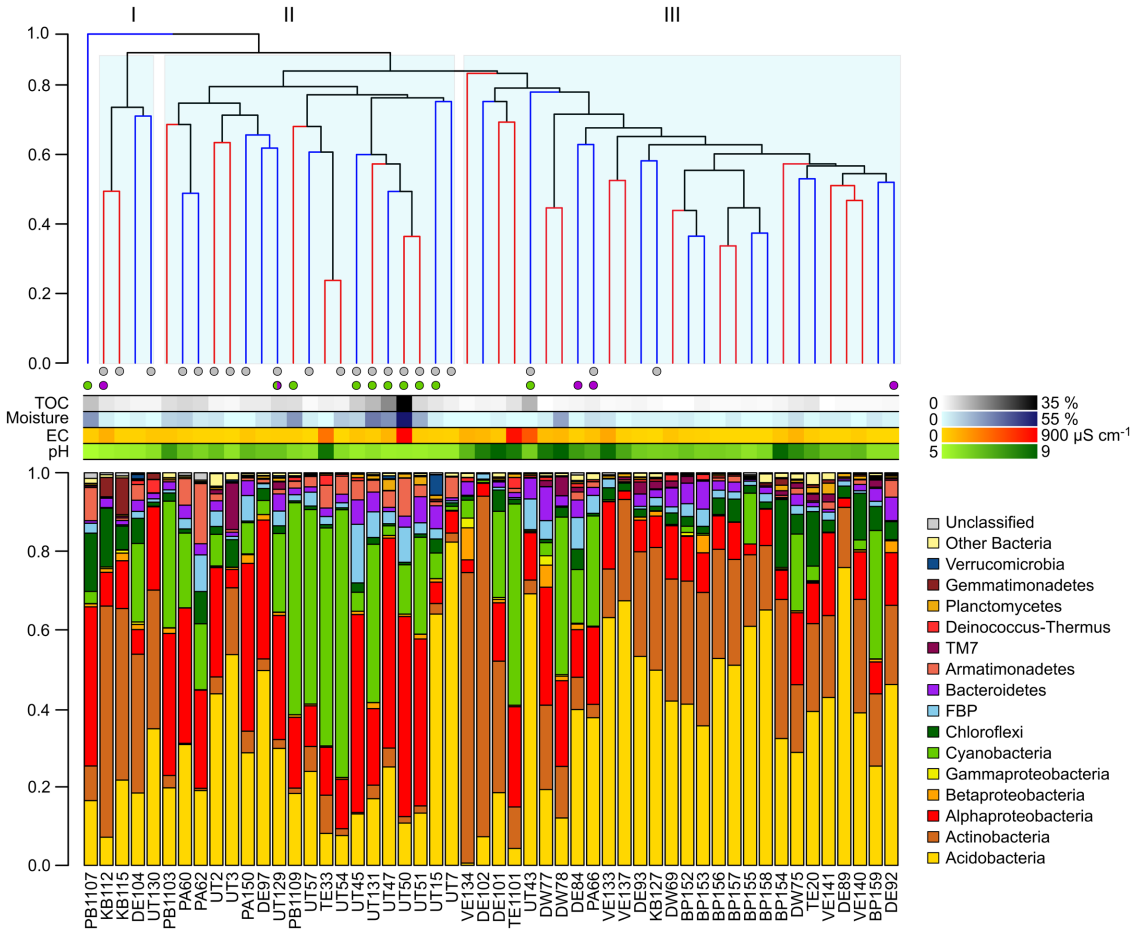

Figure 3. The dendrogram at the top shows the significant SIMPROF clusters of samples shown in black, while non-significant clusters are alternating blue or red. The gray circles underneath the dendrogram show the presence of granite (none for gneiss samples), while the green circles show the presence of macrobiota (moss, lichen, algae or a combination of these), and purple circles arthropods (mites or collembolans). Values of the significant abiotic variables are shown in the bars, with percentages of the total organic carbon (TOC) and moisture content, and the EC (μS cm^{-1}) and pH. Values of TOC were square rooted to enhance visibility. At the bottom, the barplot shows the relative abundances of the 13 best represented phyla (the Proteobacteria are represented by their classes).

