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Local-scale variation of soil bacterial communities in ice-free regions of maritime Antarctica

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ABSTRACT

The majority of biogeographic studies in Antarctic terrestrial ecosystems have focused on macroscopic eukaryote taxa. In contrast, microbial taxa have been almost neglected or examined without sufficient spatial coverage. Here, we examined the spatial distribution of soil bacterial communities and their relation to local environmental gradients in ice-free regions of the maritime Antarctic. Soils in this region were dominated by *Actinobacteria* and *Proteobacteria*, which are typically found in Antarctic soils. Notably, candidate divisions AD3 and WPS-2, which rarely occur in other biomes, were found in high abundance in acidic surface soils. Soil pH and total organic carbon (TOC) were the primary drivers of the bacterial community structure and various geochemical variables also played important roles in structuring bacterial community composition. Bacterial communities were significantly correlated to measured environmental variables, most of which are spatially structured at the local spatial scale (0.5–8 km). This suggests that spatial heterogeneity of environmental factors is an important driver of shifts in bacterial communities. Although various sets of environmental variables were related to bacterial communities, a large proportion of community variation remained unexplained. This indicates that bacterial communities in Antarctic soils may be governed by other biotic or abiotic factors which are not typically measured in other biome studies.

1. Introduction

Terrestrial ecosystems in Antarctica are characterized by extreme environmental conditions, which result in low biomass and nutrient poor environments. The mean annual temperature ranges from −10 °C on the Antarctic coast to −57 °C in the interior, and most of Antarctica is extremely dry, averaging 166 mm of precipitation per year. Terrestrial biota in the Antarctic Peninsula is largely limited to snow- and/or ice-free regions, which are located along the coastal areas at lower latitudes. The present-day boundary of many ice-free areas in the Antarctic Peninsula is a consequence of multiple glacial retreat and re-advance events that have occurred in this region since the Last Glacial Maximum (LGM) (Cofaigh et al., 2014 and references therein). Many places in the Antarctic Peninsula have become newly ice-free over recent decades because of accelerated warming in this region (Cook et al., 2005; Rignot et al., 2008).

Despite the hostile environmental conditions, terrestrial ecosystems in Antarctica are occupied by diverse macroscopic taxa, many of which have distinct biogeographical patterns. A comprehensive survey on eukaryote biodiversity identified 15 biologically distinct bioregions across the Antarctic terrestrial ecosystems (Terauds et al., 2012). Contrary to the large-scale surveys of macrofauna and even some microeukaryotes, prokaryotes have received relatively little attention. Over the past few decades, researchers have attempted to reveal which environmental constraints primarily shape microbial community composition under the extreme conditions of Antarctica rather than make regional-scale biogeographical comparisons. It is generally thought that the effects of abiotic factors on biota are more magnified in harsh and extreme environments such as Antarctica than in warmer and less hostile environments and thus they are often the primary drivers structuring the terrestrial ecosystem (Barrett et al., 2006). Stronger environmental constraints on terrestrial biota are also supported by the minimized biotic interactions in this adverse environment (Hogg et al., 2006). Several earlier studies demonstrated that local environmental gradients primarily control microbial community structure in Antarctica. For example, soil pH is among the most influential factors in determining bacterial community structure (Chong et al., 2010; Kim et al., 2015), and conductivity is reported to be one of the major factors in

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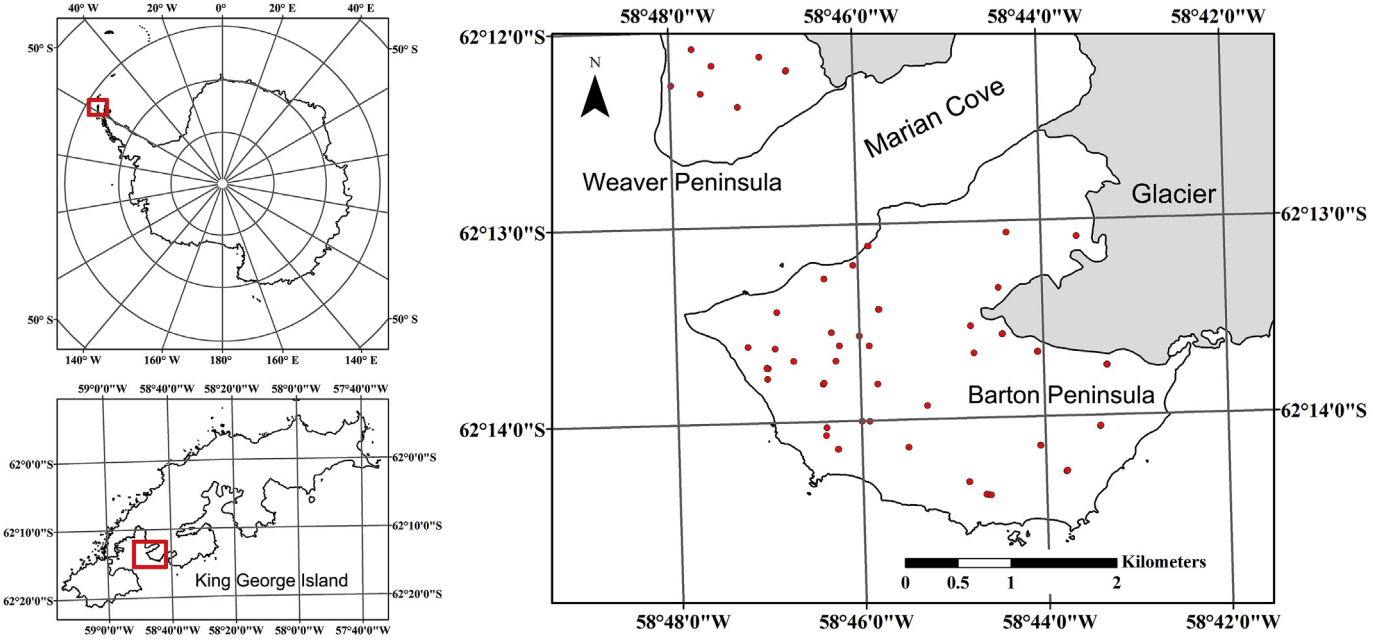

Fig. 1. Map of soil sampling locations in Barton and Weaver Peninsulas, King George Island (Antarctica).

cold and dry desert environments (Lee et al., 2012; Magalhães et al., 2012). Although many studies have focused on effects of local environmental conditions on soil bacterial community structure (Chong et al., 2010; Ganzert et al., 2011), few studies have been conducted on the spatial distribution of microbial communities due to logistical constraints or poor accessibility to sampling sites (Sokol et al., 2013; Van Horn et al., 2013). A recent review on spatial patterns of Antarctic prokaryotes collectively suggested that environmental heterogeneity rather than dispersal or historical contingency is more likely to be indicative of bacterial community assembly at micro to regional spatial scales (100 m–1000 km) (Chong et al., 2015). However, its generality was not extensively tested over a wide geographical range of the Antarctic continent and even microscale biogeographical studies at local sites are lacking.

