RESEARCH ARTICLE

Bacterial and eukaryotic biodiversity patterns in terrestrial and aquatic habitats in the Sør Rondane Mountains, Dronning Maud Land, East Antarctica

Dagmar Obbels1,†,‡, Elie Verleyen1,†, Marie-José Mano2, Zorigto Namsaraev2,3,4, Maxime Sweetlove1, Bjorn Tytgat5, Rafael Fernandez-Carazo2, Aaike De Wever1,6, Sofie D’hondt1, Damien Ertz7,8, Josef Elster9, Koen Sabbe1, Anne Willems5, Annick Wilmotte2 and Wim Vyverman1

1Laboratory of Protistology and Aquatic Ecology, Department of Biology, Ghent University, Krijgslaan 281, S8, B-9000 Ghent, Belgium, 2Centre for Protein Engineering, Institute of Chemistry, Université de Liège, Sart-TilmanB6, B-4000 Liège, Belgium, 3Winogradsky Institute of Microbiology RAS, Pr-t 60-letya Oktyabrya, 7/2, Moscow 117312, Russia, 4NRC Kurchatov Institute, Akademika Kurchatova pl. 1, Moscow, 12 31 82, Russia, 5Laboratory for Microbiology, Department of Biochemistry and Microbiology, Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium, 6Operational Directorate Natural Environment, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium, 7Botanic Garden Meise, Department Bryophytes-Thallophytes, Nieuwelaan 38, B-1860 Meise, Belgium, 8Federation Wallonia-Brussels, General Administration of the Non-Compulsory Education and Scientific Research, Rue A. Lavallée 1, 1080 Brussels, Belgium and 9Centre for Polar Ecology, Faculty of Sciences, University of South Bohemia, Institute of Botany, Academy of Sciences of the Czech Republic, Dukelská 135, 379 82, Treboň, Czech republic

*Corresponding author: Department of Biology, Ghent University, Krijgslaan 281, S8, 9000 Gent, Ghent, 9000, Belgium. Tel: +32(0)92648504; E-mail: dagmar.obbels@ugent.be
†These authors contributed equally to this paper.

One sentence summary: This is the first study to describe the diversity of both bacteria and eukaryotes in terrestrial and aquatic environments in the Sør Rondane Mountains, East Antarctica using 454 pyrosequencing analysis of partial SSU rRNA genes. Multivariate analyses revealed a general lack of differentiations according to habitat type.

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ABSTRACT

The bacterial and microeukaryotic biodiversity were studied using pyrosequencing analysis on a 454 GS FLX+ platform of partial SSU rRNA genes in terrestrial and aquatic habitats of the Sør Rondane Mountains, including soils, on mosses, endolithic communities, cryoconite holes and supraglacial and subglacial meltwater lenses. This inventory was complemented with Denaturing Gradient Gel Electrophoresis targeting Chlorophyta and Cyanobacteria. OTUs belonging to the Rotifera, Chlorophyta, Tardigrada, Ciliophora, Cercozoa, Fungi, Bryophyta, Bacillariophyta, Collembola and Nematoda...
INTRODUCTION

Antarctica is the highest, coldest, windiest and driest continent on Earth (Thomas et al. 2008). Less than 0.3% of it is ice-free, and it is in these sparse ice-free regions that the majority of the biota occurs in a variety of habitats (Convey et al. 2014). These include soils, water bodies where the ice sheet is in contact with nunataks, permanently ice-covered bedrock-based lakes and ponds, within and below rocks and in cryoconite holes in the ice sheet (Bergstrom, Convey and Huiskes 2006). The soils in these isolated, but habitable pockets of land are the most widespread habitat type (Cary et al. 2010) and similar to those in other deserts; they are poorly developed, contain a low amount of organic matter, have a low moisture content and are generally characterized by a high concentration of soluble salts (Freckman and Virginia 1997; Stomeo et al. 2012). Hence, the species inhabiting these ice-free regions are adapted to low precipitation, reduced humidity, (periodic) desiccation and osmotic stress, a low thermal capacity of the substrate, frequent freeze–thaw and wet–dry cycles, limited organic and inorganic nutrients and seasonally high levels of solar radiation (Brinkmann et al. 2007; Yergeau et al. 2007). The availability of liquid water is generally regarded as the key environmental variable controlling the distribution and activity of Antarctic terrestrial biota, and may be even more important than temperature (Kennedy 1993; Block 1996). In the wettest habitats, moss banks, microbial mats and biofilms may develop (Broady 1996; Niederberger et al. 2012). The latter two are dense communities of microorganisms believed to be responsible for much of the primary production in these extreme environments (Vincent 2000). Their matrix typically consists of mucilage in which cyanobacteria and other algal cells are embedded, together with heterotrophic and chemolithoautotrophic microorganisms, microinvertebrates and inorganic materials (Fernandez-Valiente et al. 2007). In the drier habitats, crusts may develop, which can survive prolonged dry periods and become metabolically active when water becomes available (McKnight et al. 2007; Niederberger et al. 2012). At the driest end of the humidity gradient, there are less visible signs of life, and these soils have long been considered to be ‘sterile’ (Cary et al. 2010). However, it is now well accepted that even the most arid soils with no visual presence of water or organic matter are inhabited by biological communities (Tytgat et al. 2014). Moreover, biota in the driest regions may occupy protected and more cryptic habitats (Broady 1996), such as cracks and less exposed gravelly soils, which can become humid after snow melt or remain almost completely dry throughout the entire year (Fernandez-Carazo et al. 2011). Others live underneath stones, between mineral grains or within rocks (endoliths) (Friedmann and Kibler 1980; Pointing et al. 2009).