Cyanobacteria showed only a significant positive correlation with moisture content, while no significant trends were observed for the remaining phyla.

A SIMPROF analysis of the Illumina dataset at the OTU level (Fig. 3) and the ARISA dataset (Fig. S2, Supporting Information) using the Bray–Curtis dissimilarity index showed comparable patterns and revealed a distinct clustering generally coinciding with bedrock type and the presence of macrobiota. This was confirmed by a Mantel test, which showed a significant correlation between the Illumina and ARISA datasets ($P < 0.01$, $r = 0.33$; 9999 permutations; Hellinger transformed data). The granite cluster could be further subdivided into two main subclusters based on a Bray–Curtis dissimilarity cut-off of about 0.85, which could be further divided into smaller subclusters based on a dissimilarity cut-off of 0.75 (see Supporting Information for a more comprehensive description).

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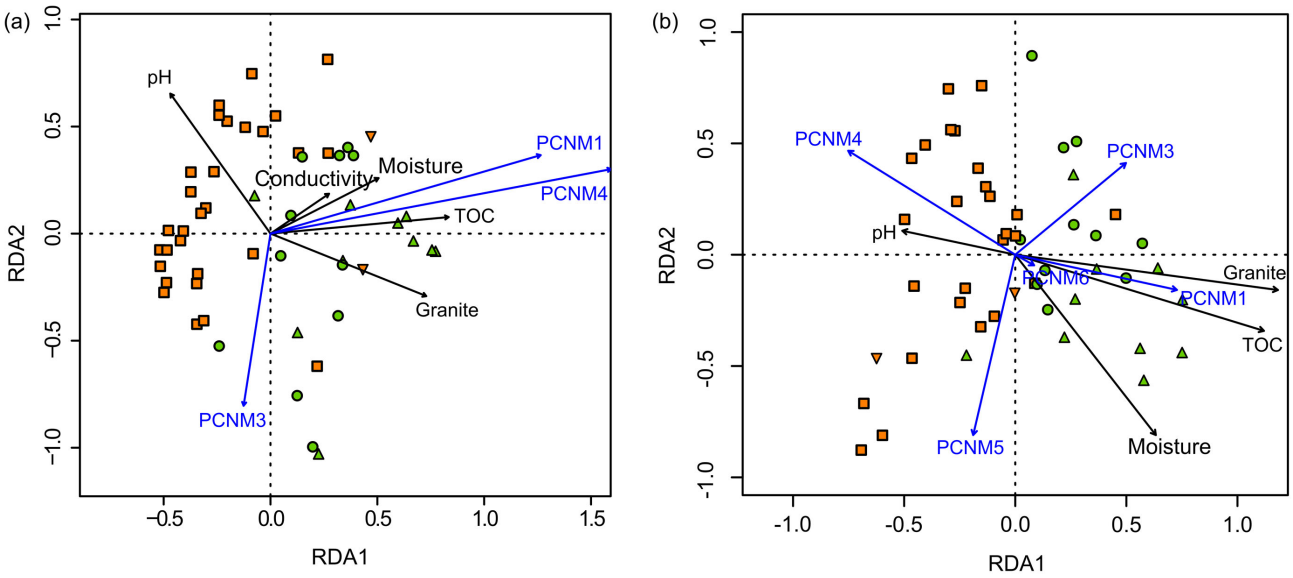

Figure 4. Redundancy analysis (RDA) plot of the 52 Illumina sequenced samples (**a**) and 49 ARISA samples (**b**). Granite derived samples are represented by green circles (mineral samples) or triangles (macrobiota samples); gneiss derived samples are represented by orange squares (mineral samples) or inversed triangles (macrobiota samples). The spatial variables (PCNMs) are indicated in blue, the environmental factors in black.