In addition, despite the potential importance of geochemistry in structuring soil microbial communities, geochemical properties (e.g., major elements, rare earth elements, etc.) have been relatively neglected in microbial community analysis compared to other edaphic variables mentioned above (Chong et al., 2010; Lee et al., 2012). Most ice-free regions in Antarctica are mainly composed of barren terrain with limited vegetation, and thus, soil microbes are likely to have stronger associations with geochemical characteristics in a given environment. Due to limited sets of variables and lack of spatial coverage, we still have only a limited integrated understanding of which environmental gradients are key drivers of microbial diversity in this environment.

In this study, we performed extensive sampling along the coastal ice-free region of the Barton and Weaver Peninsulas on King George Island and examined the spatial distribution of the bacterial community and its relation to environmental gradients. In order to fully understand the extent to which environmental variables explain the community variation, various sets of environmental variables were included in the community modelling. We also assessed the spatial scales at which bacterial communities respond to environmental gradients and tested what proportion of community variation is explained by spatially correlated environmental variables at the local spatial scale (< 8 km).

2. Materials and methods

2.1. Soil sampling and site description

King George Island (KGI) is the largest of the South Shetland Islands (SSI), stretching 540 km off the northern end of the Antarctic Peninsula. Most of the surface is covered by ice cap (Collins Ice Cap), and approximately 7% is ice-free region along the coastline (Simões et al., 1999). Soils consist of mineral and rock fragments mainly derived from igneous rocks of volcanic origin and have low levels of organic material (Bölter, 2011). The island has a cold moist maritime climate, which is characterized by a mean annual air temperature of −1.8 °C, a high relative humidity of 89%, and an annual precipitation rate of 437.6 mm water (Lee et al., 2004). Annual mean air temperature has gradually increased at a rate of 0.037 °C per year (1956–2006), and the precipitation rate also shows an increasing trend (15 mm per year) over the last couple decades (1988–2007) (Lee et al., 2008).

Progressive deglaciation has occurred on KGI due to post-LGM warming, and it receded to the present limit of ice-free land area prior to 6 Ky BP (Ingólfsson and Hjort, 2002). Late Holocene glacial re-advance was also recorded on KGI during Neoglacial cold events. More recently, rapid ice thinning and retreat of marine-terminating outlet glaciers have occurred in the last 80 years (Cook et al., 2005; Monien et al., 2011); in particular, it was reported that 7% of ice cover was lost in KGI since 1958 (Simões, 2011). Ice-free area of Barton Peninsula is relatively rich in vegetation (34.2–37.0% vegetation coverage) and is occupied mostly by cryptogamic species (Shin et al., 2014). Vegetation in Barton Peninsula consists of 62 lichens, 33 bryophytes, and two phanerogam species, being dominated by crustose lichens and three major subdominant lineages, *Usnea* spp., *Andreaea* spp., and *Sanionia uncinata* (Kim et al., 2007).

Soil samples were collected from Barton and Weaver Peninsulas, which are located at the southwest end of King George Island, during the austral summer (late December to early February) of 2010–2012 (Fig. 1). Soil samples were taken from 57 sites which are spatially distributed on the ice-free areas of Barton and Weaver Peninsulas. We randomly chose almost 20 sites each year and took the soil samples using the same sampling procedure throughout different years. At each site, three sampling locations spaced within approximately 5 m of one another were chosen and soil samples of 100 g were collected from the

surface and lower layer soils (approximately 0–3 cm and 3–10 cm depth, respectively) at each location using a large sterile spatula. Soil depth was roughly determined with bare eyes while digging the soils. We attempted to collect mineral soils from sites which were not disturbed by cryptogam communities and/or ornithogenic material in order to control for the effect of biological inputs. However, complete avoidance of organic material was almost impossible due to the substantial spatial coverage of lichens and mosses on surface soils (samples which could have potential influence of biological inputs are shown in Table S1). All samples were transported to the laboratory at King Sejong Station within 6 h and kept at −20 °C during shipment (three months on average) to South Korea.

2.2. Soil physicochemical properties

Physicochemical properties of soil samples were determined following the methods described earlier (Kim et al., 2015). Briefly, soil water content was determined by oven drying at 105 °C for 48 h. Soil pH and electrical conductivity (EC) were measured using a pH/Cond 340i multimeter (WTW GmbH, Germany) in a 1:1 and 1:5 (w/v) ratio of soil:deionized water, respectively. Total carbon (TC) and nitrogen (TN) contents were determined using an elemental analyzer (FlashEA 1112, Thermo Fisher Scientific), while total inorganic carbon (TIC) contents were analyzed using a UIC CO_2 coulometer by measuring the CO_2 gas generated by the reaction of 50 mg powdered samples with 42.5% phosphoric acid at 80 °C for 10 min. Total organic carbon (TOC) contents were calculated as the difference between the TC and TIC contents. For geochemical analyses, samples were dried at 105 °C for 2 h to remove moisture and then homogenized in an agate mortar. Pressed pellets were made for chemical determinations by X-ray fluorescence (XRF) spectrometer (PW2440, Philips). Loss on ignition (LOI) was determined after overnight heating at 950 °C. About 0.1 g of sample was completely digested in a 5:3 mixture of HF and HNO_3. The sample was dried, refluxed several times in 6.0 M HCl to remove fluorides, and redissolved in 5% HNO_3. Concentrations of trace elements (Ba, Cr, Sr, Zn, Sc, V, and Zr) and rare earth elements (other elements in Table S1) were determined by inductively coupled plasma atomic emission spectrometer (ICP-AES; Jobin Yvon 138 Ultrace) and inductively coupled plasma mass spectrometer (ICP-MS; Perkin Elmer Elan 6100), respectively. Repeated analyses of USGS rock standard powders (BCR-2, BHVO-2, and BIR-1) yielded external reproducibility better than ± 5%. Magnetic susceptibility (MS) was measured using a Bartington MS2 susceptibility meter. All the element analyses above were performed at the Korea Basic Science Institute (KBSI).

