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Soil bacterial community abundance and diversity in ice-free areas of Keller Peninsula, Antarctica

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ABSTRACT

The Antarctic is the most untouched terrestrial region of the planet but the most vulnerable to global environmental changes. In this regard, the monitoring of terrestrial environments is fundamental for the evaluation of such changes. This exploratory study aimed to provide baseline information against which to monitor changes in the Antarctic soil microbiome caused by global environmental changes or human activities and to find patterns in microbial diversity and community composition in ice-free zones of Antarctica. To achieve this goal we applied a high-throughput pyrosequencing based analysis of amplified 16S rRNA genes from twelve soil samples from the most prominent plant communities and soil types collected in the Keller Peninsula, King George Island, Antarctica. Such approach provided the variability necessary to investigate important ecological aspects of overall patterns of bacterial distribution. Quantitative and qualitative bacterial diversity measures were calculated and combined with metadata describing the samples. Overall, the soil bacterial communities in ice-free areas of Keller Peninsula were dominated by a small number of phyla found in most soils, including agricultural ones. Despite the differences in soil properties or plant cover the best predictor for the microbial community structure was the pH gradient. Our results provide evidence of no correlation between bacterial community and plant cover in Antarctic ice free zones.

1. Introduction

The Antarctic is the most untouched terrestrial region of the planet but the most vulnerable to global climate changes. Unlike other areas of the world, humans have only had a presence in Antarctica for the last c. 190 years (Headland, 2009). During these years there have been many studies focused on the biodiversity of macroorgamisms (Øvstedal and Smith, 2001; Pereira et al., 2007; Ochyra et al., 2008) and on soil genesis, properties and classification (Simas et al., 2008; Francelino et al., 2011). Most of the continent is covered by glacial ice sheets however, ice-free areas comprising less than 0.4% of the continental land mass are discontinuously distributed around the coastal margins (Ugolini and Bockheim, 2008; Cary et al., 2010). In the ice-free zones adjoining Admiralty Bay, especially in the Keller Peninsula, the distribution of plant communities shows a pattern that is closely related to aspects of the geologic configuration of the terrain and to the presence of bird colonies (Pereira et al., 2007). Those areas are directly influenced by the sea spray and have relatively high moisture content (Campbell and Claridge, 1987). The peninsula presents positive summer air temperatures, repeated freeze–thaw events and a distinctly higher precipitation than in continental areas (Ganzert et al., 2011). The extreme conditions of the Antarctic environment lead the researchers to belief that Antarctic soils contain extremely limited microbial diversity but recent literature suggest that there is a higher biodiversity within Antarctic soils than previously expected (Niederberger et al., 2008; Lee et al., 2011; Ganzert et al., 2011).

The Antarctic Peninsula is a narrow mountainous region close to 63°S where the temperatures are much less extreme than on the plateau. The Keller Peninsula is located inside Admiralty Bay, which protects the peninsula from the dominant winds that are typically strong and cold. Biodiversity in Antarctica's terrestrial and freshwater environments is low compared with equivalent habitats in other areas of the Earth. Low biodiversity means that some ecosystem functions are fulfilled by only a few species, so that levels of

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functional redundancy are low, or may even be missing (Wall and Virginia, 1999; Convey, 2007).

The history of low human activity and the low biodiversity in Antarctica's terrestrial environments makes the area unique and worthy of special consideration when attempting to determine the contributing factors to overall patterns of soil microbial diversity. Assuming that soils of the Keller Peninsula of Antarctica are among the simplest ecosystems on earth, these soils may be ideal for a full description of bacterial communities and their relationship to biotic and abiotic factors.

Here we used pyrosequencing of 16S rRNA genes to compare the bacterial communities of soil samples from ice-free areas of Keller Peninsula representing different soil types, and plant cover. Our goal was to obtain a detailed baseline description of the soil bacterial communities found in the Antarctic soils against which to compare changes in the Antarctic microbiome caused by climate changes or human activities. In addition, using Antarctica as a simple ecosystem model with low or no human influence, we attempt to determine the major factors responsible for the overall patterns of soil microbial diversity.

2. Material and methods

2.1. Study sites, soil sampling, chemical analysis and DNA isolation

Soil samples were collected on the Keller Peninsula, King George Island, Antarctica during the austral summer of 2009–2010. A total of 12 soil samples were chosen for sampling and were taken from a variety of plant communities having different soil features (Table 1). Soil was collected removing the plant cover and taking cores of 5 cm diameter and 5 cm depth. All soil was stored on ice upon collection and transported to the laboratory for extraction. The soil pH was determined in water (1:1 soil to water ratio) and the concentrations of Ca Mg, Al, K, Na, P and total organic carbon (TOC) were quantified according to Embrapa (1997). DNA was isolated from at least 1 g of mixed soil using the PowerSoil™ DNA Isolation Kit (MO BIO) as described by the manufacturer. The genomic DNA concentration and purity were determined by spectrophotometry.

An important landscape feature of Keller Peninsula is the widespread occurrence of sulfide-affected rocks, from which sulfate affected soils are formed. Generally referred to as acid–sulfate soils, they differ from the other pedons formed from basalts and andesites, due to their acid pH throughout the profile (Francelino et al., 2011). The plant communities were described according to the representative plant species or most abundant biomass and are presented in Table 1. Our sampling strategy allowed us to collect soil from the most prominent plant communities and soil types providing the variability necessary to investigate important ecological aspects of overall patterns of bacterial distribution.

