FEMS Microbiology Ecology Advance Access published December 22, 2016

Title: Aspect has a greater impact on alpine soil bacterial community structure than elevation

Running title: Soil bacterial community structure across a mountain microclimate gradient

[1]Jieyun Wu, [2]Barbara J. Anderson, [3]Hannah L. Buckley, [1]Gillian Lewis, [1,*]Gavin Lear

[1]School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland 1010, New Zealand.
[2]Biodiversity and Conservation Group, Landcare Research Ltd., Private Bag 1930, Dunedin 9054, New Zealand
[3]Department of Ecology, Lincoln University, PO Box 85084, Lincoln 7647, New Zealand.

Keywords: 16S rRNA, next-generation sequencing, micro-climate, soil microbiology, pH gradient

*Correspondence: Dr. Gavin Lear, School of Biological Sciences, The University of Auckland, 3a Symonds Street, Auckland 1010, New Zealand.
E-mail: g.lear@auckland.ac.nz
Tel: +64-9-3739799

Conflict of interest: The authors declare no conflict of interest

ABSTRACT

Gradients in environmental conditions, including climate factors and resource availability, occur along mountain inclines, providing a 'natural laboratory' to explore their combined impacts on microbial distributions. Conflicting spatial patterns observed across elevation gradients in soil bacterial community structure suggest that they are driven by various interacting factors at different spatial scales. Here, we investigated the relative impacts of non-resource (e.g., soil temperature, pH) and resource conditions (e.g., soil carbon and nitrogen) on the biogeography of soil bacterial communities across broad (i.e., along a 1,500 m mountain elevation gradient) and fine sampling scales (i.e., along sunny and shady aspects of a mountain ridge). Our analysis of 16S rRNA gene data confirmed that when sampling across distances of < 1,000 m, bacterial community composition was more closely related to the aspect of a site than its elevation. However, despite large differences in climate and resource-availability factors across elevation- and aspect-related gradients, bacterial community composition and richness were most strongly correlated with soil pH. These findings highlight the need to incorporate knowledge of multiple factors, including site aspect and soil pH for the appropriate use of elevation gradients as a proxy to explore the impacts of climate change on microbial community composition.

INTRODUCTION

Variation in climatic factors, such as temperature and precipitation, impacts the composition and diversity of a wide variety of natural biological communities, often in predictable ways (Bertrand *et al*. 2011; Knapp *et al*. 2002). Climatic gradients in conditions that occur along mountain inclines are commonly used to explore the impact of climate on community attributes, since substantial variation can occur across short geographic distances. Temperature declines by approximately 0.6 ^{o}C for each 100 m increase in elevation (McCain

and Grytnes 2010); precipitation and related ecological variables, such as soil moisture, can also vary considerably over similar spatial scales (Brown *et al*. 2012). To date, biogeographic studies along elevation gradients have concentrated almost exclusively on the community response of macroorganisms, in part because of the relative ease that larger taxa can be visually identified. These studies frequently report significant elevational gradients in community composition and richness that are suggested to be directly driven by climatic variables and particularly by the availability of thermodynamic energy, water, or combined energy-water balance (Allen *et al*. 2002; Stephenson 1990). Alternative explanations for observed diversity patterns also include the impact of deceasing land area proportional to elevation increases (Rahbek 1997) and decreased range sizes of taxa at lower elevation, according to Rapoport's Rule (Stevens 1992). Although microorganisms may be considered to be the 'unseen majority' in soil ecosystems, where they dominate numerically and comprise a large portion of the genetic diversity, the relationship between microbial communities and fine-scale variability in abiotic conditions remains poorly resolved (Grundmann 2004; Morris 1999; Vos *et al*. 2013). A better understanding of the impacts of abiotic conditions on microbial communities is necessary since they provide vital ecosystem services, including soil formation, carbon and nutrient cycling and acquisition, and are a major determinant of plant productivity and diversity (van der Heijden *et al*. 2008). Here, we explore the relationship between abiotic micro-habitat conditions and the biogeography of soil bacterial communities across a single alpine elevation gradient. Improved knowledge of micro-habitat variability in soil bacterial community composition and diversity across elevation gradients will aid in developing and testing hypotheses regarding the response of these vital communities to global climate change.