2.3. DNA extraction, 16S rRNA gene pyrosequencing, and bioinformatic analysis

Soil DNA was extracted from 0.3 g of each soil sample using a FastDNA® SPIN Kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. Extracted DNA was amplified targeting the V1−V3 region of the 16S rRNA gene using barcoded fusion primers, 27F (5′-AC-AGAGTTTGATCMTGGCTCAG-3′) and 519R (5′-X-AC-GWATTACCGCGGCKGCTG-3′), where 'X' represents 8-bp error-corrected barcodes and 'AC' is a 2-bp linker sequence (Hamady et al., 2008). The PCR conditions were as follows: initial denaturation for 5 min at 94 °C, followed by 25 cycles of denaturation (1 min, 94 °C), annealing (1 min, 50 °C), and extension (1 min 30 s, 72 °C). The PCR mixture consisted of a total volume of 50 μL, 10 pmol of each primer, 1.5 U of Taq polymerase (GeneAll, Korea), 2/25 volume of dNTP mix, and 1/10 volume of 10X buffer provided with the enzyme. The 454 adapter sequence was incorporated into the purified PCR product, and 16S amplicon sequencing was performed by DNALink Inc. (Seoul, Korea) using the 454 GS FLX Titanium Sequencing System (454 Life Sciences, Roche Applied Science, Penzberg, Germany). Raw sequence reads were processed following the 454 SOP in mothur v.1.40.5 (Schloss et al., 2009). Flowgrams were denoised using the mothur-implemented Pyronoise, and the denoised sequences were then aligned against the SILVA pre-aligned reference dataset using the NAST (Nearest Alignment Space Termination) algorithm. Both ends of the alignments were trimmed to make alignment positions fully overlapped among sequence reads. Putative chimeras were identified using the *de novo* UCHIME chimera detection algorithm, and chimera-free sequences were taxonomically assigned to genus-level phylotypes against the EzBioCloud 16S database (Yoon et al., 2017) using Naïve Bayesian Classifier with a confidence threshold of 80%. Sequences assigned to archaea, eukarya, chloroplasts and mitochondria were removed. Quality-filtered sequences were clustered into operational taxonomic units (OTUs) at 97% similarity cutoff and singleton OTUs were omitted to avoid introducing sequencing bias into the following community analyses. The level of bacterial richness and diversity among samples was compared at the equal sample coverage (0.628) using the estimateD function in the iNEXT package (Hsieh et al., 2016) with R v.3.5.0 software (Table S2).

The average rRNA operon (*rrn*) copy number has been suggested as a community-level trait in the life history strategies of microbes (Green et al., 2008), and in particular it was recently shown that a decrease in average *rrn* copy number is a consistent feature of bacterial communities throughout succession (Nemergut et al., 2016). Bacterial *rrn* copy number was estimated using PICRUSt v.1.1 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States), a bioinformatics software designed to predict the functional composition of metagenomes from 16S rRNA gene data and a reference genome database (Langille et al., 2013). PICRUSt predictions of 16S copy numbers are well correlated with actual annotations, and the prediction is not systematically biased by the availability of reference genomes or copy number annotations for close relatives (Langille et al., 2013). Unlike other functional genes, annotations of 16S copy numbers per genome from the integrated microbial genome (IMG) database are used as input. We further curated the IMG-derived pre-calculated input file by comparing the ribosomal RNA operon copy number database (*rrn*DB) v5.4 (Stoddard et al., 2015) to increase taxa coverage and prediction accuracy. Taxonomic classification was performed based on Greengenes database v.13.5, and the resultant BIOM file was analyzed using the web-based Galaxy version of PICRUSt. Copy number information for the bacterial 16S rRNA gene was used to calculate 'the weighted mean *rrn* copy number' (Nemergut et al., 2016). The weighted mean *rrn* copy numbers were obtained by multiplying copy numbers by relative abundance of each OTU and summing these values within a sample. Raw sequence data were submitted to the NCBI Sequence Read Archive (SRA) database with an accession number of PRJNA379750.

2.4. Statistical analyses

Bray−Curtis dissimilarities were calculated using the Hellinger-transformed OTU matrix and visualized using non-metric multidimensional scaling (NMDS). Permutational multivariate analysis of variance (PERMANOVA) and analysis of similarity (ANOSIM), implemented in PRIMER v6 (Clarke and Gorley, 2006), were used to determine if bacterial community structure differed significantly between different groups (i.e. different regions and soil layers). The vectors of environmental variables were fitted onto the ordination space using the envfit function in the vegan package (Oksanen et al., 2013) with R v.3.5.0 software, and the significance of each correlation was tested based on 999 permutations. We employed distance-based redundancy analysis (dbRDA) for constrained ordination using the capscale function in the vegan package (Oksanen et al., 2013) with R v.3.5.0 software. Prior to dbRDA, the distribution of each variable was plotted, and normalizing transformations were performed on skewed variables according to the 'Draftsman plot' result in PRIMER v6 (Clarke and Gorley, 2006). For example, TC, TN, C/N ratio, $CaCO_3$, TOC, kurtosis (the degree of peakedness in the grain-size frequency curve), Sr, Zn, and Mo