Cluster I (Fig. 3) showed a relatively high abundance of Actinobacteria, with the samples sharing several distinct OTUs (notably Intrasporangiaceae, including the genus *Terracoccus* and related unclassified taxa), and a relatively low abundance of Proteobacteria and the near absence of Cyanobacteria compared to the other samples within the granite cluster. Granite cluster II contained several distinct acidobacterial Chloracidobacteria and Solibacteres (including Candidatus *Solibacter*) OTUs, and also the only two Koribacteraceae OTUs, one of which was identified as Candidatus *Koribacter*. Furthermore, a relatively large number of Armatimonadetes and Gemmata (Planctomycetes) OTUs were present. Nearly, all moss and lichen containing samples were restricted to this cluster. Markedly, five gneiss derived samples belonged to the granite cluster, of which two Perlebandet samples, with sample PB1109 containing lichens. The third Perlebandet sample, PB1107, was the overall outlier, yet, still grouped more closely within the granite cluster and, interestingly, also contained moss and lichens. Two other gneiss samples originated from the eastern Duboisbreen region, and a last one from Teltet, which showed the highest overall similarity with an Utsteinen sample.

While samples on gneiss spanned a wider gradient in pH, they appeared to be more similar in terms of OTU composition (Fig. S3, Supporting Information). They displayed generally higher proportions of Acidobacteria and Actinobacteria, and lower numbers of Proteobacteria and Cyanobacteria. One granite sample, KB127, showed notable similarity with the gneiss samples and in the field gneiss was visibly present, suggesting that this sample may at least partly consist of gneiss. The gneiss cluster III displayed a more gradual splitting of its members into small (2–3 sample) groups, but two larger subclusters were present, one of which contained nearly all Brattnipane samples, while the other seemed to be at least partly driven by several Chloroflexi OTUs.

A redundancy analysis of both the Illumina and ARISA datasets showed that TOC, followed by pH, EC (only for the Illumina data), bedrock type and moisture content significantly ($P < 0.001$) explained variation in the community structure (Fig. 4a and b), while TC was not significant. An RDA with a subset of 45 samples for which elevational data was also present did not show a significant effect of elevation (data not shown). Spatial variables explained a larger part of the variation in the ARISA dataset (five significant PCNM variables) compared to the Illumina dataset (three variables). The importance of the different abiotic variables in explaining patterns in OTU composition was further confirmed by the variation partitioning analysis. This revealed that ~18% of the observed variance could be explained by the selected abiotic and spatial variables and that geographical distance by itself accounted for 3% of the observed variance, while the abiotic variables and the overlap of the abiotic and spatial variables accounted for 9.4% and 5.6%, respectively. Bedrock by itself significantly explained 1.5% of the variance and an additional 4.5% through the overlap with the other abiotic variables.

Moisture content showed a weak but significant positive correlation with the richness and diversity indices and number of OTUs (Table 2). Moreover, the presence of moss resulted in a significantly higher inverse Simpson value (One-way ANOVA; $F = 5.7$; $P < 0.05$).

DISCUSSION

Factors structuring bacterial community structure

The multivariate analyses of both the Illumina and ARISA datasets revealed congruent patterns and showed that the total organic carbon content and related to this the presence or absence of macrobiota (i.e. algae, mosses and lichens), pH, bedrock type, moisture content, and, also EC for the Illumina dataset, significantly, explained part of the variation in the bacterial communities. The variation partitioning analysis further revealed that 9.4% of the variation could be uniquely explained by the abiotic variables, 3% by geographical distance and 5.6% by the overlap between both groups of predictors. It follows that the measured environmental factors partly shape the bacterial communities in this part of the Sør Rondane Mountains. These

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significant variables were also shown to exert an influence on bacterial assemblages in terrestrial habitats in other Antarctic ice-free regions. For example, the effects of pH and EC on bacterial communities have been well documented in soils from the Transantarctic Mountains and the MDV (e.g. Magalhães *et al.* 2012; Van Horn *et al.* 2013; Geyer *et al.* 2014; Kim *et al.* 2015), and the influence of soil organic matter was also demonstrated in some studies from the latter region (Niederberger *et al.* 2008, 2015; Geyer *et al.* 2014). By contrast, the effect of the moisture content on the bacterial community composition or diversity in Antarctic soils appears to be less conclusive. For example, the moisture content had a clear effect on the bacteria in some soils from the MDV as shown by Niederberger *et al.* (2008, 2015) and Geyer *et al.* (2014), while it appeared to be marginally important in other soils from the region (Wood *et al.* 2008), as well as in those in the Maritime Antarctic (Newsham, Pearce and Bridge 2010) and the Transantarctic Mountains (Magalhães *et al.* 2012).