If the factors driving the biogeographic distribution of microbial taxa along mountain elevation gradients are fundamentally similar to those affecting communities of macroorganisms, then equivalent patterns should be observed, such as declines in species richness at increased elevation. However, the inconsistent nature of the patterns so far observed across mountain elevation gradients for microbial taxa, imply that related variation in bacterial communities can be complex and follow no single rule; authors describe decreases in diversity/richness (Bryant *et al*. 2008), no trend (Fierer *et al*. 2010), or unimodal patterns (Singh *et al*. 2012) in bacterial community composition with increased elevation. Similarly, if elevational gradients in community diversity and richness are universal, then we would expect the same environmental drivers (i.e., temperature and availability of water) to be key predictors of these fundamental bacterial community attributes.

Temperature and precipitation significantly affect bacterial community structure and processes, including respiration and enzyme activity (Fierer *et al*. 2003; Rinnan *et al*. 2009; Zeglin *et al*. 2013). Therefore, where patterns in soil bacterial community attributes are not tightly related to gradients in climatic conditions across elevation gradients, we expect that alternative factors such as variation in soil type and management are likely to be the key drivers of community assembly (Fierer and Jackson 2006). However, the relative impact of edaphic factors on alpine bacterial communities remains poorly understood, particularly within single mountain elevation gradients where we would expect the uniform geology and land use attributes to allow variation in natural climatic and environmental factors, rather than anthropogenic factors, to be a stronger determinant of microbial community composition (Singh *et al*. 2014). It is important to note that even where land management and edaphic variables are controlled or otherwise accounted for, additional site attributes can contribute to observed environmental patterns in bacterial community composition and diversity. For

example, climatic variation across mountain environments is related not only to site elevation, but also to aspect, slope and shading (Titshall *et al*. 2000), which can vary considerably over distances of only a few metres. Many studies have noted the effect of slope and aspect in determining soil temperature (Davies *et al*. 2006; Thomas *et al*. 2001; Weiss and Weiss 1998), evaporative demand (Bennie *et al*. 2008), soil moisture (Carter and Ciolkosz 1991; Schaetzl and Anderson 2005), soil chemistry (Hunckler and Schaetzl 1997; Miller *et al*. 2004; Thompson and Kolka 2005) and nutrient cycling (Gilliam *et al*. 2015; Sariyildiz *et al*. 2005; Sidari *et al*. 2008) principally via modifying the amount of solar radiation received. Although the relationships between fine-scale variation in site slope and aspect have been poorly investigated for microbial communities, Sidari *et al*. (2008) observed significant correlations between soil microbiological activity and aspect-induced microclimatic differences in the content and composition of soil organic matter. For these reasons, we chose to quantify variation in soil bacterial community attributes along a *c*.1500 m mountain incline encompassing a broad microhabitat gradient, as well as examining local-scale variation related to site aspect.

The availability of growth-limiting resources, especially nitrogen and carbon (Mason *et al*. 2014; Zinger *et al*. 2009) impact the abundance, diversity and composition of many communities, including plants (Edgar 2013; Kalra and Maynard 1991; Rahbek 2004), bacteria (Calleja-Cervantes *et al*. 2015; Zhang *et al*. 2015) and fungi (Lauber *et al*. 2008). For example, Shen *et al*. (2015) observed a significant relationship between soil carbon, nitrogen, and variation in bacterial community composition across a climatic gradient in alpine tundra. Variation in carbon and nitrogen concentrations and fluxes are frequently correlated with variability in temperature and precipitation (Altschul *et al*. 1990; Huber *et al*. 2007; Knapp *et al*. 2002; Weltzin *et al*. 2003) and related to both increasing microbial metabolism and