2.2. 16S rRNA amplification and pyrosequencing

Twelve independent PCR reactions were performed for each soil sample with the primers 338R and 27F for the amplification of the V1–V2 hypervariable regions of the 16S rRNA gene. PCR was performed with the GoTaq PCR core system (Promega, Madison, WI, USA). The mixtures contained 5 μl of 10× PCR buffer, 200 mM dNTPs, 100 mM of each primer, 2.5 U of Taq polymerase and approximately 100 ng of DNA template in a final volume of 50 μl. The PCR conditions were 94 °C for 2 min, 30 cycles of 94 °C for 45 s; 55 °C for 45 s; and 72 °C for 1 min extension; followed by 72 °C for 6 min. The 16S rRNA gene fragments were sequenced using 454 GS FLX Titanium (Lib-L) chemistry for unidirectional sequencing of the amplicon libraries. Barcoded primers were used to multiplex the amplicon pools so they could be sequenced together and computationally separated afterward. To do this, 8-base barcodes were added to the 5′-end of the reverse primers using the self-correcting barcode method of Hamady et al. (2008). The primers were attached to the GS FLX Titanium Primer A (5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG-3′) and Primer B (5′-CCTATCCCCTGTGTGCCTTGGCAGTC-3′) sequences, modified for use with GS FLX Titanium emPCR Kits (Lib-L) and a two-base linker sequence was inserted between the 454 adapter and the 16S rRNA primers to reduce any effect the composite primer might have on PCR efficiency. The PCR products for each of the 12 samples were purified and combined in equimolar ratios with the quantitative DNA binding method (SequalPrep Kit, Invitrogen, Carlsbad, CA, USA) to create a DNA pool that was used for pyrosequencing from the A-Key adaptor. All raw sequences were submitted to the NCBI Sequence Read Archive (SRA) under the accession number SRA048722.2.

2.3. Sequence read analysis

The raw sequences obtained were processed using QIIME (Caporaso et al., 2010) with default parameters. Briefly, the multiplexed reads were first filtered for quality and assigned to the starting soil samples. The filtering criteria included a perfect match to the sequence barcode and primer, 200 bp in length, no more than two undetermined bases, and a least 60% match to a previously determined 16S rRNA gene sequence (Hamady et al., 2008). Additionally, to identify potentially chimeric sequences, the dataset were subject to the ChimeraSlayer implemented in Mothur (Schloss et al., 2009). The output fasta file was used for building a table with the Operational Taxonomic Unit (OTU) abundance of each sample and the taxonomic assignments for each OTU. To do this, the sequences were clustered into OTUs based on the relatedness of the sequences (97% similarity) and a representative sequence from each OTU was selected. These representative sequences were subjected to the RDP naïve Bayesian rRNA Classifier (Wang et al., 2007), which attaches complete taxonomic information from domain to species to each sequence in the database with 80% taxonomy confidence and an *e*-value of 0.001. The representative set of sequences was also used for align the sequences against a reference database and to build a phylogenetic tree necessary for downstream measurements.

2.4. Defining similar and dissimilar groups of soil bacterial communities

Principal Coordinate Analysis (PCoA) was performed to find clusters of similar groups of samples. PCoA is an ordination method based on multivariate statistical analysis that maps the samples in different dimensions and reflects the similarity of the biological communities. A matrix using the UniFrac metric (weighted and unweighted) for each pair of environments was calculated. The distances were turned into points in space with the number of dimensions one less than the number of samples. The first three principal dimensions were used to plot a three-dimensional graph. To test whether the results were robust to sample size we used a sequence-jackknifing technique in which the PCoA clusters were regenerated using a subset of 500 sequences randomly selected from each soil for 100 replicate trials. The Jackknifed PCoA was performed using QIIME (Caporaso et al., 2010).

To corroborate the results obtained by the PCoA we estimated the diversity of each sample using Faith's index of phylogenetic diversity (Faith, 1992). For this measurement we calculated the diversity metrics for a randomly selected subset of 5000 sequences per soil, as alpha diversity indexes are correlated with the number

Table 1
Soil chemical analyses and plant richness for soil samples from ice-free areas of Keller Peninsula, Antarctica.