The variation partitioning analysis further revealed that bedrock type by itself also explained a significant portion (1.5%) of the variation in community structure, independent from that explained by the other environmental factors, including TOC content and hence the presence of macroscopic vegetation cover. Moreover, bedrock type, possibly in combination with (micro)climatic conditions also appeared to affect the presence of vegetation as evidenced by the higher abundance of lichens, mosses and algae on granite soils compared with those on gneiss (Fig. 3). However, this also resulted in an imbalanced sampling design as only two macrobiota, containing samples situated on gneiss bedrock, were available after the campaigns of 2009–10 and 2010–11. This in turn complicated the assessment of potential bedrock-related differences in the presence of biota on local environmental conditions. More in particular, while the pH and the TOC concentrations were significantly different between the granite and gneiss samples on mineral soils (Table S2, Supporting Information), this could not be statistically assessed for the macrobiota containing samples. All in all, however, our sampling design had no negative effect on the multivariate analyses. The ordination and variation partitioning analyses suggest that bedrock type by itself, but likely also in combination with its potential effect on the presence of macrobiota, exerts an influence on the bacterial communities in soils from the Sør Rondane Mountains (Fig. 4). This is on the one hand surprising, given that the gneiss is of local origin and largely has the same mineral composition as the granite (Shiraishi *et al.* 1997), but the pressure of metamorphism causes the minerals to line up, giving gneiss a distinct-banded appearance (Fossen 2010). This was confirmed by preliminary lead isotopic analysis of individual feldspars from gravels at several sites, in combination with cosmogenic surface exposure dating analysis of granite–syenite boulders, which suggested that the mineral composition of the gneiss bedrock in the Sør Rondane Mountains is indeed similar to that of the granite (S. Roberts, unpublished data). On the other hand, and despite these similarities in mineral composition, the banded texture of gneiss makes it more vulnerable to physical and chemical weathering (Fossen 2010). More in particular, the darker bands with mafic minerals (biotite, amphibole and pyroxene) in gneiss can act as a transport zone of liquid water, which potentially results in the release of base cations (Best 2002). This can explain the significantly higher pH of the weathering products in the gneiss derived soils (Table 1; S2, Supporting Information). Although we lack geochemical data to support this, we tentatively suggest that these chemical differences might in part explain the observed dissimilarities in the bacterial community structure between both bedrock types (e.g. Gray *et al.* 2014; Bryce *et al.* 2016). This is analogous to other studies which revealed that differences in mineral and (trace) element composition or soil texture are important factors in shaping bacterial communities in Antarctica and elsewhere (Barton *et al.* 2007; Carson *et al.* 2009; Mitchell *et al.* 2013; Yarwood *et al.* 2014; Kim *et al.* 2015). Interestingly, while communities on gneiss-derived soils generally differed from those on granite-derived soils, two samples from Perlebandet (gneiss) grouped in a significant cluster composed of granite samples (Fig. 3). The factors underlying this are unclear, but they might be related to the particular gneiss subtype of Perlebandet (augen gneiss), the presence of marble bedrock (Shiraishi *et al.* 1997), or other local, potentially unmeasured environmental conditions. In summary, bedrock type appears to be an important driver in Antarctic soils, as was for example also demonstrated in other oligotrophic environments (Barton *et al.* 2007).