decreasing energy use efficiency in warmer conditions, providing water availability is not limiting. Hence, while climatic factors such as temperature are predicted to be strong independent determinants of bacterial community composition and metabolism (Fierer *et al*. 2003; Kessler 2000), resource availability is expected to have additive and interacting impacts on bacterial communities across broad altitudinal ranges. For instance, Wang *et al*. (2011) observed decreasing bacterial community richness with increasing elevation, which they correlated with an increase in carbon availability at higher altitudes. Here, we quantify how fine- and coarse-scale gradients in resource and non-resource factors (e.g., temperature, soil pH) are related to soil bacterial community composition and richness across a range of sample site elevations and aspects across a single mountain ridge.

We sought to test three hypotheses. First, we hypothesised that variation in bacterial community composition would correlate with elevation and that bacterial taxonomic richness would decline with increased elevation (Elevation H_1), as is frequently reported for macro-organisms (Aubry *et al*. 2005; Carneiro *et al*. 2013; Rahbek 2004). Second, since environmental conditions, particularly temperature, are strongly impacted by aspect, we hypothesised significant differences in bacterial community composition, and a decline in bacterial taxonomic richness, on shadier compared to sunnier aspects of the mountain ridge (Aspect H_2). Finally, we hypothesised (Environment H_3) that non-resource environmental conditions, such as soil temperature and moisture, would be a more important determinant of bacterial community attributes than environmental resource factors, such as concentrations of soil C, N and P. Evidence of a significant role for non-resource, and particularly climatic factors, in determining bacterial community composition and richness would imply that the biogeography of bacterial communities is fundamentally similar to that of macroorganisms.

MATERIALS AND METHODS

We conducted a survey of soil bacterial 16S rRNA gene data collected across a continuous ridge leading to the peak of Mount Cardrona, New Zealand. We compared communities across both sunny and shady sides of the mountain ridge and ridgeline, at 100 m elevation intervals across an elevation gradient of almost 1,500 m to test the influence of aspect relative to elevation on bacterial community richness and composition (i.e., Elevation H_1 & Aspect H_2). Our final hypothesis (Environment H_3) was tested in combination with the collection of both soil resource (e.g., concentrations of soil C, N, P, and the average biomass of aboveground plants) and non-resource environmental data (e.g., soil pH, temperature and moisture).

Site Description and Sample Collection

To assess the impact of microclimate variability on soil bacterial community attributes, we collected a total of 405 soil samples from a continuous ridge on the north-eastern side of Mt. Cardrona, New Zealand (44.85° S, 168.95° E; Figure 1). We identified 15 elevation bands (Ebands) located at 100 m elevation intervals from 500 m to 1900 m along the ridge. Since the slope of the ridge at 1300 m is very shallow, this Eband was separated into two (i.e. one at the upper edge of the ridge called E1301 and another at the lower edge called E1300). Hence, samples were collected from a total of 16 Eband locations.

In addition to elevation, microclimate conditions are impacted by variability in site aspect. For this reason, each Eband encompassed five clusters of soil samples. One of the five clusters (R0) at each Eband was located on the centre of the leading ridge line, two (SU1, SU2) to the north (warmer/sunnier side), and two (SH1, SH2) to the south (cooler/shadier side). All of these clusters were geographically equidistant (i.e., collected at 25 m intervals).

A single sample cluster was collected at the summit of the mountain at 1936 m. Within each sampling cluster, five individual soil samples were collected across a 1.5 m transect using a soil corer to 10 cm depth allowing us to also quantify the fine-scale variability in bacterial community composition and relative richness at each sample location. Hence, there are 405 samples in total (16 Ebands x 5 sample clusters x 5 site samples + 5 summit samples = 405). All soil samples were contained in labelled ziplock bags to avoid contamination, and stored at -20 ^{o}C within 24 h of collection.