Because of these extreme environmental conditions, in combination with the isolated nature of the continent and regional extinction in response to glacial-interglacial cycles, the biological activity in Antarctic terrestrial habitats is dominated by microscopic organisms, mosses, lichens, fungi and a few invertebrates including microarthropods and nematodes (Convey et al. 2008; Cowan, Christoffersen and Powell 2012). The long evolutionary history of the latter taxa in geographical isolation and the survival of biota in isolated ice-free refugia resulted in a high incidence of endemism among these organisms (Convey et al. 2008). Although there is also increasing evidence for endemism among microorganisms in Antarctica (De Weyer et al. 2009; Vyverman et al. 2010), the vast majority of the ice-free regions remain understudied in terms of their microbial biodiversity. This is particularly true for inland nunataks in the largely inaccessible mountain chains in East Antarctica (e.g. the Sør Rondane Mountains and Wohltat Massif) and the Transantarctic Mountains, although recent studies revealed that the habitats in these regions contain a relatively large diversity, including previously undiscovered taxa (e.g. Fernandez-Carazo et al. 2011; Peeters, Ertz and Willems 2011; Tytgat et al. 2014). For example, in the Sør Rondane Mountains, a cultivation-based study of bacteria in two samples revealed the presence of five major phyla, namely Actinobacteria, Bacteroidetes, Proteobacteria, Firmicutes and Deinococcus-Thermus, covering 35 different genera, which included a considerable number of potentially new species and genera distributed over 24 genera belonging to different phylogenetic groups (Peeters, Ertz and Willems 2011). De-naturing Gradient Gel Electrophoresis (DGGE) and microscopy of the cyanobacterial community structure in 10 samples taken in the vicinity of the construction site of the Princess Elisabeth station before it was built revealed the presence of 10 morphotypes and 13 Operational Taxonomic Units (OTUs) belonging to the Oscillatoriales, Chroococcales, Nostocales and Stigonematales (Fernandez-Carazo et al. 2012); 46% of the OTUs appeared to be restricted to Antarctica. For microeukaryotes, biodiversity data are still largely lacking for these regions. Even for more conspicuous and rather well-known organisms such as lichens, a new species (Trachelia antarctica Ertz, Apton, G. Thor & Øvstedal) was described from the construction site of the Princess Elisabeth station where 48% of the lichen flora is made up of Antarctic endemics (Ertz et al. 2014).

Here, we aimed to provide a comprehensive inventory of the eukaryotic and bacterial community structure and diversity in a variety of habitats in the Northern part of the Sør Rondane Mountains (Dronning Maud Land, East Antarctica), by using high parallel tag sequencing on a Roche 454 GS FLX+ platform with general bacterial and eukaryotic primers for parts of the SSU rRNA genes. By doing so, we aimed to assess differences in bacterial and eukaryotic community structure between aquatic and terrestrial habitats in the region. The habitats studied were microbial crusts, soil and gravel, endolithic communities, cryoconite holes, water lenses where the ice meets bedrock, benthic...
samples from a bedrock-based lake, water samples from melted supraglacial lake ice and epiphytic communities on mosses and lichens. In addition, for the two most dominant photoautotrophic groups in the soils, namely Cyanobacteria and green algae, DGGE analysis based on group-specific primers complemented the pyrosequencing inventories, and provided a more in-depth view on their diversity in the most widespread habitat type in the region.

MATERIALS AND METHODS
Site description and sample collection

The Sør Rondane Mountains are a 220 km long, east–west trending mountain range situated c. 200 km from the coast (Fig. 1). This range forms part of a chain of mountains surrounding the East Antarctic continent from the Bourg Massif in Western Dronning Maud Land to the Yamato Mountains in Eastern Dronning Maud Land (Mackintosh et al. 2014). The average air temperature measured by the automatic weather station near the Belgian Princess Elisabeth Station (71°57’S, 23°21’E and 1372 m a.s.l.) in the period between February 2009 and October 2014 equals −18°C, with an average minimum of −24.4°C in August (the coldest month), and an average of −7.8°C in January (the warmest month; Gorodetskaya et al. 2013). The dominant wind direction is from the east, whereas winds from the SE are somewhat less frequent (Pattyn, Matsuoka and Berte 2009). The areas sampled in this study are the Utsteinen nunatak and the adjacent ridge Vengen, Teltet, Vikinghøgda, Perlebandet, Duboisbreen, Svidlandfjellet and Yuboku Valley (Fig. 1).

A total of 53 samples were selected from the BELARE expeditions between 2007 and 2012 (Table S1, Supporting Information; Pictures supplementary material). During the field season of 2007-08 (before the start of the construction of the Princess Elisabeth Station), 14 samples of microbial crusts, 10 gravel samples, three filters from defrosted ice, three soil samples, one biofilm and one sample from guano of petrel on gravel were collected. During the field seasons of 2010–11 and 2011–12, additional samples were taken from soils as well as less widespread habitats. In total, 10 soil samples were added, as well as three.

Figure 1. Map of the Sør Rondane Mountains with an indication of the sampling locations.
samples from endolithic habitats, two from epiphytic communities on mosses, samples from two bedrock-based permanently ice-covered lakes, one from a meltwater lens where the ice sheet meets the bedrock and samples from two cryptonite holes. Samples of soil and gravel were collected in sterile falcon tubes of 15 and 50 ml or sterile Whirlpack® bags. Sub-glacial habitats (cryptonite holes and lakes) were accessed with an ice drill. The sediments from the ice-covered lake were sampled using a custom-made corer. All the samples were frozen upon arrival at the station and transported and kept frozen prior to analysis.

Next-generation sequencing analyses of the eukaryote and bacterial communities

A total of 22 samples were selected for Next-generation sequencing analysis on a 454 GS FLX+ platform (Table S1, Supporting Information). Extracellular DNA was removed following Corinaldesi, Danovaro and Dell’Anno (2005). Genomic DNA was isolated as described by Zwart et al. (1998). This included a bead-beating method with phenol extraction and ethanol precipitation. After extraction, the DNA was purified on a Wizard column (Promega). For the bacteria, the V1–V3 variable regions of the 16S rRNA were amplified using fusion primers containing the 454/Roche adaptor, a unique 454/Roche MID and the general forward prokaryote primer PA according to Edwards et al. (1989) or the general reverse prokaryote primer BKLF (5′-GTATTACCGCGGCTGCTGGCA-3′), which resulted in a fragment of 557 bp. (Cleenwerck et al. 2007). For the eukaryotes, the V4 region was amplified using a forward fusion primer containing the 454/Roche adaptor, a unique 454/Roche MID’s and a general forward eukaryote primer TAReuk454FWD1 (Stoeck et al. 2010) and a reverse fusion primer with TAREUKREV3 as the general eukaryotic primer (Stoeck et al. 2010), resulting in a fragment of 446 bp.