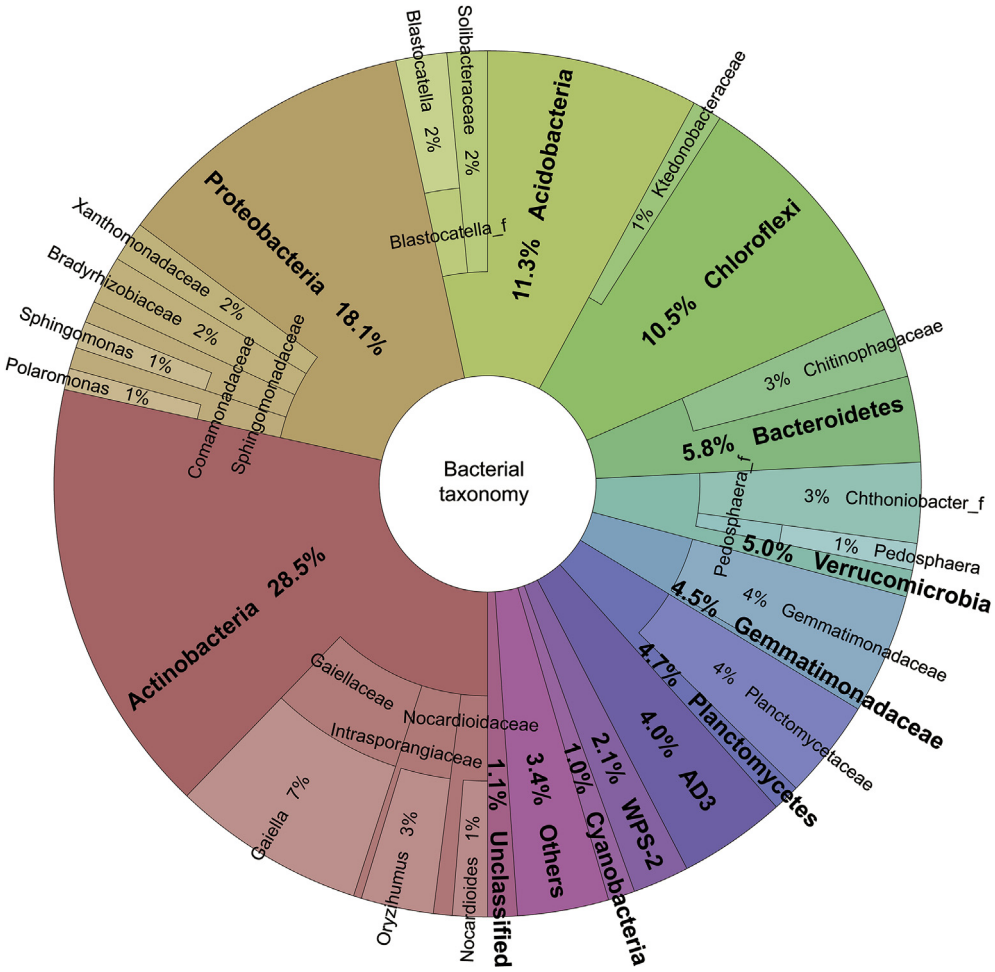

Fig. 2. Krona chart of bacterial taxonomy at multiple taxonomic ranks (phylum, family, and genus levels). Averaged values of each taxon across all samples are shown, and only abundant lineages (> 1.0% in relative abundance) are listed. The inner circle represents the phylum level, while family and genus levels are displayed in the middle and outer circles, respectively.

were log-transformed; MS (magnetic susceptibility), CaO, K_2O, Cu, Nb, Cs, and Th were square-root transformed; and Al_2O_3, MnO and SiO_2 were power transformed. Depth was used as the dummy variable. Values below the analytical detection limits were set to half the value of the detection limit. Multicollinearity between variables was tested using Spearman's rank correlation in the varclus function in the nmle package with R v.3.5.0 software. Highly correlated variables (Spearman's $\rho^2 > 0.70$) were excluded, resulting in 49 out of 70 variables being retained, and the remaining co-varied variables were further omitted by measuring the variance inflation factor (VIF) over the course of model selection.

For spatial analysis, we used a spatial eigenfunction approach, Moran's eigenvector maps (MEMs), to generate spatial variables across study sites (Dray et al., 2006). MEMs represent multiscale spatial explanatory variables (spatial eigenfunctions) generated by spectral decomposition of spatial relationships among data points. The MEM framework is a powerful multivariate method which allows spatially explicit ecological modelling of beta diversity by introducing spatial components explicitly into community analysis (Dray et al., 2012). We first tested various types of connectivity matrices (e.g., Gabriel graph, Delaunay triangulation graph, minimum spanning tree, k-nearest neighbor, and relative neighborhood) and the best MEM model was obtained using the connectivity matrix of 'Relative neighborhood' weighted by the Euclidian distance function. We then partitioned the variation of response data (bacterial community structure) with respect to geochemical, edaphic, and spatial components (Borcard et al., 1992). Prior to the variation partitioning, we independently applied forward selection procedures with double stopping criteria (Blanchet et al., 2008) to the geochemical, edaphic, and MEM variables to select the set of variables that best explained the community variation. The significance of each fraction was tested based on adjusted R^2 values with 999 permutations. Variation partitioning result was plotted using an area-proportional Venn diagram in the eulerr package with R v.3.5.0 software.

To identify unmeasured or unmeasurable ecological processes through the analysis of spatial structure or residuals, we followed the 'space as a surrogate' approach of McIntire and Fajardo (2009) and the worked example of Dray et al. (2012). This approach allowed us to assess two major things; 1) the spatial scales at which bacterial community variation explained by measured environmental variables is spatially structured, and 2) at which spatial scales the remaining residuals are still spatially structured when environmental variables are factored out. The MEM framework was used to test the multiscale components of spatial structures in each of three components: the original Hellinger-transformed OTU abundance table (Y), the table fitted by environmental variables (F), and the residuals not explained by environmental variables (R). We performed redundancy analysis (RDA) to identify community variation explained by environmental variables, and partial residual analysis (PRA) was used to uncover community variance unexplained by measured environmental variables. Smoothed scalograms were generated by projecting the site scores on the first and second axes of each ordination analysis onto the spatial scales (ranging

from local to micro spatial scales by grouping significant MEMs into seven successive spatial scales). Definitions of spatial scales represented in MEMs are as follows: local scale (0.5–8 km) and microscale (< 0.5 km). All significant MEMs were represented in a decreasing order in each scalogram. The R^2 values (the proportion of variance explained by a given spatial scale) in each scalogram should be uniformly distributed across the spatial scales if there is no spatial structure (Ollier et al., 2006). All statistical analyses were performed using versatile packages in R v3.5.0 (www.r-project.org). NMDS axis scores were used to make an interpolated map using the inverse distance weighting (IDW) method in ArcGIS v10.4.