Environmental conditions

We used ibutton temperature loggers (Maxim Profile, USA) to collect real-time temperature data (below ground temperature at 10 cm depth) from each sampling cluster (n=81) at Mt. Cardrona in the summer (from February to March in 2014). Soil moisture was measured gravimetrically (i.e., soil samples were weighed before and after being placed in an oven at 105^{o}C for 96 hours.) (Rayment and Lyons 2011). Soil pH was measured by the Landcare Research Environmental Chemistry Laboratory using method 106i. Briefly, a 1:2.5 suspension of soil:water was stirred vigorously then left to stand overnight before measurement with a pH electrode. Full descriptions of each method are available from http://www.landcareresearch.co.nz/resources/laboratories/environmental-chemistry-laboratory.

Resource availability

Soil physiochemical properties from each sample were also analysed by Landcare Research Environmental Chemistry Laboratory using standard procedures to determine concentrations of organic C and total N (method 114), and Olsen P (method 124). Ammonium and nitrate were extracted using a modified version of method 118. Briefly, 2M KCl was used in a 1:10

soil: extractant ratio and the resulting slurry turned end-over-end for one hour, followed by filtration of the sample prior to analysis. Aboveground plant biomass from each sampling location, was also removed from a 20 × 20 cm grid centred on the soil core. Plant matter was dried at 60^{o}C and weighed (Rayment and Lyons 2011).

DNA Sequence Analyses

DNA was extracted from individual samples using MOBIO PowerSoil-htp 96 well Soil DNA Isolation Kits (MOBIO Laboratories Inc., USA) according to the manufacturer's protocol, but using a TissueLyser II disruption system (QIAGEN, Germany) to agitate sample solutions (30 revolutions per second, 2 minutes, twice).

To characterise bacterial community composition, the DNA from each soil sample was amplified and sequenced on an Illumina MiSeq sequencer (Illumina Inc., USA) following a standard protocol (Illumina 2013). Briefly, DNA fragments were amplified using modifications of the primers 341F (5'-**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CCTACGGGNGGCWGCAG-3') and 785R (5'-**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GACTACHVGGGTATCTAATCC-3'). These primers target the V3 and V4 region of bacterial 16S rRNA genes to provide a good combination of domain and phylum coverage (Klindworth *et al*. 2013) but are modified to also include Illumina adapter overhang nucleotide sequences (bold) required for downstream DNA sequencing. DNA was amplified using the standard protocol, which follows the thermocycling procedure: (1) 95 ^{o}C for 3 min; (2) 25 cycles of 98 ^{o}C for 30 s, 55 ^{o}C for 30 s, 72 ^{o}C for 30 S; (3) 72 ^{o}C for 5 min. To ensure the accuracy of our sequencing approach to identify the correct taxa within samples, we also amplified the DNA of a 20-

species ‘Microbial Mock Community’ (Community ‘B’ from BEI Resources, catalogue No. HM-782D, USA). Following amplification, PCR products were individually purified using AMPure XP reagents (Beckman Coulter, USA) and a Biomek 4000 liquid handling workstation (Beckman Coulter Inc., USA), according to the manufacturer’s instructions. The concentration of purified amplicons was finally measured and recorded using a Qubit® dsDNA HS Assay Kit (Life technologies, USA) before submission to New Zealand Genomics Ltd. for sequencing. Briefly, the procedure followed by the sequencing facility, prior to DNA sequencing, was to attach a combination of Nextera XT A and B barcode dual indices (Illumina Inc., USA) to the DNA from each sample. This approach ensured that the DNA from each sample could be identified by its unique DNA barcode. After the DNA from up to 192 samples was pooled for DNA sequence analysis, sequence data were demultiplexed (i.e. assigned to the samples from which they originated) by the DNA sequencing facility. Our sequence analysis of the mock community DNA data (not shown) detected only DNA from the 20 species in the original mock community, suggesting that the barcoding and sequencing results of the Illumina Miseq platform were reliable. All of raw sequences have been uploaded onto the SRA-NCBI database (BioProject ID: PRJNA338717).