The PCR mixture for both eukaryotes and bacteria contained 1 μl of template DNA, 2.5 μl of dNTP’s (2 μM each) (Geneamp, Applied Biosystems, Foster City, United States), each deoxynucleoside triphosphate at a concentration of 200 μM (Invitrogen, Waltham, United States), 0.25 μl Fast-Start High Fidelity Polymerase of 5 U μl−1 (Roche, Indianapolis, United States) and 2 μl of the forward and reverse fusion primer targeting the 16S rRNA gene in more detail, part of the 18S rRNA gene was amplified using primers Euk528F (5′-CCGGTTGAATTCAGGTC-3′) and CHLOO2r (5′-CTTGGAGCCGCAACTTGC-3′) according to Zhu et al. (2005) (410 bp) (Table S1, Supporting Information). In addition, a nested PCR protocol with primers described by Diz et al. (2001) was applied. The specific Chlorophyta primers Euk1A (5′-CTGTGTTGATCTCCGAG-3′) and CHLOO2r, and the general primers Euk1A and Euk 516r-GC (5′-ACCAAGCTTGGCCCTCC-3′) (520 bp) were used in the first and second PCR, respectively. In the first PCR, a high specificity was obtained, and in the second PCR, the fragment was shortened to obtain better profiles by DGGE (Diz et al. 2001; Verleyen et al. 2010). PCR amplification procedures were performed with a T1 Thermocycler (Biometra, Westburg, Leusden, The Netherlands). Each mixture contained 2 μl of template DNA, primers at a concentrations of 0.5 μM (Invitrogen, Waltham, United States), each deoxynucleoside triphosphate at a concentration of 200 μM (Applied Biosystems), 400 ng of bovine serum albumin (BSA), 5 μl of 10x PCR buffer (100 mM Tris-HCl (pH9)), 500 mM KCl, 2.5 μl of Taq DNA polymerase (Applied Biosystems, Foster City, United States). The PCR mixtures were adjusted to a final volume of 50 μl with sterile HPLC-water. The PCR programs were performed consisting of denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min and a primer extension at 72 °C for 3 min. This was followed by 10 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min, primer extension at 72 °C for 3 min and a final extension at 72 °C for 20 min. The PCR program for the eukaryotes was identical to that of the bacteria, except the amount of cycles, which were 18 in the first part of the PCR and 17 in the last part and the annealing temperature for the touchdown PCR started at 57 °C and was 48 °C in the second part of the PCR. The PCR products were purified with a High Pure PCR Product Purification Kit (Roche, Indianapolis, United States). The quantity of DNA was tested on a Qubit (Life Technologies) using a DS DNA assay, and the length of the amplicon was tested on a Bioanalyzer (Agilent Technologies, Santa Clara, United States) using a DNA 1000 kit. Based on these results, the samples were pooled equimolar and amplicon sequencing was performed in the forward direction on a Roche 454 GS FLX+ platform at Beckman Coulter Genomics. The obtained reads were processed using Mothur version 1.27.0, generally following Schloss et al. (2009) and the Mothur SOP (http://www.mothur.org/wiki/454_SOP; version of 6 November 2012) with some modifications to improve confidence levels as described in Tytgat et al. (2014). In short, the data were denoized using Mothur’s PyroNoise (Quince et al. 2009), the minimal required sequence length was set at 200 nucleotides (nt) and to avoid poor sequences quality, no ambiguous bases and sequences with homopolymers longer than eight nt were allowed. De novo chimera detection was done with Uchime (Edgar et al. 2011), after which sequences were grouped into OTUs at the 97% identity level using the furthest neighbor clustering algorithm implemented in Mothur. Using the Wang classifier algorithm (Wang et al. 2007), the OTUs of the bacteria were mapped against the Greengenes database 13.5 (DeSantis et al. 2006) and those of the eukaryotes against PR2 (version of May 2013) (Guillou et al. 2013).

Green algal diversity studied using DGGE

DNA extraction and purification followed the protocol described above. In order to study the diversity of green algae in more detail, part of the 18S rRNA gene was amplified using primers Euk528F (5′-CCGGTTGAATTCAGGTC-3′) and CHLOO2r (5′-CTTGGAGCCGCAACTTGC-3′) according to Zhu et al. (2005) (410 bp) (Table S1, Supporting Information). In addition, a nested PCR protocol with primers described by Diz et al. (2001) was applied. The specific Chlorophyta primers Euk1A (5′-CTGTGTTGATCTCCGAG-3′) and CHLOO2r, and the general primers Euk1A and Euk 516r-GC (5′-ACCAAGCTTGGCCCTCC-3′) (520 bp) were used in the first and second PCR, respectively. In the first PCR, a high specificity was obtained, and in the second PCR, the fragment was shortened to obtain better profiles by DGGE (Diz et al. 2001; Verleyen et al. 2010). PCR amplification procedures were performed with a T1 Thermocycler (Biometra, Westburg, Leusden, The Netherlands). Each mixture contained 2 μl of template DNA, primers at a concentrations of 0.5 μM (Invitrogen, Waltham, United States), each deoxynucleoside triphosphate at a concentration of 200 μM (Applied Biosystems), 400 ng of bovine serum albumin (BSA), 5 μl of 10x PCR buffer (100 mM Tris-HCl (pH9)), 500 mM KCl, 2.5 μl of Taq DNA polymerase (Applied Biosystems, Foster City, United States). The PCR mixtures were adjusted to a final volume of 50 μl with sterile HPLC-water (Sigma, Aldrich, Diegem, Belgium). The PCR program followed Zhu et al. (2005) for the first set of primers with an annealing temperature of 58 °C. The PCR program for the nested PCR from the green algae started with a denaturation step of 5 min at 94 °C. In a next step, 25 cycles were performed consisting of denaturation at 94 °C for 30 s, annealing at 58 °C for 45 s and a primer extension at 72 °C for 10 min. After the Chlorophyta-specific amplification and purification with a QiaQuick PCR purification kit (QiaGen, Hilden, Germany), 1 μl of PCR product was transferred to a new 50 μl reaction mixture containing the general primers described above but no BSA and only 1 μl of Taq DNA polymerase (Applied Biosystems, Foster City, United States). The PCR program started with a denaturation step at 94 °C. In a next step 20 cycles were performed which consisted of denaturation at 94 °C for 30 s, annealing at 56 °C for 45 s and a primer extension at 72 °C for 130 s, with a final extension at 72 °C for 30 min. The final PCR product was again purified with the QiaQuick PCR purification kit (QiaGen, Hilden, Germany).