3. Results

3.1. Bacterial community profile

In total, 277,186 high-quality bacterial sequences were clustered into 24,353 bacterial OTUs, and singleton OTUs accounted for 44.2% of the total OTUs. Soil samples in this region were dominated by *Actinobacteria* (28.5% on average), followed by *Proteobacteria* (18.1%), *Acidobacteria* (11.3%), and *Chloroflexi* (10.5%) (Fig. 2). Other phyla such as *Bacteroidetes* (5.8%), *Verrucomicrobia* (5.0%), *Planctomycetes* (4.7%), *Gemmatimonadetes* (4.5%), candidate phylum AD3 (4.0%), and candidate phylum WPS-2 (2.1%) were also abundant across all samples to a lesser extent (Fig. 2). Interestingly, two unknown bacterial lineages, candidate divisions AD3 and WPS-2, were overrepresented in this region (0.0–35.0% and 0.0–8.0% in relative abundance, respectively). They occurred at a wide range of abundances in acidic soils but were almost absent or present at lower abundance above pH 7 (Fig. S1). At the genus level, *Gaiella* belonging to *Actinobacteria* was the most dominant genus (7.2% on average) across all samples, and *Oryzihumus* (*Actinobacteria*) (2.7%), *Blastocatella* (*Acidobacteria*) (1.9%), *Nocardioides* (*Actinobacteria*) (1.3%), and *Sphingomonas* (*Proteobacteria*) (1.0%) were also consistently found in higher abundance (Fig. 2). No environmental variables showed strong correlations with bacterial richness or diversity (Pearson's $|r| < 0.30$, all $P > 0.05$).

3.2. Environmental factors determining bacterial community composition

We fitted vectors of environmental variables onto the ordination space to examine which predictor variables are mainly associated with bacterial community structure in this region. Among various edaphic variables, soil pH was the single dominant factor in structuring bacterial communities (envfit $R^2 = 0.71$, $P < 0.001$), and TOC was the next most significant edaphic variable ($R^2 = 0.36$, $P < 0.001$) (Fig. 3A). Soil pH was strongly correlated to NMDS axis 1 scores (Pearson's correlation $r = 0.83$, $P < 0.001$), which corroborates the mapping result of NMDS axis 1 scores (Fig. 3C). When geochemical variables (major elements, trace elements, and rare earth elements) were fitted onto the ordination space, various elements were significantly correlated to bacterial community composition, and the direction of most fitted vectors was parallel to the NMDS axis 2 (Fig. 3A). For example, Ce ($R^2 = 0.30$, $P < 0.001$), Ba ($R^2 = 0.22$, $P = 0.003$), and Be ($R^2 = 0.22$, $P = 0.003$) were positively correlated with NMDS axis 2, whereas MnO ($R^2 = 0.43$, $P < 0.001$), Co ($R^2 = 0.40$, $P < 0.001$), MgO ($R^2 = 0.30$, $P < 0.001$), Cr ($R^2 = 0.27$, $P = 0.002$), and Fe_2O_3 ($R^2 = 0.27$, $P = 0.002$) were negatively correlated with the equivalent axis. When glacial striae were overlaid onto the map, the mapping result of NMDS axis 2 scores was overall spatially structured following the deglaciation trajectory (Fig. 3D). We further tested a possible relationship between a bacterial life history trait and community compositional changes along the NMDS axis 2. The average *rrn* copy number of bacterial communities decreased with increasing NMDS axis 2 scores (Pearson's $r = -0.52$, $P < 0.001$) (Fig. S3). There was no significant difference in bacterial community structure between Barton and Weaver Peninsulas (PERMANOVA and ANOSIM, all $P > 0.05$) and between upper and lower soil layers (PERMANOVA and ANOSIM, all $P > 0.05$).

3.3. Spatial variation in bacterial community composition

The best set of spatial variables (11 MEM variables) was created through the MEM framework and all significant MEMs had positive Moran's *I* statistic values (Fig. S2). We partitioned the variation of the bacterial communities with respect to three explanatory components each (edaphic, geochemical, and spatial variables) and their combined effects. Variation partitioning analysis revealed that 31.3% of the total community variation was explained by the whole set of edaphic ($R^2_{adj} = 0.164$, $P < 0.001$), geochemical ($R^2_{adj} = 0.191$, $P < 0.001$), and spatial variables ($R^2_{adj} = 0.159$, $P < 0.001$) (Fig. 3B). Geochemical variables explained the most, to a lesser extent but edaphic and spatial variables also significantly contributed to the total community variation. The unique contribution of each component to the total variation accounted for almost one third of each component, which indicates that the majority of component variations are jointly explained by combined effects. Almost half of the edaphic ($R^2_{adj} = 0.078$) and geochemical ($R^2_{adj} = 0.084$) components were correlated with the spatial component. Almost one-third of the variation explained by each component was jointly explained by three components together ($R^2_{adj} = 0.055$). This collectively suggests that a considerable proportion of both edaphic and geochemical variables are spatially structured. Although various sets of environmental variables were included in the community model, a large proportion of community variation remained unexplained (residuals accounted for 68.7% of the total variation).