Sample ID	Location	TOC ($g\,kg^{-1}$)	Ca + Mg ($cmol_c\,kg^{-1}$)	Al ($cmol_c\,kg^{-1}$)	Na ($cmol_c\,kg^{-1}$)	K ($mg\,kg^{-1}$)	P ($mg\,kg^{-1}$)	pH (H_2O)	Flowering plants	Mosses	Lichens	Plant biomass
S1	62°03′44.8″S 58°24′49.8″W	35	28	0.0	0.4	78	44	8.4	*Deschampsia antarctica* Desv.; *Colobanthus quitensis* (Kinth.) Bartl.	*Sanionia uncinata* (Hedw.) Loeske; *Hennediella antarctica* (Angstr.) Ochyra & Matteri	–	High
S3	62°03′48.1″S 58°24′42.6″W	32	26	0.0	0.4	64	54	7.6	*Deschampsia antarctica* Desv.	*Syntrichia magellamica* Zander; *Sanionia uncinata* (Hedw.) Loeske	–	High
S10	62°03′58.0″S 58°24′49.9″W	18	15	0.1	1.2	132	156	6.5	*Deschampsia antarctica* Desv.	*Sanionia uncinata* (Hedw.) Loeske; *Andreaea gainii* Cardot	*Psoroma cinnamomeum* Malme	High
S12	62°04′29.5″S 58°28′54.4″W	18	25	0.1	0	70	106	6.5	–	*Schistidium* SP.	*Leptogium puberulum* Hue	Low[a]
S13	62°04′31.1″S 58°24′42.3″W	35	30	0.0	0.4	5	43	6.8	–	*Sanionia uncinata* (Hedw.) Loeske	–	Low
S24	62°04′44.3″S 58°23′59.8″W	28	29	0.1	0.6	84	110	8.0	–	*Sanionia uncinata* (Hedw.) Loeske; *Syntrichia magellamica* Zander; Bryum pseudotriquetum (Hedw.) P. Gaertn., B. Mey. & Scherb.	–	Low
S32	62°03′55.5″S 58°24′54.3″W	152	64	0.0	1	81	102	7.8	*Deschampsia antarctica* Desv.; *Colobanthus quitensis* (Kinth.) Bartl.	*Sanionia uncinata* (Hedw.) Loeske	–	High
S4	62°03′ 51.3″S 58°24′47.0″W	29	9	0.4	0.6	6	181	6.1	*Deschampsia antarctica* Desv.	*Sanionia uncinata* (Hedw.) Loeske	–	High
S14	62°04′37.5″S 58°24′46.8″W	80	24	0.9	0.9	226	138	5.5	–	*Polytrichastrum alpinum* (Hedw.) G. l. Sm.; *Sanionia uncinata* (Hedw.) Loeske	–	High
S17	62°04′46.2″S 58°25′09.9″W	34	21	1.8	0.6	124	101	5.2	–	*Polytrichum piliferum* Hedw.; *Sanionia uncinata* (Hedw.) Loeske	*Cladonia* SP.	High
S19	62°04′19.3″S 58°25′00.1″W	28	14	0.4	1	85	162	6	–	*Polytrichastrum alpinum* (Hedw.) G. l. Sm.; *Sanionia uncinata* (Hedw.) Loeske	–	High
S27	62°05′09.2″S 58°24′50.1″W	26	24	0.4	0.6	109	165	6.1	–	*Polytrichastrum alpinum* (Hedw.) G. l. Sm.; *Sanionia uncinata* (Hedw.) Loeske	–	High

TOC, total organic carbon.
[a] Discontinuous vegetation covering less than 3% of the area.

of sequences collected (Lemos et al., 2011). To support the difference between groups observed by the PCoA, we applied a *t*-test.

2.5. Network-based analysis

To examine the co-occurrence of OTUs in the soil samples we applied a network-based analysis. The network allows for the visualization of the OTUs that are either unique or shared by specific groups of soil samples. Connections were drawn between samples and OTUs, with edge weights defined as the number of sequences from each OTU that occurred in each sample. To cluster the OTUs and soil samples in the network the spring embedder algorithm layout was used. In this algorithm the nodes act like steel rings that exert mechanical forces creating an attractive force between nodes that are far apart, and a repulsive force between nodes that are close together (Shannon et al., 2003). In the resultant graph, samples that share more OTUs cluster closer together. The network diagram was visualized with Cytoscape (Shannon et al., 2003) with two kinds of nodes; OTU-nodes, grouped using 97% similarity cutoff, and soil sample nodes.

2.6. Finding the bacteria responsible for the difference between communities

To determine whether specific clusters of bacteria differ between environments, an exact Chi-square test (based on 50,000 Monte Carlo iterations) was performed to get a *p*-value for the null hypothesis that there was no difference between all possible pairwise combinations of samples. The *p*-values were ordered and processed to find a false discover rate (FDR) of less than 1%.

The test was performed using the OTU table obtained with QIIME and running an R script implemented in PANGEA (Giongo et al., 2010). The datasets were first normalized to get equal sample sizes between soil samples. The percent of relative change of bacteria that differed statistically by the test (p-value ≤ 0.01; FDR ≤ 0.01) were summarized in a table.

2.7. Phylogenetic tree building

Unclassified sequences (below the Domain level) that differed between neutral and acidic soils were aligned against a set of closest known bacterial relatives using ClustalW (Larkin et al., 2007) and the evolutionary analysis was conducted in MEGA5 (Tamura et al., 2011). The reference sequences were chosen as the closest classified bacterial relative obtained by blast search against the NCBI 16S ribosomal RNA sequences database and excluding uncultured/environmental sample sequences. Maximum Likelihood fits of 24 different nucleotide substitution models were calculated and the model with the lowest AICc score (Akaike Information Criterion, corrected), which are considered to describe the substitution pattern the best, was chosen (see Table S2). The evolutionary history was inferred by using the Maximum Likelihood method based on General Time Reversible (GTR) model (Nei and Kumar, 2000). A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories) and the rate variation model allowed for some sites to be evolutionarily invariable (+I). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 44 nucleotide sequences. All positions containing gaps and missing data were eliminated.

3. Results

3.1. Taxonomy classification

After filtering the 454 reads by base quality and removing reads smaller than 200 bases, a total of 158,039 sequences were obtained from the 12 soil samples collected in the Keller Peninsula.