The presence of PCR products was determined by analyzing 5 μl of product on 2% agarose gels, staining with ethidium bromide, and comparison with a molecular weight marker (Smartladder, Eurogentec, Seraing, Belgium). Equal amounts of...
PCR product (700 ng) were applied to the DGGE gels and three standard lanes were analysed in parallel to the samples. Electrophoresis was performed at 60 °C for 16 h at 75 V. To obtain a matrix of the relative intensity of each band in all samples, the gels were analysed using the software package BioNumerics 5.1 (Applied Maths BVBA, Kortrijk, Belgium). All the different and strong bands were excised and sequenced after re-extraction and amplification.

Sequencing of the 18S rRNA gene fragments was performed with an ABI-Prism sequencing kit (PE Biosystems, Foster City, United States) using primer EUK1A and EUK516r for Chlorophyta specific 18S and an automated sequencer (ABI 3130XL). A nucleotide BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi; 2015) was performed in order to obtain sequences with the greatest significant alignment. The accession numbers for GenBank are KU127506-KU127575.

Cyanobacterial diversity studied using DGGE

The DNA of 18 samples (Table S1) was extracted following a modified protocol based on the method of Zhou, Bruns and Tiedje (1996). The extraction method was optimized to enable the DNA extraction from terrestrial cyanobacteria surrounded by thick sheaths, as the study of Fernandez-Carazo et al. (2012) had shown that though cyanobacteria with a thick sheath were observed by microscopy, they were not detected at the molecular level. Two steps were added to destroy this protective layer. The environmental sample was grounded several times using a micropestle (Eppendorf, Hamburg, Germany) in 250 μL of 0.5 M of EDTA with 0.25 g of glass-beads (0.17-0.18 mm diameter; Braun Biotech, Melsungen, Germany). After grinding, 500 μL of 0.5 M of EDTA was added and the tube was continuously vortexed horizontally for 20 min. The tubes were centrifuged for 3 min at 16 000 g (Centrifuge 5424, Eppendorf) to eliminate the EDTA. The pellet was resuspended in 100 μL of sterile water and 200 μL of a 0.2 M NaOH/1% SDS solution; which was added by gently mixing by inversion. The tube was incubated for 15 min at 70 °C. Subsequently, 150 μL of a cold potassium acetate solution (pH 5.5; 3 M) was added and the tube was gently mixed and placed on ice for 5 min. After centrifugation at 16 000 g to separate the pellet containing the cyanobacterial cells and the supernatant containing low quantities of DNA, the DNA was extracted from the pellet as described by Zhou, Bruns and Tiedje (1996). In addition, the DNA was precipitated from the supernatant by adding 800 μL of 95% ethanol. After 2 min at room temperature, the tube was centrifuged at 16 000 g and the pellet was washed with 300 μL of 70% ethanol and air-dried. The DNA extracted from the pellet and from the supernatant was merged for the following experiments. The extracted DNA was purified with the Wizard DNA clean-up system (Promega, Madison WI) and was eluted by adding 50 μL of TE -4 (10 mM TRIS-HCl pH 8; 0.1 Mm EDTA pH 8). The PCR products were obtained after two successive PCR reactions to amplify the V3–V4 variable region of the 16S rRNA as described by Fernandez-Carazo et al. (2012) with the primers 16S378F/16S781R and sequenced using 16S378F (GIGA, Liège, Belgium) using an ABI 3730 x1s DNA analyzer (Applied Biosystems, Foster City, USA). When several bands from the same samples showed the same sequences, we selected the longest one with the best quality and sequenced it in forward with the primer 16S781R (see Fernandez-Carazo et al. 2012) for more information about different bands with the same sequence. The sequences were deposited into Genbank with the following accession numbers (KT887889–KT887949). The new sequences were analyzed with the ones determined by Fernandez-Carazo et al. (2012) for the same Antarctic region and aligned with the two most similar strain sequences and the most similar uncultured sequence found by SeqMatch of the RDP (Cole et al. 2014). A distance tree was constructed by the Neighbor-joining method (Saitou and Nei 1987) using TREECON (Van de Peer and De Wachter 1994) on partial sequences of 326 bp and a bootstrap analysis was performed that involved the construction of 500 resampled trees. Sequences sharing more than 97.5% of similarity were grouped into OTUs using MOTHUR using the average neighbor method (Schloss et al. 2009).

Statistical analyses

Statistical analysis was only performed on the 454 sequencing data, due to the presence of mixed band classes and different band classes with the same sequence in the DGGE data. In all further analyses, only OTUs with more than five sequences in the dataset were considered. Species accumulation curves were calculated in R (version 3.1.2 Pumpkin Helmet) using the package vegan (version 2.2-1) (Oksanen et al. 2015). Similarity profile routine analyses (SIMPROF; Clarke et al. 2008) in R using the clustvec package (version 1.1) (Whitaker and Christman 2014) was run on Hellinger transformed sequence abundance data to assess the differences in community structure between the samples. 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. First, a Bray–Curtis similarity matrix was constructed and subsequently used to undertake a group-average cluster analysis. Second, to ascertain the level of structure present in the groups.
formed by each dendrogram, a SIMPROF test with 10 000 simulations and the stopping rule specified at the 5% significance level was run.

RESULTS
Eukaryotic biodiversity and community composition

The Roche 454 analysis of the 22 samples resulted in 384 447 sequences and 391 OTUs when only OTUs with more than five sequences were considered. Species accumulation curves of all samples combined containing all OTUs, including singletons, did not level off, suggesting either that not all the diversity in the habitats had been captured or that the data contained a large amount of spurious OTUs with a low abundance (Fig. S1, Supporting Information). Species accumulation curves containing OTUs with more than five sequences did level off (Fig. S1, Supporting Information). Following the classification scheme of PR2, which is mainly based on Adl et al. (2012), six supergroups were present with at least 4641 sequences in the samples, namely Alveolata, Amoebozoa, Archaeplastida, Opisthokonta, Rhizaria and Stramenopila (Fig. 2). An OTU belonging to the proposed taxonomic group of Hacrobia was present with only seven sequences. Ciliophora was the only Division in the Alveolata, and Lobosa within the Amoebozoa. Chlorophyta (including Trebouxiophyceae, Chlorophyceae and Mamiellophyceae), Streptophyta (mosses) and one OTU of Rhodophyta were the three Divisions within the Archaeplastida. The Opisthokonta contained the Fungi, the Metazoa and one OTU belonging to the Choanoflagellida. Within the Fungi, Basidiomycota was the dominant class and mainly contained members of the Tremellomycetes. Ascomycota and Chytridiomycota were present but less dominant.