We further used the MEM framework to assess spatial scales at which bacterial community variation is modeled by environmental factors, and to determine whether the remaining spatial structure, which is not explained by measured variables, is significant. If any such remaining spatial pattern exists, we further assessed the spatial scales at which it was spatially structured. Both Y (the initial OTU abundance table) and F (the OTU table fitted by environmental variables) showed similar shapes of spatial distribution, both of which are mainly structured by local-scale spatial components (i.e. smoothed MEM 1) ($R^2_{max} = 0.47$, $P < 0.001$ and $R^2_{max} = 0.50$, $P < 0.001$ for axis 1 and 2 of the table Y; $R^2_{max} = 0.57$, $P < 0.001$ and $R^2_{max} = 0.48$, $P < 0.001$ for axis 1 and 2 of table F) (Fig. 4). The first axis of the residual table (R, residuals which are not explained by measured environmental variables) was explained by middle-scale spatial components (i.e. smoothed MEM 4) ($R^2_{max} = 0.13$, $P = 0.025$) and the second axis was mainly explained by local-scale spatial pattern ($R^2_{max} = 0.13$, $P = 0.016$).

4. Discussion

4.1. Bacterial community profile

Predominance of *Actinobacteria, Proteobacteria*, *Acidobacteria* and *Chloroflexi* in this region is in line with results from a couple of former studies performed in other places across the Antarctic Peninsula (Yergeau et al., 2007, 2009), including Fildes Peninsula (Wang et al., 2015) and Keller Peninsula (Roesch et al., 2012). The relative abundance of *Bacteroidetes* and *Firmicutes* was relatively low across samples in this region as compared to previous reports. For example, members of *Bacteroidetes* were predominant in moss-covered soils on Livingston Island (Ganzert et al., 2011), and *Firmicutes* was one of the dominant phyla in rhizosphere soils of flowering plants on Admiralty Bay (Teixeira et al., 2010). This discrepancy may be ascribed to the difference in sampling area in that soil samples in this study were taken mainly from unvegetated sites which were not disturbed by other biotic materials (e.g. rhizosphere, ornithogenic, or seal-covered sites). It is also consistent with our previous finding showing the predominance of *Bacteroidetes* and *Firmicutes* in the ornithogenic soils of neighboring sites (Kim et al., 2012).

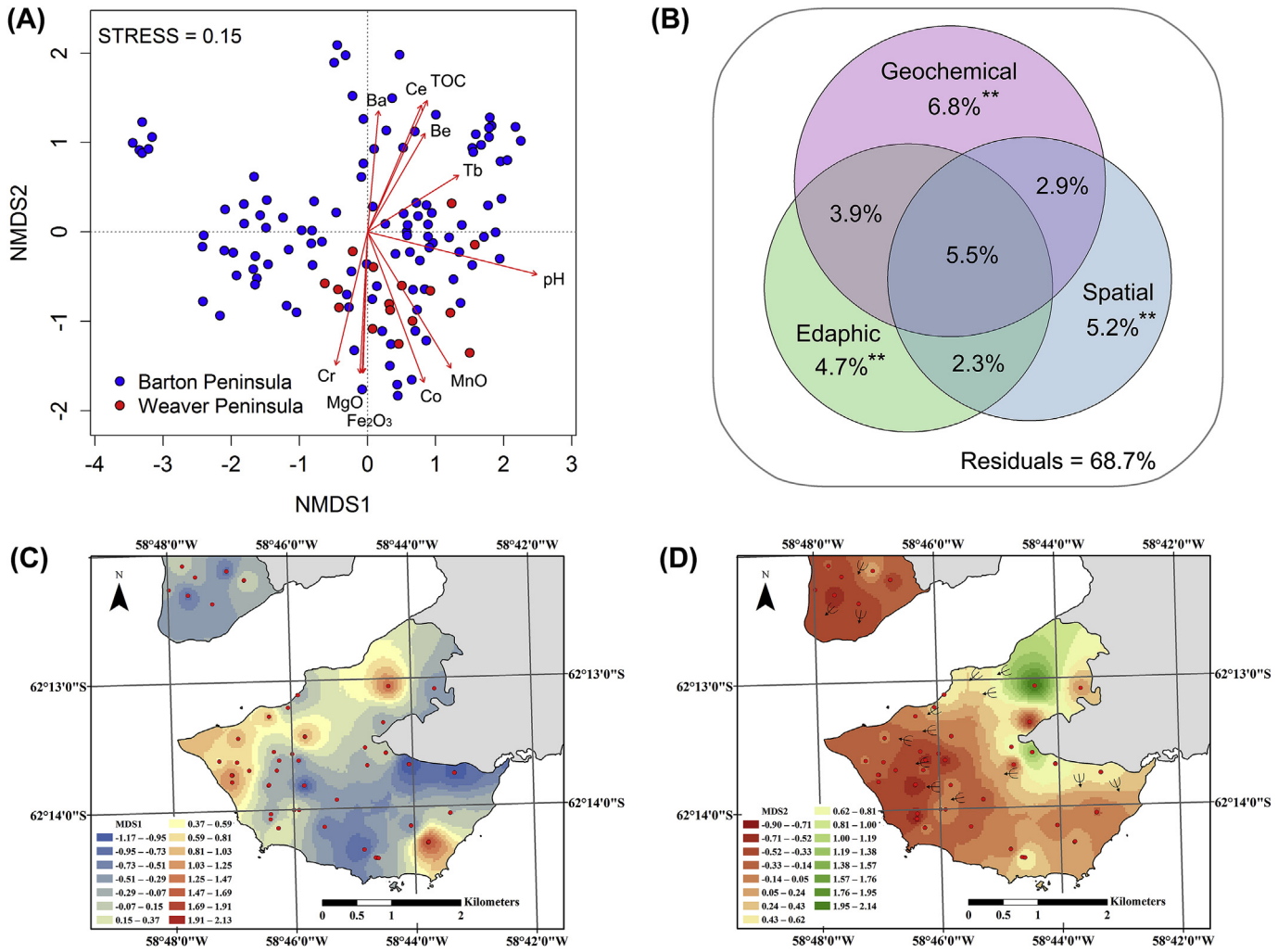

Fig. 3. (A) NMDS plot representing the relative similarities of bacterial community composition among samples. Statistically significant vectors of environmental variables (envfit $R^2 > 0.20$, $P < 0.01$) were overlaid onto the ordination space. **(B) Venn diagram representing variation partitioning results of bacterial community data with respect to edaphic, geochemical, and spatial variables**. Adjusted R^2 values were used to explain each fraction of variation, and empty fractions have small negative adjusted R^2 values. All testable fractions (excluding joint fractions) were significant when tested by Monte Carlo 999 permutations (denoted as **, $P < 0.01$ in all cases). **Maps of bacterial community similarities based on (C) NMDS axis 1 and (D) NMDS axis 2 scores.** Glacial striations with Roche moutonnee are displayed in (D) following the geomorphic map of Seong et al. (2009).