The sequences were assigned to the closest bacterial relative using the RDP naïve Bayesian rRNA Classifier (Wang et al., 2007) with 80% taxonomy confidence and an *e*-value of 0.001. Across all 12 samples, 123,255 (78%) were able to be classified below the domain level. On average, each individual sample was represented by 10,271 classifiable sequences. Of the classifiable sequences, 16 phyla were identified across the sample set found in all samples (listed in Table S1 in the Supplemental material). The dominant phyla/sub-phyla were Bacteroidetes, Acidobacteria, Betaproteobacteria, Alphaproteobacteria, and Actinobacteria, representing approximately 22%, 17.7%, 17.7%, 11%, and 7% of the sequences that could be classified below the domain level, respectively.

3.2. Clusters of similar bacterial community

The dataset was used to find clusters of similar bacterial communities by applying a Jackknifed Principal Coordinate Analysis (PCoA) (Fig. 1A and B). The weighted and unweighted PCoA analyses showed that the soil bacterial communities clustered in two distinctive groups using both weighted distance metric, which accounts for changes in the relative abundance of taxons, and unweighted distance metric, which accounts for presence/absence of taxons. The Jackknifed PCoA is capable of giving an estimate of sampling bias by resampling a smaller dataset (500 sequences in our experiment) and generating a graph made up by the mean values obtained. Around the mean values, ellipses that represent the IQRs (measure of statistical dispersion obtained by sequencing jackknifing) are drawn. If the ellipses are small, the same result would likely to be achieved with a different set of sequences from the same environment, but if the ellipses are large a different result might be expect. The IQRs for these point locations were extremely small showing that the results were robust to sample size and evenness. Thus, the composition of the bacterial community differed whether or not the abundance of taxa was considered. Two well-defined clusters were observed in our tests for both unweighted UniFrac distance metric, and weighted UniFrac distance metric. Biotic and abiotic environmental factors like the diversity of plant communities, soil composition and chemical features can be associated with the differences found between these two groups. However the most significant association found was related to the soil pH (see Table 2).

The group I comprised a cluster of five soil samples with a mean pH of 5.8 while the group II comprised a cluster of seven soil samples from areas with a mean pH of 7.4. Using the unweighted metric, relatively little variation is explained by the first three axes. On the other hand, the first three axis of the weighted metric

Table 2
Correlations between the ordination score of the first axis of the weighted PCoA (which explains 43% of the variance in the original data) and key environment parameters.

Environmental parameters	Model type			
	Linear		Quadratic	
	r^2	p	r^2	p
TOC ($g\,kg^{-1}$)	0.001	0.954	0.145	0.492
Ca + Mg ($cmol_c\,kg^{-1}$)	0.139	0.231	0.270	0.242
Al ($cmol_c\,kg^{-1}$)	0.591	0.003	0.748	0.002
Na ($cmol_c\,kg^{-1}$)	0.218	0.125	0.363	0.131
K ($mg\,kg^{-1}$)	0.108	0.108	0.161	0.453
P ($mg\,kg^{-1}$)	0.311	0.059	0.316	0.180
pH (H_2O)	0.681	0.001	0.739	0.002
Flowering plants	0.017	0.017	0.108	0.595
Mosses	0.001	0.944	0.096	0.633
Lichens	0.001	0.981	0.001	0.999
Plant richness	0.018	0.673	0.110	0.590