In addition to bedrock type alone, the presence of moss or lichen also resulted in very distinct bacterial assemblages. More in particular, the bacterial community structure was highly similar between the samples containing moss and lichen, leading to the presence of a significant cluster containing these samples within the one composed of the samples situated on granite (Figs 3 and 4). This is, on the one hand, likely be due to increased availability of organic nutrients (debris, exudates) resulting in a larger proportion of heterotrophs, but possibly also due to differences in microclimatic conditions, such as a reduced wind exposure and increased humidity and heat retention (e.g. Cannone and Guglielmin 2009). Moreover, moss and lichen, containing samples in soils from both bedrock types, shared a relatively high number of OTUs compared to samples from their respective mineral soils (Fig. S4, Supporting Information). However, the number of taxa above OTU level was comparable between samples containing macrobiota and mineral soils, although, for example, relatively more Proteobacteria lineages were present in the former. Interestingly, while Firmicutes were found to be the most dominant phylum in the rhizosphere of vascular Antarctic plants (Teixeira *et al.* 2010) and highly abundant in MDV samples with a higher moisture content (Van Horn *et al.* 2013), they were nearly absent from our dataset (only 126 sequences) and mainly restricted to two mineral gneiss samples. Similarly, relatively a few Deinococcus–Thermus were recovered from the soils without vegetation (2233 sequences, of which 1156 identified as *Truepera*). This is in contrast to dry mineral soils from Victoria Land in which they are abundantly present (Niederberger *et al.* 2008; Pointing *et al.* 2009).

While vegetation cover appears to be important for structuring the bacterial communities, OTU richness was not significantly related to the TOC content (Table 2). Interestingly, similar conclusions were drawn from a clone library survey from ornithogenic soils in the MDV (Aislabie *et al.* 2009). By contrast, Geyer *et al.* (2013) found a productivity–diversity relation in a variety of MDV soils using T-RFLP. We did, however, notice a significant trend of increasing OTU-richness with increasing moisture content, and Shannon and inverse Simpson diversity indices were significantly higher in samples with a higher moisture content too. This is however in disagreement to what Newsham, Pearce and Bridge (2010) as well as Stomeo *et al.* (2012) found in Mars Oasis (West Antarctic Peninsula) and MDV soils, respectively. This difference in the effect of the moisture content on bacteria in the soils in the Sør Rondane Mountains might be related to the generally very low moisture content in mineral soils or the wide gradient covered in the present study.

In addition to the abovementioned environmental variables, spatial distance appeared to explain a significant, yet relatively

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minor (3%, three out of the six PCNM variables) part of the variation in community structure in the Illumina dataset. Variation partitioning further revealed that the overlap between geographic distance with abiotic variables was more important than distance itself, which was also observed in the MDV and the Transantarctic Mountains (Geyer *et al.* 2013; Sokol *et al.* 2013; Chong, Pearce and Convey 2015). Nevertheless, some spatial autocorrelation is apparent since samples in close proximity tend to be more alike, which was clearly evident in the samples from Brattnipane. More striking however is the observation that the communities in the (northern) nunataks differed substantially from those situated on similar bedrock samples in the main mountain range to the south. This suggests limited dispersal capacities, most likely in combination with microclimatic differences as also noted in other areas from the Antarctic (see Chong, Pearce and Convey 2015 for a review). Indeed, the different regions of the main mountain range showed a relatively high similarity, which may be partly linked to their closer geographic connectedness, possibly facilitating dispersal of organisms over short distances in combination with the severe influence of the nearby polar plateau.