Interestingly, we observed a high abundance of two unknown bacterial lineages, candidate divisions AD3 and WPS-2, in this region. They are known to be globally distributed mainly in soils of cold regions but often rare at local sites (Costello, 2007). In general, AD3 dominates deeper soil layers with low carbon contents, while WPS-2 increases in relative abundance towards the soil surface (Costello, 2007). Recently, a high abundance of AD3 (5.1% on average, with a maximum of 17%) and WPS-2 (9.3% on average, with a maximum of 25%) was reported on surface soils of Mitchell Peninsula, East Antarctica (Ji et al., 2016). Despite their global occurrences, almost nothing is known about the physiology and ecological roles of AD3 and WPS-2 in nature because there are no cultured representatives and no genome sequences available. It has been reported that their dominance in relative abundance shifts towards the surface layer (0–10 cm soil depth) when soils are covered by snow in an alpine tundra, USA (Costello, 2007), which is in accordance with the surface soil dominance in two ice-free regions (Barton and Mitchell Peninsula) of the Antarctic.

We additionally found that their existence may be associated with soil pH. They occurred at a wide range of abundances in acidic soils but are almost absent or present at lower abundance above pH 7 (Fig. S1), suggesting that they can withstand or thrive under acidic conditions in cold regions. Their preference for acidic environments is also commonly observed in the Arctic. For instance, they are one of the dominant lineages in the acidic environment of the glacier foreland of Styggedalsbreen (Norway) (Mateos-Rivera et al., 2016), whereas they are almost absent in alkaline environments such as the glacier forelands of Austre Lovénbreen (Svalbard) (Kim et al., 2017) and Duke River Glacier (Canada) (Kazemi et al., 2016). Further study is needed to determine why these lineages are abundant in acidic soils across a wide range of alpine and polar regions and why they favor the soil-to-snowpack interface.

4.2. Environmental factors determining bacterial community composition

We found that soil pH is the single dominant factor in shaping bacterial community structure in this region. Soil pH has been suggested as one of the best predictors of bacterial community composition across the globe, including Antarctica (Chong et al., 2015; Lauber et al., 2009; Smith et al., 2010; Van Horn et al., 2013). It has been reported that a large area of Barton Peninsula is covered by acid sulfate soils (pH 2.8–6.0) depleted in bases, and soils adjacent to andesitic materials have close to neutral pH values (pH 6.4–8.1) (Bockheim, 2015). For example, exposed terrain on the southwestern coast of Barton Peninsula is dominated by brownish sulfidic soils, which corroborates the spatial