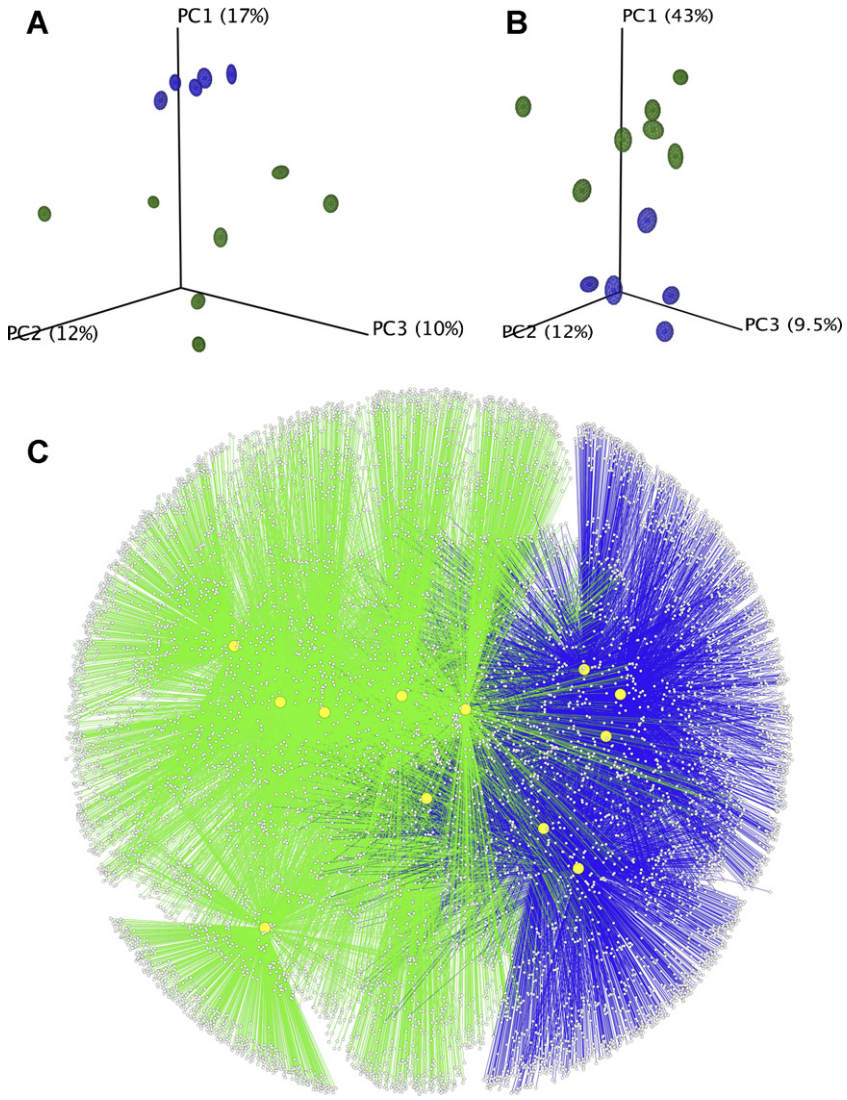

Fig. 1. Clusters of bacterial communities and the co-occurrence of OTUs within the soil samples from Keller Peninsula, King George Island, Antarctica. (A) Jackknifed PCoA plots with unweighted UniFrac distance metric, which accounts for presence/absence of taxons. (B) Jackknifed PCoA plots with weighted UniFrac distance metric, which accounts for changes in the relative abundance of taxons. The clusters were generated using a subset of 500 sequences from each environment for 100 replicate trials. The positions of the points are the average for the jackknife replicates and are displayed with a network around the points representing the statistical dispersion in each axis. Clusters of soils represented in blue belong to the group I (acidic soils) and cluster of soils represented in green belong to group II (neutral soils). (C) Network based analysis of soil bacterial communities. White circles are representative sequences from each OTU clustered based on the relatedness of the sequences (97% similarity). Yellow circles are soil samples. Each sample is connected with the OTUs through edges color-coded according to the groups of samples in which pH ranged from 5.2 to 6.1 (blue) and soil samples in which the pH ranged from 6.5 to 8.4 (green).

accounted for 74.5% of the variation in the data – indicating that the overall differences between the groups are more related to the abundance of specific OTUs than to their presence or absence. The Principal Component 1 was pulled out and regressed against the metadata from Table 1. The regression confirmed that the groups are separated by a pH gradient. In the weighted analysis, PC1 (accounting for 43% of variance) correlates strongly to the pH gradient ($r^2 = 0.68$; $p = 0.0009$) and soil aluminum ($r^2 = 0.59$; $p = 0.0035$) (Table 2), which is also related to pH. However no correlation was observed between the values of the first axis of the weighted PCoA and flowering plants, lichens, mosses or plant richness.

To corroborate the results obtained by the PCoA we also calculated a phylogenetic distance (Faith's PD) for each soil sample. The average PD for the group I was 67.1 and the average for the group II was 74.2 and this difference was significant (p-value = 0.069 at 95% confidence interval).

Once we recognized the microbial communities that differed with respect to their abiotic environments and established that the soil microbial assemblage patterns were related to soil pH, we further explored the co-occurrence of OTUs in the soil samples using a network-based approach.

3.3. Co-occurrence of OTUs

To visualize the phylogenetic information and how the OTUs are partitioned between samples the data were summarized in a spring-embedder network (Fig. 1C). The network was color coded according to the groups observed by the PCoA analysis. While the PCoA showed the groups of similar bacterial communities, the network analysis provided a visual display of shared versus unique OTUs. With the network-based analyses we mapped soil microbial community composition and structure and displaying microbial partitioning across soil samples

The soil microbial network consisted in 8051 nodes and 15,514 edges. Overall, the soil bacterial communities from neutral soils were more similar to each other then those of the acidic soils. The

results are consistent with the PCoA and together these results support an association between the soil pH and microbial community membership and provide evidence of no correlation between bacterial community and plant cover in Antarctica.

To determine whether specific clusters of bacteria differ between the two groups of soil samples detected by the PCoA, and by the network analysis an exact Chi-square test (based on 50,000 Monte Carlo iterations) was performed to get a *p*-value for the null hypothesis that there was no difference between all possible pairwise combinations of samples. On the basis of the exact Chi-square test, 22 bacterial taxa were found to have statistically significantly higher abundances in the acidic soil samples compared to the neutral samples (Table 3). Of these, Sphingobacteriales, Gp4 and Gemmatimonas were the most abundant with 9.72%, 6.42% and 4.22%, respectively, of the total number of sequences. Furthermore, seven bacterial taxa were found to have statistically significantly higher abundances in the neutral soil samples. Of these, the most abundant were Gp3 and an unclassified family of Burkholderiales making up 7.36% and 5.30%, respectively, of the total number of sequences. Besides the classifiable sequences we also detect 23 unclassified OTUs that presented different abundances between the acidic soils and neutral soils (Table 3). Of those, 15 were significantly more abundant in acidic soils (group I) whereas 8 were significantly higher in neutral soils (group II).

3.4. Identification of novel rRNA genes

The automated tools used for finding the closest bacterial relative does not re-construct trees but instead fits an OTU into known phylogenetic groups if the similarity matches a criteria of identity. In attempt to identify those OTUs that differed statistically between the samples and did not match our classification criteria, we generated an alignment of each of our unclassified sequences that differed between neutral and acidic soils (as observed by the chi-square test – Table 3) against a set of closest known bacterial relatives and built a phylogenetic tree (Fig. 2). We did not find any sequence that branches apart of the domain Bacteria confirming that our sequences were not chimeric and in fact belonged to the domain Bacteria. On the other hand, at least 6 OTUs (OTUs – 4284, 2735, 155, 4010, 6925) did not cluster with any known phylogenetic group. As we were analyzing short reads (300 bases) the tree might not be robust enough to identify novel rRNA genes however, it is plausible that our soil samples harbored new candidate bacterial divisions and those OTUs presented a significant contribution to the variation found between our samples.