Bioinformatic Analyses

Paired end read DNA sequence data were merged and quality filtered using the USEARCH sequence analysis tool (Edgar 2013). After quality filtering, dereplication was performed so that only one copy of each sequence is reported; ‘singleton’ sequences represented by only one DNA sequence in the database were removed. Sequence data were then checked for chimera sequences and clustered into groups of operational taxonomic units based on a sequence identity threshold equal to or greater than 97% (hereafter referred to as 97% OTUs) using the clustering pipeline UPARSE (Edgar 2013) as described in Ramirez *et al*. (2014).

Next, prokaryote phylotypes were classified to corresponding taxonomy by implementing the RDP classifier routine (Wang *et al*. 2007) in QIIME v. 1.6.0 (Caporaso *et al*. 2010) to interrogate the Greengenes13_8 database (McDonald *et al*. 2012); all sequences of chloroplast and mitochondrial DNA were removed. Finally, the DNA sequence data were rarefied to 5,500 randomly selected reads per sample and three samples per site.

To confirm the similarity of key bacterial taxa identified in the present study to those found at other study sites, we used the Nucleotide database from NCBI to search for closely related DNA sequences for inclusion in our phylogenetic analysis, which was completed using Geneious software (version 7.1.6; Biomatters Ltd, New Zealand). Briefly, we aligned all sequence data using the Geneious alignment tool, estimated evolutionary distances with a Jukes-Cantor Distance Model and constructed unrooted consensus phylogenetic trees from these distances using neighbor-joining. Bootstrap confidence levels were estimated from 10,000 replicated alignments of the data.

Quantitative Data Analyses

To assess the variation in bacterial community richness and composition, our analyses used either bacterial taxon richness (the relative number of different 97% OTUs at each site) or mean compositional similarity (calculated by constructing a Bray-Curtis distance matrix from the relative bacterial abundance data). Spatial patterns in bacterial taxon richness and composition were plotted using the heatmap function within the R package 'gplots'. Multivariate sample data were related to explanatory matrices of spatial and environmental data using distance-based redundancy analysis (db-RDA) and a forward selection procedure with the 'capscale' and 'ordistep' functions in the 'vegan' package in R (Oksanen *et al*. 2015). Variance partitioning procedures using db-RDA outlined in Borcard *et al*. (2011) were

performed to indicate how much total variation in the bacterial community data be explained by groups of either (i) soil 'resource' variables, for example, concentrations of soil C, N and P, and above-ground plant biomass, which can both remove and input nutrients into the soil (ii) 'non-resource environmental' variables, that is soil temperature, moisture content, and pH, (iii) a combination of resource and non-resource environmental variables, and (iv) unexplained variance. The variance partitioning procedure computes canonical R^2 values analogous to the adjusted R^2 values produced in multiple regression (Peres-Neto *et al*. 2006). The components of variation associated with bacterial community variability at sample elevations and aspects were quantified and visualised using distance-decay curves within the R package 'vegan' (Oksanen *et al*. 2015). We plotted multivariate regression trees using the 'randomForest' package in R (Liaw and Wiener, 2002), to identify which individual resource and non-resource environmental soil attributes correlate most closely with the observed variation in bacterial community composition and richness across the study site. Finally, a correlation 'heatmap' was used to visualise the strength of correlation between each environmental factor using the R package 'corrplot'.