Figure 2. (a) A SIMPROF analysis of the 454 data showing the significant clusters (blue and red lines) based on the relative abundances of the eukaryotic OTUs. The names of the endolithic samples are indicated in red, the cryoconite holes in light blue, the lakes in dark blue, the soil and epilithic samples in khaki and the epiphytic moss samples in light green, (b) bar plots for each sample showing the seven most abundant eukaryotic supergroups following the classification in PR-2 and (c) bar plots with the Opisthokonta further subdivided into the main classes.
More than 85% of the Metazoan sequences belonged to the Rotifera. Tardigrada, Nematoda and Arthropoda (Collembola) were the other Metazoan groups. Cercozoa was the only group belonging to the Rhizaria. Within the Stramenopila, Bacillariophyta was the most abundant group, while Chrysophyta and the understudied groups Bicomoea and Oomyecota were subdominant.

The SIMPROF analyses revealed no clear clustering according to habitat type, which is likely related to the relatively large number of OTUs (46%) shared between both aquatic and dry terrestrial habitats. However, the sediment sample from the ice-contact water body (11PER001) was significantly different from the other habitats (Fig. 2). This was not only because of the presence of one specific OTU belonging to Embryophycaceae but also due to the relatively low amount of sequences (6315) and the absence of particular taxa that were abundant in the majority of the other samples, such as one OTU related to the Prasioflagelates and the tardigrade Dipsascon. Two other aquatic samples (11SVI000 and 11SVI005) also grouped together in a significant cluster and were characterized by the absence of these taxa. These bedrock lakes formed a larger cluster with one of the cryoconite hole samples (11UN005). The other cryoconite sample (11UN006) clustered with soil and epilithic samples. The latter two habitats were generally characterized by the presence of the four most common OTUs related to Rotifera, Chlorophyta and Tardigrada, and clustered with epiphytic and endolithic samples. Interestingly, the three endolithic samples (10DUB026, 11UN007 and 11PER001) clustered in three different significant groups. One of them (10DUB026) grouped together with a sample from the Utsteinen nunatak (10UN002) probably because of a diatom species being abundant in both samples. The second endolithic sample (11UN007) formed a significant cluster with a sample (10UR081) from a relatively wet microenvironment where a lichen crust was present due to the presence of a number of OTUs belonging to Rotifera which were shared. The third endolithic sample (11PER001) formed a significant cluster on its own and was characterized by the presence of an unclassified OTU of the Bacillariophyta, and OTUs related to the Collembola (Arthropoda) and Streptophyta.

Green algae

Analysis of the excised DGGE bands using the primers by Zhu et al. (2005) revealed one band class which corresponded to multiple OTUs. The occurrence of these ‘mixed band classes’ was much higher than using the primers from Díez et al. (2001), for which all the band classes were mixed. The DGGE analysis with the primers from Díez et al. (2001) targeting the chlorophytes, which are the most abundant eukaryotic phototrophs, resulted in 30 different band classes in the 22 soil samples. Around 63% of the band classes were sequenced. In total, 3.8% of the sequences had less than 97% similarity with any of the sequences in the NCBI database, and 23.1% had > 97% similarity with uncultured eukaryotes (Table S2, Supporting Information). Eight band classes were > 97% related to uncultured eukaryotes but could not be assigned to a higher taxonomic level. A total of 72.7% of the band classes of which more than one band was sequenced appeared to be mixed. The analysis revealed the presence of green algae with 99% similarity with taxa belonging to the Trebouxioiphyceae, Chlorophyceae and Ulvophyceae (see Table S2, Supporting information, for a list of the genera encountered).

Ten of the 22 samples analyzed by DGGE with the primers of Díez et al. (2001) were additionally analyzed by DGGE with the primer set from Zhu et al. (2005), which resulted in 21 different band classes of which only one was mixed. An average of 5.5 bands per sample and a maximum of ten were obtained. Almost 48% of the bands were rare and occurred only in one or two samples. Sixteen out of the total of 60 bands were sequenced and subsequently used in a BLAST search. Around 43.8% of the sequences had less than 97% similarity to known sequences in the NCBI database (Table S3, Supporting information). A total of 25% of all the sequences had more than 97% similarity, but were related to uncultured taxa.

Diversity and community composition of bacteria

The pyrosequencing analysis revealed the presence of 167 711 sequences of good quality in the 22 samples, which resulted in 1952 OTUs when only OTUs with more than five sequences in the dataset were considered. Similar to the eukaryotes, the species accumulation curve did not level off when all sequences were retained while it did when only OTUs with > 5 sequences were analyzed. The sequences belonged to 23 phyla, namely the Acidobacteria, Actinobacteria, Armatimonadetes, Bacteroidetes, BRC1, Chlorobi, Chloroflexi, Cyanobacteria, FBP, Firmicutes, Fusobacteria, Gemmatimonadetes, MVP-2, Nitrospirae, OD1, Planctomycetes, Proteobacteria, Thermi, TM6, TM7, verrucomicrobia, and WPS-2. Cyanobacteria was the dominant phylum, with the orders of the Oscillatoriales, Chroococcales, Pseudanabaenales, Nostocales and Synechococcales. The phyla Proteobacteria, Bacteroidetes, Acidobacteria, the candidate phylum FBP, Actinobacteria, Deinococcus-Thermus and Armatimonadetes were the other phyla containing an OTU with a relative abundance exceeding 0.5%.

The SIMPROF analysis revealed the existence of a significant cluster which was solely composed of aquatic samples, including the three lakes and one of the cryoconite holes (Fig. 3). The two bedrock-based lakes (11SVI000 and 11SVI005) are dominated by two OTUs that belong to Leptolyngbya, a filamentous cyanobacterial genus. A total of 33.3% of the OTUs occurred in both terrestrial and aquatic samples. Within the terrestrial samples and similar to the eukaryotes, no clear clustering could be observed according to habitat type. For example, the endolithic samples grouped in three different clusters, which were characterized by the absence or low abundance of the most abundant OTU that was related to the cyanobacterium Phormidium. One of the endolithic samples (11UN007) clustered with a wet soil sample on a lichen (11PER013) and one on a moss (11PER007) due to the occurrence of OTUs related to the Armatimonadetes and the Actobacteraceae (Alphaproteobacteria). One of the other endolithic samples formed a significant cluster with an epilithic sample (11PER001), again due to the shared presence of OTUs belonging to the Chloracidobacteria (Acidobacteria). The third endolithic sample (10DUB026) is dominated by cyanobacterial OTUs belonging to the Chroococcales and formed a significant cluster with samples (10UR081 and 10UR082) which shared unclassified OTUs belonging to other Cyanobacteria, the Acidobacteria, the Actinobacteria and the candidate division FBP. Except for the cluster composed of aquatic samples, every significant cluster contained at least one soil or epilithic sample.