4. Discussion

This exploratory study aimed to obtain a detailed baseline description of the soil bacterial communities found in the Keller Peninsula against which to compare changes in the Antarctic microbiome caused by climate changes or human activities. The Keller Peninsula is still one of the least known parts of Antarctica in terms of microbial diversity. The soils from this region are distinctly different from those of other Antarctic climatic zones because of the warmer temperatures (annual average temperature for the last 66 years has been 2.1 °C – source http://antartica.cptec.inpe.br/) and higher water availability (Simas et al., 2008). The combination of these factors allows the growth of flowering plants, mosses and lichens. Only 49.5% of Keller Peninsula present greater soil development, and the vegetation cover occurs on less than 3% of the total area (Francelino et al., 2011). The sampling sites were chosen considering the vegetal formations, plant biomass and absence of plant cover (bare soil). We obtained a database of 158,039 sequences from soil samples taken from the most significant ecological niches of the Keller Peninsula. To the best of our knowledge, this dataset is the first microbial inventory from the soils of Keller Peninsula and is valuable to the further monitoring of the evolution of bacterial communities during future global climate changes.

Most soils, including agricultural ones, seem to be dominated by only a small number of phyla, namely Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes and Firmicutes (Janssen, 2006; Roesch et al., 2007; Fierer et al., 2009; Lauber et al., 2009; Uroz et al., 2010). Although organisms in environments at the extremes of temperature are presumably under strong selective pressures, our results shown that the soils in ice-free areas of Keller Peninsula are dominated by these same phyla. This indicates that at least at a coarse level of taxonomic resolution it is possible to find some degree of ubiquity in soil samples. Studying biogeography of soil bacterial communities in the Arctic and comparing the diversity with soils from a wide range of temperate biomes, Chu et al. (2010) found a common diversity structure within soil bacterial communities around the globe. Those results suggest that patterns in soil microbial diversity are structured according to local variation. However it is also important to recognize that the methods we were using for this high throughput approach (and the methods applied in the studies cited above) were not robust enough to identify all taxa inhabiting the soil sample. Most of our problems were inherent to PCR bias that appears to exclude some phyla during amplification, mainly Verrucomicrobia (Bergmann et al., 2011), and due to the barcoded primers used (Berry et al., 2011). In this regard, new studies using different sets of primers may find variations in the abundance of those phyla.

Bacteria from the Sphingobacteriales order were the most abundant in all soil samples (see Tables 3 and S1). Fulthorpe et al. (2008) studying the bacterial phylogenetic structure of soils from four distinctly different sites in South and North America found that the *Chitinophaga* (which belong to the Sphingobacteriales order) was among the most abundant or the second most abundant genus in all four soils with 7.5–13.8% of the total bacterial sequences in these soils. The authors used a primer that target the V9 region of the small subunit of the bacterial ribosomal RNA gene. In this work with Antarctic soils, we used a primer targeting the V3 region so this observation is probably not associated with primer specificity and the use of different sets of primers reveal that the abundance and ubiquity of this taxa is expected to be correct. Janssen (2008) screened thirty-two libraries of 16S rRNA genes of members of the domain Bacteria, prepared from a variety of soils from Europe, Australia, North and South America and also found high abundance of members of the Sphingobacteriales among the three most abundant in soils.

In addition to the aspects of overall bacterial distribution in soils from Keller Peninsula, we attempt to determine the major factors responsible for the overall patterns of soil microbial diversity. The greatest advantage of this study is the low or no human influence under the Antarctic environment. Here we present evidences of a strong influence of pH as the environmental factor that drives the overall patterns of soil microbial diversity in ice-free areas of Keller Peninsula. Qualitatively, we found no clear relationship between soil bacterial diversity and any other variables tested (e.g. plant richness, plant species or bare soil). Altogether the results suggest that despite the differences in soil properties or plant cover the best predictor for the microbial community structure in ice-free areas of Keller Peninsula was the pH gradient. Other studies already had shown that soil pH could largely explain diversity and richness of soil bacterial communities (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010). However, most of these studies had used soil samples from sites under influence of human activity. Our results present the same pattern in an ecosystem under no human

Table 3
List of the closest bacterial relative whose abundances differ statistically ($p \leq 0.01$; FDR ≤ 0.01) between neutral and acidic soils.