Bacterial community composition

In general, the main phyla observed are globally dominant in soils (Janssen 2006) and also more or less the same as those reported from other Antarctic terrestrial studies (Yergeau *et al.* 2007b; Pointing *et al.* 2009; Teixeira *et al.* 2010; Van Horn *et al.* 2013; Bakermans *et al.* 2014; Kim *et al.* 2015), namely Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Verrucomicrobia, Bacteroidetes, Armatimonadetes, Gemmatimonadetes and Planctomycetes, and additionally candidate division FBP and Cyanobacteria (Fig. S1, Supporting Information). However, half of our samples were dominated by Acidobacteria. This was particularly the case in gneiss derived soils with a very low TOC concentration, which might be related to the slow growth rate of Acidobacteria, which usually thrive in oligotrophic conditions (Fierer, Bradford and Jackson 2007). Nearly, all acidobacterial reads were assigned to the class Chloracidobacteria (Subdivision 4 of the Acidobacteria), which represented ∼95% of all Acidobacteria sequences. The phylum Acidobacteria as a whole displayed a relatively low diversity (only 145 OTUs), while having 32% of the total reads. Similar high relative abundances of Acidobacteria have recently been reported from Victoria Land, where they appeared to be associated with the relatively richer—albeit still oligotrophic—soils, characterized by a higher pH (Van Horn *et al.* 2013; Kim *et al.* 2015). Among the Acidobacteria, Chloracidobacteria are considered to be aerobic anoxygenic photoheterotrophs, which contain distinctive bacteriochlorophylls (Bchl *a* and *c*) (Bryant *et al.* 2007). Although we cannot confirm the actual presence of bacteriochlorophylls *a* and *c* in our samples, the ability to use solar energy in the Antarctic as a means to generate ATP would be advantageous (Dana, Wharton Jr. and Dubayah 1998), and might indeed lead to their high relative abundance in many of our samples. Tahon *et al.* (2016) found evidence for a large diversity of light capturing *pufM* genes in some of these samples, at least suggesting the possibility of exploiting this energy source by soil organisms. In the SRM, contrary to the Chloracidobacteria, OTUs from the Acidobacteriaceae (genus *Terriglobus* and unclassified OTUs) were restricted to moss-containing samples, while Koribacteraceae were nearly completely restricted to granite samples with macrobiota. Members of the Solibacterales showed an increased relative abundance on granite derived soils, with relatively high carbon content. It is known that Candidatus *Solibacter* is a chemoorganotroph that is able to use complex organic carbon sources such as cellulose, and stabilizes its environment through biofilm production (Ward *et al.* 2009). Candidatus *Solibacter* was also the most recovered genus from a metagenomics study of moraine soils in Mars Oasis, a biodiversity hotspot in Maritime Antarctica (Pearce *et al.* 2012).

In addition to Acidobacteria, Cyanobacteria were also widespread (75% of the samples). However, they showed a relatively low abundance compared with aquatic habitats (Tytgat *et al.* 2014; Obbels *et al.* 2016). Their presence has important ecological consequences, because while nitrogen is thought to be a major limiting factor in Antarctic terrestrial systems, it is generally assumed that N-fixation in Antarctica is mainly performed by Cyanobacteria (Cowan *et al.* 2011), either free-living or as cyanobionts in lichens (Seneviratne and Indrasena 2006; Yergeau *et al.* 2007a). It is also assumed that other free-living organisms rarely fix nitrogen in these environments due to energetic constraints (Raymond *et al.* 2004). However, also within the Actinobacteria, certain species (e.g. *Arthrobacter*, *Frankia*, *Rothia* and *Corynebacterium*) have N-fixation potential (Sellstedt and Richau 2013). Considering the relatively high abundance of Actinobacteria in our soils, and that *Arthrobacter* and unclassified Frankiaceae related OTUs were among the most represented Actinobacteria, these taxa can potentially be important N-fixers in addition to Cyanobacteria in these terrestrial environments. For Cyanobacteria, it is hypothesized that taxa belonging to the Nostocales order particularly play a major role in nitrogen fixation in similar ecosystems (Cowan *et al.* 2011). Indeed, a clone library study by Tahon *et al.* (2016) from the Utsteinen area showed that only *nifH* sequences from *Nostoc*-related organisms were recovered. In our dataset, an OTU showing 99.1% similarity to the recently described heterocystous genus *Toxopsis* (Lamprinou *et al.* 2012) in the Nostocales order was the fifth most relative abundant OTU (after four Chloracidobacteria OTUs). However, it was recovered from only 19 samples and seemed closely associated with the presence of moss, lichens, a high TOC content and granite soils. Overall, ten cyanobacterial OTUs were among the 30 most abundant OTUs and appeared to be closely related to either *Nostoc*, several *Phormidium* spp., *Microcoleus vaginatus* CJ1-U2-KK1 and *Crinalium* PCC9333.

In conclusion, Acidobacteria (Chloracidobacteria) and Actinobacteria (Actinomycetales) dominated mineral soil samples situated on gneiss derived bedrock, while Proteobacteria (Sphingomonadaceae), Cyanobacteria, Armatimonadetes and candidate division FBP-dominated soil samples with a high total organic carbon content that were mainly situated on granite derived bedrock. It follows that the structure of soil microbial communities in the Sør Rondane Mountains is mainly controlled by (i) the availability of moisture and TOC, which is related to the presence of mosses or lichens, and (ii) bedrock type, and related to this weathering rates and substrate stability.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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