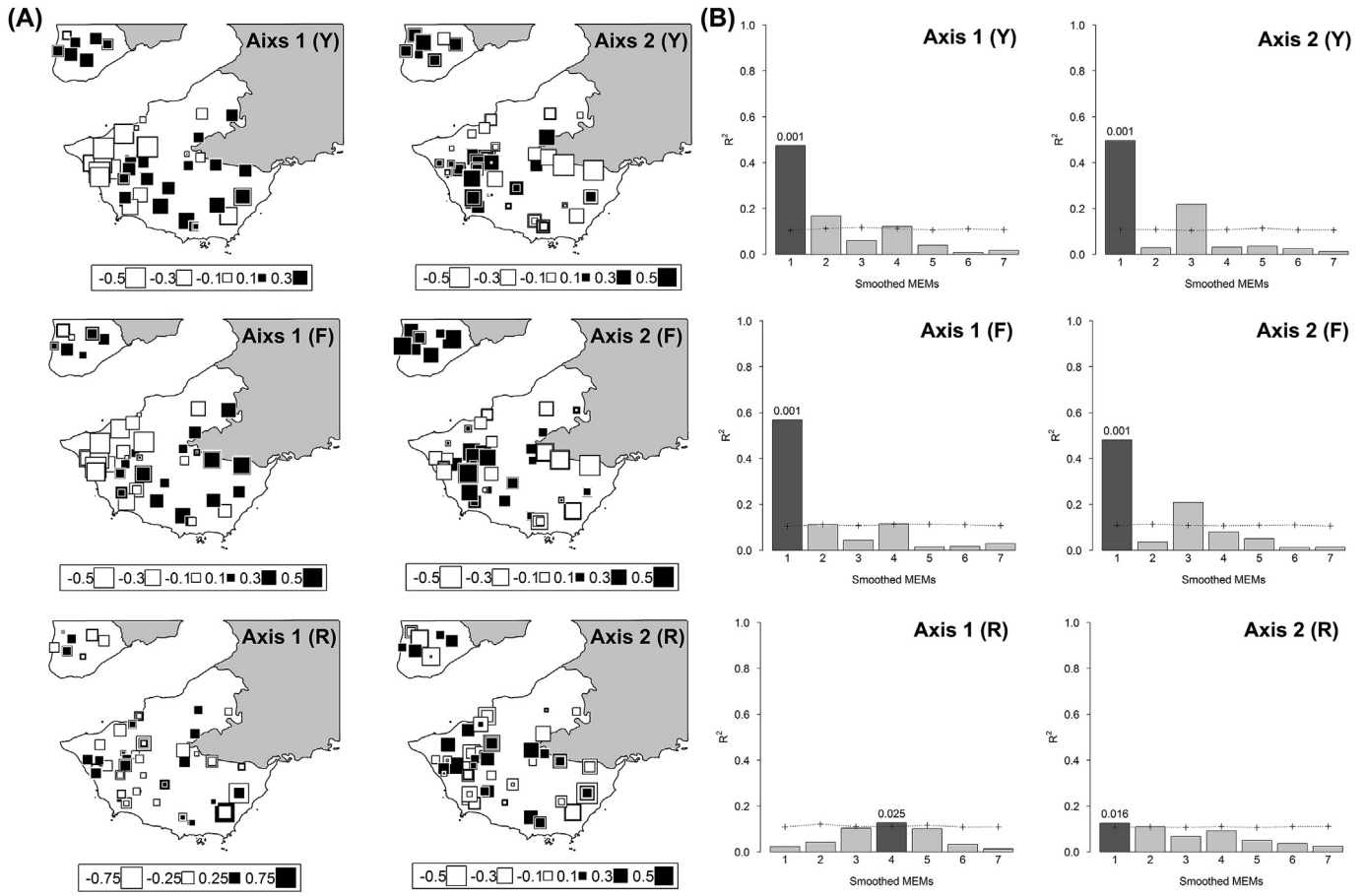

Fig. 4. (A) Maps of the site scores of first and second axes and (B) the corresponding smoothed scalograms for the MEMs. Eleven MEMs were assembled into seven spatial groups. Each R^2 value on the y-axis represents the proportion of variance explained by each spatial scale. The 95% confidence limit is shown by the dashed lines. The significance of the scale with highest R^2 (bars with dark gray) was tested using 999 permutations of the observed values.

distribution of bacterial communities. Interestingly, despite the wide range of soil pH (pH 3.2–8.9) in this region, there was no relationship between bacterial richness and soil pH, which is contradictory to previous results observed in other biomes (Lauber et al., 2009; Chu et al., 2010; Tripathi et al., 2012). Another unusual pattern found in this region is the lack of correlation between pH and the relative abundance of *Acidobacteria*, which generally increase towards acidic soils in other biomes (Jones et al., 2009). These unusual patterns might be unique to terrestrial ecosystems of low-latitude Antarctic coastal margins.

Strong associations between earth elements and bacterial community structure have been reported in several regions of Antarctica. For example, copper (Cu) and/or lead (Pb) contents have been suggested as major determinants of soil bacterial community structure (Chong et al., 2012; Kim et al., 2015; Lee et al., 2012). However, inferring a possible relationship between each individual element and bacterial assemblages is difficult due to the scarcity of information on the physiology of metal-tolerant or -sensitive soil microbes under natural environments. A possible scenario is that shifts in bacterial community structure may be associated with elemental composition changes in exposed terrain driven by deglaciation or disappearance of snow cover. Two sets of elements lay on the ordination space in opposite directions along the NMDS axis 2, and the spatial distribution of mapped NMDS axis 2 scores paralleled glacial striae remaining on outcrops.

The decreasing pattern of *rrn* copy numbers of bacterial communities along the NMDS axis 2 may be another supporting evidence of bacterial community succession on newly exposed terrain. The *rrn* copy number is associated with many physiological processes such as growth rate, sporulation, and stress response, and is thus regarded as a key functional trait in microbial life history strategies (Klappenbach et al., 2000; Yano et al., 2013). Microbes with higher *rrn* copy numbers are known to respond more rapidly to resource availability (Shrestha et al., 2007). It was recently suggested that the average *rrn* copy numbers are relatively higher in early successional stages than in the later stages, and that they monotonically decrease during ecosystem succession (Nemergut et al., 2016). The decrease in average *rrn* copy numbers during succession was also supported by a finding in the glacier foreland of the High Arctic (Kim et al., 2017). However, this 'trapped in time' hypothesis should be tested in the future, together with surface exposure ages of the sampled area.

4.3. Spatial variation in bacterial community composition

The variation partitioning analysis revealed that only 31.3% of total community variation was explained by measured environmental variables, which are commonly used to identify environmental drivers of microbial community structure in other biomes. A possible reason for this low proportion of explained variation may be that aboveground vegetation was not accounted for in this model. Even if it is expected that biotic interactions are minimized under harsh environments of Antarctica (Hogg et al., 2006), interactions between cryptogam communities and soil bacterial assemblages may have non-negligible contributions to the total community variation, considering the extensive ground cover of lichen and moss communities in the maritime Antarctic. Interactions among bacterial species could also play a role in structuring bacterial communities given that antibiotic resistance genes and phage signals were detected in the Antarctic soil metagenomes (Wei et al., 2015). Furthermore, unmeasured climatic or temporal variables such as microclimatic factors, terrain exposure age, and snow

cover duration could also increase the proportion of variance explained by the model.

The proportion explained by environmental variables is not substantial, but soil bacterial community structure in this region is to a lesser degree shaped by environmental gradients (edaphic and geochemical variables), which are spatially structured at the local scale (0.5–8 km), and the micro-scale spatial component (< 0.5 km) plays a negligible role in structuring the soil bacterial community. Environmental variables accounted for more than 80% of the total explained variation in the model. This large explained proportion of environmental effects suggests that contemporary selection is the major driver of bacterial biogeographic patterns in this area, which is consistent with the recent comprehensive comparison results (Hanson et al., 2012). A minor role of historical processes may be ascribed to the small study area, because historical processes (including dispersal limitation) are often more evident at larger geographic scales (Hanson et al., 2012). A nonsignificant micro-scale component was also observed in a biogeographical study of soil bacterial communities across the Transantarctic Mountains, Antarctica (Sokol et al., 2013). The authors found evidence of cyanobacterial biogeography at the local scale (< 3 km), but there was no detectable biogeographical pattern for whole soil bacterial assemblages at the equivalent spatial scale.

5. Conclusions

Our study demonstrated that a large proportion of bacterial community variation in maritime Antarctica is unexplained by measured environmental variables, although comprehensive sets of variables were fitted into the community model. This indicates how little we know about environmental drivers of bacterial community structure in Antarctic soils. A certain but not substantial proportion of bacterial community variation was explained by environmental variables (i.e., soil pH and TOC), most of which are spatially structured at the local scale (0.5–8 km). We further found that not only edaphic components but also geochemical properties play important roles in structuring bacterial community composition. Candidate divisions AD3 and WPS-2 were overrepresented in this region, and bacterial richness was not correlated with soil pH, which is unique to this region. Ice-free habitats are expanding in the Antarctic Peninsula due to recent climate change. Examination of microbial communities and their relation to environmental factors will help to better predict terrestrial ecosystem changes in response to future climate change in Antarctica.

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Appendix A. Supplementary data

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