Closest bacterial relative	Acidic soils	Neutral soils	Fold difference
	% of all sequences		
Sequences classified below the domain level			
Acidobacteria; Acidobacteria; Acidobacteriales; Acidobacteriaceae; Gp3	3.64	**7.36**	2.0
Acidobacteria; Acidobacteria; Acidobacteriales; Acidobacteriaceae; Gp4	**6.42**	3.06	2.1
Acidobacteria; Acidobacteria; Acidobacteriales; Acidobacteriaceae; Gp6	1.60	**2.22**	1.4
Actinobacteria; Actinobacteria; Actinobacteridae; Actinomycetales; Micrococcineae	**2.06**	1.60	1.3
Actinobacteria; Actinobacteria; Actinobacteridae; Actinomycetales; Micromonosporineae	**0.60**	0.18	3.3
Bacteroidetes	**1.80**	1.18	1.5
Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae; Flavobacterium	**1.66**	0.04	41.5
Bacteroidetes; Sphingobacteria; Sphingobacteriales	**9.72**	5.34	1.8
Bacteroidetes; Sphingobacteria; Sphingobacteriales; Crenotrichaceae; Terrimonas	**1.38**	0.30	4.6
Bacteroidetes; Sphingobacteria; Sphingobacteriales; Flexibacteraceae	**1.68**	0.36	4.7
Bacteroidetes; Sphingobacteria; Sphingobacteriales; Flexibacteraceae; Niastella	**1.72**	0.64	2.7
Bacteroidetes; Sphingobacteria; Sphingobacteriales; Saprospiraceae	**0.66**	0.04	16.5
Chloroflexi; Anaerolineae	**0.62**	0.02	31.0
Chloroflexi; Anaerolineae; Caldilineae; Caldilineales; Caldilineacea	**0.16**	0.00	8.0
Chloroflexi; Chloroflexi; Chloroflexales	**1.02**	0.14	7.3
Gemmatimonadetes; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; Gemmatimonas	**4.22**	3.00	1.4
OP11; OP11 genera incertae sedis	**0.12**	0.00	6.0
Proteobacteria	2.26	**2.86**	1.3
Proteobacteria; Alphaproteobacteria	1.40	**2.90**	2.1
Proteobacteria; Alphaproteobacteria; Rhizobiales	1.08	**2.62**	2.4
Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae	**1.06**	0.58	1.8
Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae	**2.48**	0.64	3.9
Proteobacteria; Betaproteobacteria	2.82	**3.46**	1.2
Proteobacteria; Betaproteobacteria; Burkholderiales	3.28	**5.30**	1.6
Proteobacteria; Betaproteobacteria; Burkholderiales; Incertaesedis5	**0.64**	0.14	4.6
Proteobacteria; Betaproteobacteria; Nitrosomonadales; Nitrosomonadaceae; Nitrosospira	**0.26**	0.06	4.3
Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae	**0.14**	0.00	7.0
Proteobacteria; Gammaproteobacteria	**0.62**	0.30	2.1
Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae	**1.74**	1.36	1.3
Sequences that could not be classified below the domain level			
Uncultured Bacteria OTU ID – 4284	**0.53**	0.03	19.1
Uncultured Bacteria OTU ID – 6231	**0.12**	0.01	15.0
Uncultured Bacteria OTU ID – 2735	**0.11**	<0.00	5.3
Uncultured Bacteria OTU ID – 5499	**0.10**	<0.00	4.9
Uncultured Bacteria OTU ID – 2626	**0.06**	<0.00	3.1
Uncultured Bacteria OTU ID – 155	**0.07**	<0.00	3.3
Uncultured Bacteria OTU ID – 8009	0.35	**2.68**	7.7
Uncultured Bacteria OTU ID – 6624	**0.05**	<0.00	2.3
Uncultured Bacteria OTU ID – 3072	**0.11**	0.01	13.6
Uncultured Bacteria OTU ID – 7204	**0.05**	<0.00	2.6
Uncultured Bacteria OTU ID – 5613	**0.17**	<0.00	41.4
Uncultured Bacteria OTU ID – 152	**0.14**	<0.00	34.3
Uncultured Bacteria OTU ID – 3112	**0.09**	<0.00	4.3
Uncultured Bacteria OTU ID – 4010	**0.15**	<0.00	7.7
Uncultured Bacteria OTU ID – 6606	**0.10**	<0.00	5.0
Uncultured Bacteria OTU ID – 6925	0.01	**1.18**	103
Uncultured Bacteria OTU ID – 133	**0.21**	<0.00	11.0
Uncultured Bacteria OTU ID – 178	0.01	**0.42**	29.7
Uncultured Bacteria OTU ID – 192	0.02	**0.20**	8.6
Uncultured Bacteria OTU ID – 418	<0.00	**0.12**	40.6
Uncultured Bacteria OTU ID – 523	<0.00	**0.10**	35.0
Uncultured Bacteria OTU ID – 798	0.08	<0.00	4.0
Uncultured Bacteria OTU ID – 1638	0.03	**0.47**	16.5

Those percent of sequences in bold indicate the soil type, which is higher for that bacterial relative.

influence. In agreement with our results, Teixeira et al. (2010), studying the rhizospheres of two vascular plants that are found in Antarctic ecosystems, *Deschampsia antarctica* Desv (Poaceae) and *Colobanthus quitensis* (Kunth) BartI (Caryophyllaceae), observed that the bacterial community structure was very similar in microbial diversity and composition irrespective of the plant species or sampling location.