Cyanobacteria

For cyanobacteria, which were the most abundant bacterial photoautotrophs, 18 soil samples were studied in detail using DGGE with specific primers, including nine samples already analyzed by Fernandez-Carazo et al. (2012) but using another DNA extraction method (Table S5, Supporting information). The guano sample (07UNE054) was also tested but did not yield any DGGE bands.
The modified protocol enabled us to obtain the sequences of one or two OTUs not yet observed from eight out of nine samples, which included taxa with large polysaccharidic sheaths (e.g. OTU7 corresponding to *Cyanothce aeruginosa*, OTU14 corresponding to *Chroococcidiopsis* sp. and OTU16 corresponding to *Hassalia/Tolypothrix* sp.). However, the modified protocol did not always improve the diversity estimation, as it could not recover OTU6 (uncultured unicellular) and OTU18 (*Stigonema*). In total, the sequences were grouped into 18 OTUs. This added another six OTUs to the dataset described in Fernandez-Carazo et al. (2012) (see Table S4 and 5, Supporting Information; Fig. S2, Supporting Information, for an overview and the taxonomic affiliation and the geographic distribution of the OTUs). The richness per sample ranged from one to six OTUs. Forty-four percent of the OTUs were rare and occurred in only one sample (Table S4, Supporting Information). Six of the eighteen OTUs only included sequences from uncultured organisms, fourteen OTUs have been found outside Antarctica while three OTUs seemed to be restricted to the Antarctic (Table S4, Supporting Information).

**DISCUSSION**

**General eukaryotic diversity**

The Roche 454 sequencing using universal eukaryotic primers of terrestrial and aquatic communities from the Northern part of the Sør Rondane Mountains revealed the presence of similar organisms at the higher taxonomic level as those reported from comparable habitats elsewhere in Antarctica (Convey et al. 2008, 2014) and in ice-free regions in Dronning Maud Land (see Velasco-Castrillon, Gibson and Stevens 2014, for a review). Among metazoans, the most abundant sequences were from Rotifera, and to a lesser extent from Nematoda, Tardigrada and Collembola. These are all groups known to be important microinvertebrates in Antarctic terrestrial communities (Convey et al. 2008). However, while mites are known to be present in nunataks in Dronning Maud Land (Tytgat subm; this issue) and similar habitats in other Antarctic regions (Convey and Stevens 2007), they were not detected in our samples. This may be due to (i) the lack of samples in the present study containing visible communities of mites and hence their potentially low densities in our samples and the small probability of finding them in our DNA extract and/or (ii) the sensitivity of the primer used. Ciliates and Cercozoa were the most abundant heterotrophic protists, which is in agreement with earlier studies of microbial mats from High Arctic and Antarctic freshwater ecosystems (Hodgson et al. 2010; Jungblut et al. 2012). Among the Fungi, the classes Basidiomycota, Ascomycota and Chytridiomycota were represented. All lichens and lichenicolous fungi previously found in Utsteinen were members of the Ascomycota (Ertz et al. 2014). Green algae photo-bionts from lichens are known to belong to genera from the Trebouxiophyceae and Ulvophyceae (Ovstedal and Smith 2009), and although taxa could not be identified up to the genus...
level using the pyrosequencing approach, the DGGE analysis revealed the presence of several putative green algae photobiont genera such as Trebouxia, Prasiola and Diplophyra (Thiis et al. 2011; Table S2 and 3, Supporting Information). As to our knowledge, Basidiozyma and Chytriomyzota are not lich-nized in the region; OTUs belonging to these taxa may in-clude free-living, endosymbiotic or parasitic life forms. Chloro-phytes, diatoms, and to a lesser extent Cryptophytes, were the dominant microeukaryotic photautotrophic organisms. This again agrees well with previous inventories of microbial mat samples (Jungblut et al. 2012) and soils (Niederberger et al. 2015) from Continental Antarctic regions. One moss species belonging to the genus Grimmia was also present in the epiphytic samples. Interestingly, we also detected OTUs related to the Pseudodendromonadales, which was particularly abundant in one of the lakes and one of the cryoconite holes. This taxon is a poorly studied member of the Stramenopila and generally reported from aquatic ecosystems, including saline lakes, ombrotrophic pools and coastal seawaters (Cavaller-Smith and Chao 2006). To our knowledge, this is the first report of the presence of mem-bers of the Pseudodendromonadales in Antarctica, which calls for more studies to assess the distribution of taxa belonging to this order in these habitats.

When focusing on the Chlorophyta, which are the most abundant eukaryotic photoautotrophs in the region, taxa belong-ing to the classes Chlorophyceae, Trebouxiphyceae, Ulvo-phyceae and Mamiellophyceae were identified. The latter class was represented by the genus Monomastix, which was only de-tected using the 454 analysis and was largely restricted to the aquatic samples, being particularly abundant in one of the cryo-conite holes. Based on the DGGE analysis using specific primers in a selection of soil samples, a total of 12 genera could be identified, including two sequences belonging to the genera Rosenungiella and Xylochloris (Guiry and Guiry 2016, AlgaeBase), which were previously unknown to Antarctica. The other gen-era were previously reported in Antarctica (see Table S6, Sup-porting Information, for detailed information). In contrast to these cosmopolitan genera, Hemichloris has never been found outside Antarctica (Tschermak-Woess et al. 2006). In our samples, this genus occurred in a cavity of a boulder filled with gravel and water. The two species belonging to this genus were previously reported from cryptendolithic and endolithic samples from Victoria Land (Tschermak-Woess et al. 2006). Three se-quences assigned to uncultured eukaryotes were also related to taxa found in Antarctica; one in a moss pillar in Hotoke lake (Syowa Oasis; Nakai et al. 2012), one in a microbial mat in Or-ange Pond (McMurdo Ice Shelf, Victoria Land; Jungblut et al. 2012) and the third one in soils near the Brazilian Antarctic station (NCBI match, unpublished). The remainder of the uncultured eu-karyotes was reported from outside Antarctica, suggesting that based on this part of the 18S rRNA, the chlorophyte flora in Antarctica is composed of both endemic taxa as well as cos-mopolitan taxa. However, it is very likely that the relatively small fragment of the 18S rRNA gene used (410 bp) here is insufficient to discriminate taxa for assessing patterns in endemism. For ex-ample, a study based on chloroplast DNA genes in Prasiola crispa revealed that this taxon consists of several cryptic species in Antarctica (Moniz et al. 2012), but these cannot be discriminated based on the fragment amplified for DGGE. Additional inven-tories using different genes or metagenomics approaches, en-abling more fine-grained taxon delineation, are needed to fully understand the distribution of chlorophytes but also of other taxa in Antarctica.