Due to limitations of the experimental design, PCR and sequencing bias and the number of samples analyzed in our experiment, it is important to be cautious in data interpretation and extrapolating information from results obtained. It is also important to notice that we are not attributing the total bacterial variation found in our work to the soil pH. The pH was the most significant variable that correlates to the microbial composition in ice-free areas of Keller Peninsula however, it not explain the total variation. Although our results point to apparently no relationship (or at least not detectable by the methods applied in this work) between soil bacterial communities and plants, it is well known that roots release hydrogen or bicarbonate ions into the soil, causing the pH in the rhizosphere to decrease or increase (Marschner et al., 1982). Also, other researches already found significant influence of vegetation over microbial composition. Angel et al. (2010) using a molecular fingerprint technique found significant differences in the microbial community composition largely explained by precipitation gradient combined with vegetation cover. Mitchell et al. (2010) studied the vegetation composition and soil chemistry as predictors of the

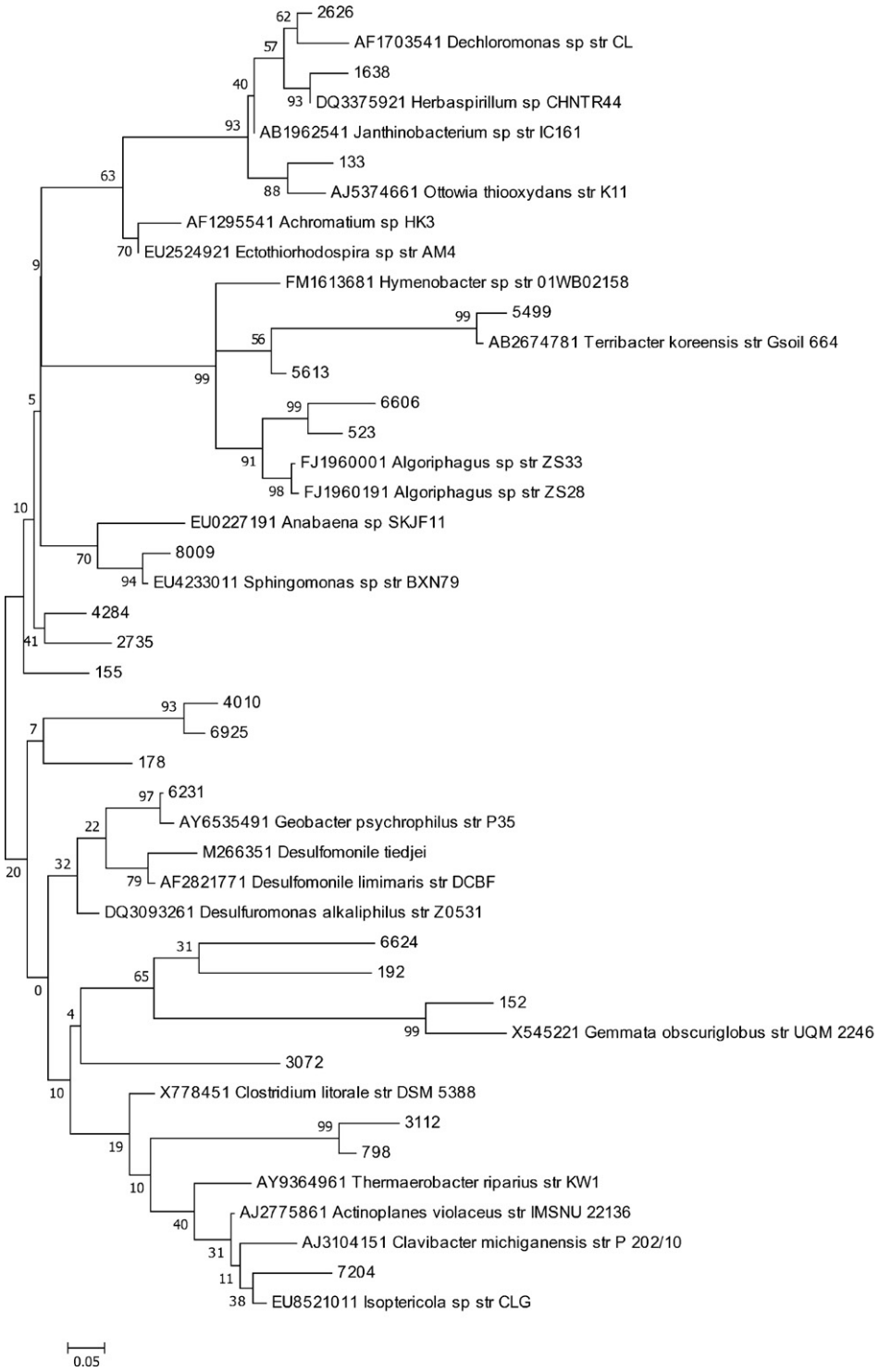

Fig. 2. Molecular Phylogenetic anaylsis of the 16S rRNA sequences that were found to be different between neutral and acidic soils but were not classified below the domain level by the RDP naïve Bayesian rRNA Classifier. The reference sequences were chosen as the closest classified bacterial relative obtained by blast search against the NCBI 16S ribosomal RNA sequences database and excluding uncultured/environmental sample sequences. The evolutionary history was inferred by using the Maximum Likelihood method. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

soil microbial community and concluded that the vegetation composition predicted the soil microbial community at least as well as the soil chemical data. Those works were performed comparing different ecosystem types while we are working in a local scale. Apparently the microbial composition seems to be unique to each climatic region.

5. Conclusions

The strategy applied here allowed us to detect different patters in bacterial community structure in soil samples from the Keller Peninsula, King George Island, Antarctica. At a coarse level of taxonomic resolution, these patterns mirror those found in most

soils, including agricultural ones. Rather than vegetal composition or plant richness the major factor responsible for the overall patterns of soil microbial diversity was the soil pH, which revealed that, in the Antarctic environment, plant diversity was not the best predictor of soil microbial patterns.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2012.04.009.

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