The other sequences obtained using the primers from Diez (2001) also revealed the presence of sequences belonging to the Rhizaria and Alveolata. Interestingly, the sequence belonging to the Alveolata is related to Sarcoyctica, a parasite found in mammals and birds, including the Wilson’s storm petrel (Oceanites oceanicus) and the Antarctic petrel (Thalassoica antarctica) (Bark-boa and Palacios 2009), bird species previously reported from the study region (Belare field report, Scarman). The sample (07UN0054), in which this sequence was detected, was taken at a breeding site of snow petrels (Pagodroma nivea) (CEE 2007), so possibly this species can also be infected by this parasitic protist.

### General bacterial diversity

Although the 454 analysis of the bacteria resulted in less than half the amount of sequences compared to the eukaryotes, at least five times more OTUs were discovered for the bacteria. This is probably due to the higher bacterial diversity, or alternatively because of the higher discriminative power of the 16S rRNA gene compared with the 18S rRNA marker gene (Pawlowski et al. 2012). Among the bacteria, the 454 analysis revealed that OTUs belonging to the phylum Cyanobacteria were dominant and contained the most common orders previously reported from Antarctica (Jungblut et al. 2012), including the Oscillatori-ales, Chroococccales, Pseudanabaenales, Nostocales and Syne-chococcales. The latter order is reported for the first time in the Sør Rondane Mountains (Fernandez-Carazo et al. 2012). Proteobacteria appeared to be the second most dominant bacterial phylum. This is similar to observations in bacterial communities associated with cyanobacteria in the McMurdo Dry Valleys also in continental Antarctica (de la Torre et al. 2003; Chang et al. 2013); soils in Signy Island, Alexander Island and Anchor Island near the Antarctic Peninsula and in the sub-Antarctic (including, South Georgia and the Falkland islands); and permafrost in the Arctic (Yergeau et al. 2007; Cary et al. 2010; Berkamens et al. 2014). The dominance of Proteobacteria is however in con-trast with dry soils from the McMurdo Dry Valleys where this phylum appeared to be less abundant than the Acidobacteria, Actinobacteria and Bacteroidetes (Cary et al. 2010). Bacteroidetes is the third most abundant phylum in our dataset. Interestingly, on Livingston Island, it was found to be more abundant in moss covered soils than in the surrounding unvegetated soils (Ganz-ert et al. 2011). This is in accordance with one of our epiphytic samples (10UR066), which appeared to have the second highest number of Bacteroidetes sequences. However, in the other epiphytic sample (11UNN007), Bacteroidetes sequences are much less abundant, suggesting that besides the presence of mosses other factors are also important for enabling the dominance of this and other phyla. For example, in Wright Valley in the McMurdo Dry Valleys, the abundance of Acidobacteria appeared to be negatively correlated with water availability (Zeglin et al. 2011). This phylum is the dominant taxon in one of the endolithic samples (10DUB026), but is also relatively abundant in one of the cryoconite holes (11UN006) questioning a direct link between water availability and the abundance of this phylum in the Sør Rondane Mountains. The candidate phylum FBP is another relatively abundant phylum, which was also found in cryptoendolithic communities dominated by cyanobacteria in the McMurdo Dry Valleys (de la Torre et al. 2003). In our dataset, it is the most abundant phylum in three epithiic samples. Mem-bers belonging to the Actinobacteria, which is a metabolically diverse phylum, are present in all samples. This phylum was also previously reported to be dominant in edaphic microbial communities in the McMurdo Dry Valley, and in soils from Victoria Land, the Falkland Islands, Signy Island, and Alexander Island (Yergeau et al. 2007; Chong et al. 2012; Lee et al. 2012; Stomeo et al. 2012).
When focusing on the Cyanobacteria using the group specific primer set targeting the V3–V4 region of the 16S rRNA gene and merging our data with Fernandez-Carazo et al. (2012), the 19 samples analyzed revealed the presence of 18 OTUs, which is ~40% of the total number of terrestrial cyanobacterial OTUs found in Antarctica till 2010 (Namsaraev et al. 2010). Hence, the addition of nine samples to the dataset of Fernandez-Carazo et al. (2012) and the use of an improved DNA extraction method revealed the presence of six additional OTUs. Repeated sampling of similar habitats (biofilms and crusts on gravel and rocks) around the Ustinenen nunatak has yielded a quite similar diversity (mainly consisting of OTU19, OTUS, OTU1 and less frequently OTU8). In total, 19% of the cyanobacterial OTUs are at present only found in the Antarctic region, which is lower compared the previous study in the same region (46% in Fernandez-Carazo et al. 2012) (Table S5, Supporting Information). This observation could be explained by the increased number of sequences presently stored in the NCBI database compared with 2011. Interestingly, OTUs 5 and 6 were only found in the Antarctic, while OTU7 is cosmopolitan. OTU1 can be identified as *Phormidium* priestleyi, and its presence seems restricted to polar and alpine biotopes. Other OTUs showed a more widespread distribution confirming previous observations in Antarctica (e.g. Jungblut, Lovejoy and Vincent 2010; Verleyen et al. 2010). The presence of *Microcoleus autumnalis* in the majority of samples is remarkable. This shows that this taxon is very resilient to many environmental conditions. It can be found in Antarctic aquatic microbial mats, seepages and wet soils. Based on a molecular clock analysis, Strunecky et al. (2013) inferred that this genus was present on the Antarctic continent since before the Gondwana break up.

**Differences in community structure between the different habitats**

The multivariate analysis of the eukaryotic dataset revealed that the taxonomic composition of the samples grouped independently from the habitat type (aquatic versus dry terrestrial habitats) and that—with a few exceptions—none of the OTUs with more than five sequences appeared to be habitat-specific. Rotifers appeared to be the most abundant group (in terms of the amount of sequences) in the majority of the samples and occurred in every habitat type studied. Also the second and third most abundant OTUs (both belonging to Chlorophyta) occurred in all habitat types. By contrast, among the OTUs attaining more than 0.1% relative abundance in the entire dataset, some appeared to be restricted to the aquatic habitats, such as an unclassified rotifer and a Stramenopila OTU belonging to the Pseudodendrononadales. Others were restricted to the terrestrial samples, such as one belonging to the nematode genus *Panagrolaimus* and a chlorophyte of the Chlamydomonadales. However, because of the presence of the most abundant OTUs in every habitat type and nearly all samples, no clear clustering consistent with habitat type (aquatic versus terrestrial systems) could be observed in the eukaryotes.

In contrast, for the bacteria, the aquatic samples appeared to be significantly different from the terrestrial ones, in part due to the presence of OTUs related to the filamentous cyanobacteria *Leptolyngbya* (Pseudanabaenales) in the aquatic samples. This genus also dominates microbial mats in coastal Antarctic lakes (e.g. Verleyen et al. 2010; Strunecky et al. 2013) and was reported from other inland Antarctic locations, such as in Forlidas Pond and Lundstrom Lake, where it occurred in the water bodies themselves as well as in the catchment area of these formerly larger lakes (Hodgson et al. 2010; Fernandez-Carazo et al. 2011). The samples from the drier soils are, in contrast, dominated by Bacteroidetes, Acidobacteria, Proteobacteria, Deinococcus-Thermus or the candidate phylum FBP. This agrees well with McMurdo Dry Valley soils where Bacteroidetes and Deinococcus-Thermus are also frequently observed (Cary et al. 2010). It follows that liquid water availability is indeed one of the most important environmental factors determining the presence of particular bacterial taxa in these terrestrial habitats. This agrees well with studies in the McMurdo Dry Valley soils where soils were investigated along a moisture gradient (Niederberger et al. 2015) and where the bacterial community structure varies according to different levels of moisture (Fell et al. 2006). However, all in all, a relatively large amount of OTUs in both bacteria, but particularly in eukaryotes (33% and 46%, respectively) was shared between aquatic and terrestrial habitats. Moreover, within the latter only a few OTUs appeared to be restricted to either endolithic, soil and moss samples. This suggests that a relatively high number of the terrestrial and lacustrine eukaryotes in Antarctica are generalists, which have a broad tolerance and opportunistically occupy the different available habitats. This is probably related to their relatively low richness, which probably results in less competition than in cases where other species compete for a shared part of the fundamental niche leading to smaller realized niches of the individual taxa. The finding that several taxa occupy both aquatic and terrestrial habitats has also important consequences for understanding the biogeography of Antarctic terrestrial organisms. More in particular, the high incidence of endemism in some regions (Convey and Stevens 2007) and the pronounced biogeographic provincialism recently observed in a variety of taxonomic groups (Terauds et al. 2012) can only be explained by the survival of biota in glacial refugia during Pleistocene and Neogene ice ages, in combination with the long-term isolation of the content and dispersal limitation (Convey et al. 2014). However, recent comprehensive summaries of the deglaciation history of Antarctica (Mackintosh et al. 2014) have revealed that only a few regions remained ice-free during the Last Glacial Maximum and probably also during preceding ice ages. Particularly coastal regions, currently containing the majority of the lacustrine systems in Antarctica, appear to be overprinted by the terrestrial ice sheets during glacial-interglacial cycles (Mackintosh et al. 2014) probably leading to regional extinctions. It follows that taxa currently inhabiting the majority of these ice-free regions recolonized these areas after survival either (i) in high-altitude nunataks further to the South, (ii) in the sparse regions that remained ice-free during glacial maxima such as the Larsenmann Hills (Hodgson et al. 2001, 2006) or (iii) as recently hypothesized in supraglacial ecosystems such as cryoconite holes (Stanish et al. 2013). Interestingly, the high amount of eukaryotes shared between different habitats suggests that taxa occurring in terrestrial ecosystems can indeed potentially survive in for example cryoconite holes. However, the high amount of taxa shared between different terrestrial and aquatic habitats should be confirmed based on other genes enabling a higher taxonomic resolution, because the 18S rRNA gene might be too conservative to detect species-level differences in community structure.

**Sequences new to science**

A striking feature in all the groups studied, except for the cyanobacteria, is the presence of a relatively large number of sequences with a similarity of less than 97% with known sequences stored in public databases. For the green algae, this is the case for 4% and 44% of the sequences obtained with the
primers from Díez et al. (2001) and Zhu et al. (2005), respectively, while for the cyanobacteria studied using DGGE, this was only found for one out of the 18 OTUs (OTU8). For the 454 analysis with general eukaryotic and bacterial primers, this is almost 36% and 9%, respectively. These high numbers can be explained by the incompleteness of the public databases. The high number of unknown sequences thus underscores the need for further in-depth characterization of Antarctic habitats using state-of-the-art sequencing techniques, and a set of primers targeting different genes (Yergeau et al. 2007; Chong et al. 2013).

CONCLUSIONS
The 454 analysis of different habitats present in the Sør Rondean Mountains revealed that OTUs belonging to the Rotifera, Chlorophyta, Tardigrada, Ciliophora, Nematoda, Collembola, Bryophyta, Cercozoa and Bacillariophyta dominated the eukaryotic communities. Chlorophytes were the dominant photoautotrophs and a DGGE analysis of soils samples revealed the presence of eleven genera, of which two are reported for the first time in Antarctica, namely Rosenvingiella and Xylocloë. For the bacteria, Cyanobacteria, followed by Proteobacteria, Bacteroidetes, Acidobacteria and Actinobacteria were the most abundant bacterial phyla. Multivariate analyses revealed no clear clustering according to habitat type (lakes versus terrestrial) in the eukaryotes, while aquatic communities in the bacteria were significantly different from those in the epilithic, endolithic and epiphytic habitats. However, in both bacteria and particularly in the eukaryotes a relatively large amount of OTUs was shared between terrestrial and aquatic habitats. In most groups studied, a relatively large number of sequences was present with a similarity of less than 97% with known sequences stored in public databases. We conclude that additional taxonomic inventories of inland Antarctic locations using multiple primers sets, targeting different taxonomic groups and habitats, are needed to fully document the biodiversity of terrestrial ecosystems in the region and to assess potential source sink dynamics between different habitats.

SUPPLEMENTARY DATA
Supplementary data are available at FEMSEC online.

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