Environmental variables were analysed individually using univariate analysis of variance (ANOVA), with *P*-values obtained by permutation. For these data, there were only two relevant factors: Aspect and elevation. Where appropriate, we also used paired or unpaired *t*-tests to detect differences in environmental variables between groups of samples collected at different elevations and aspects. The pairwise Bray-Curtis distance matrix calculated from the bacterial community data was analysed using permutational multivariate analysis of variance (PERMANOVA; Anderson 2001; McArdle and Anderson 2001). Significant differences in bacterial community composition identified by the PERMANOVA procedure may be caused by average differences in compositional similarity among groups, or alternatively, by

differences in the within-group multivariate dispersion among groups. To quantify variation in bacterial community data within groups of study sites (e.g., to compare average community similarity of sample data collected within each elevation band), we used multivariate dispersion (MVDISP) index values, which calculate average Bray-Curtis distances among sample data. Permutational analysis of multivariate data dispersion (PERMDISP) routines were then used to confirm if within-group differences in multivariate dispersion varied significantly across elevation and aspect. MVDISP, PERMANOVA and PERMDISP routines were performed in PERMANOVA+, an add-on of the PRIMER6 package (Plymouth Marine Laboratory, UK). The PERMANOVA routine (i.e. permutational ANOVA) was similarly used for the statistical analysis of univariate data (e.g., taxonomic richness or soil carbon concentrations). We repeated our analyses for subsets of the bacterial community data to study variation in the relative abundance of the ten most abundant bacterial families, since dominant taxa typically have a larger influence on bacterial community composition.

Samples located on the sunnier aspect of the mountain ridge at an elevation of 900 m were heavily impacted by localised grazing and also by elevated concentrations of soil nitrogen. Thus, not surprisingly, the composition of bacterial communities in these samples differed markedly from that found at other sites across the elevation gradient (Supplementary Figure S1). For this reason, data from these two sample sites were not used in further analyses.

RESULTS

We identified approximately 17,000 distinct bacterial operational taxonomic units (or OTUs of 97% DNA sequence similarity) from 2.2 million rarefied 16S rRNA gene sequences, representing 487 bacterial families across the study site.

Elevation and aspect patterns

Multivariate analysis of the bacterial community data (Figure S1) revealed that across the entire study site, the gradient in elevation had a greater impact on both bacterial community composition and taxon richness than aspect (as assessed by the square root of the component of variation attributable to these factors in the PERMANOVA model; Supplementary Table S1, all $P < 0.001$). All interactions analysed in the model were statistically significant ($P < 0.001$). To further explore the hypotheses that bacterial community composition correlated with gradients in elevation (H1) and aspect (H2) we used spatial heatmaps to visualise the nature and extent of variability in community composition and richness (Figure 2). Bacterial community composition changed markedly across the elevation gradient (Figure 3; $y = 0.0003x + 0.30$, $R^2 = 0.99$). Bacterial community composition changed less in response to differences in aspect than to differences in elevation across the study site. The average difference in bacterial community composition comparing samples collected from sunnier (SU2) versus shadier aspects of the mountain ridge (SH2) that were separated by a fixed distance of 100 m, was 0.56 Bray-Curtis units. The same average difference in bacterial community composition (i.e., ~0.55 Bray-Curtis units) was only observed when comparing samples differing in elevation by 900 m or greater along the mountain ridge (Figure 1c). This difference in elevation corresponded to a flat surface distance of 4 km. This suggests that, comparing sample sites separated by fixed distances across the study site (e.g., ~100 m), variation in bacterial community composition changed more rapidly with variation in site aspect than site elevation. No clear pattern in bacterial community richness was observable across the whole elevation gradient. However, at higher elevations (i.e., above approximately 900 m), bacterial taxon richness was greater on the sunnier side of the mountain (i.e., 1263,

97% OTUs were detected per sample, on average), compared to on shadier aspects of the mountain ridge (mean 97% OTU richness = 1174; *t*-test *p*-value = 0.005).

We wished to confirm whether observed differences in bacterial community composition were caused by true variation in average bacterial community composition (i.e., the location of bacterial community data in multivariate space), or differences in community dispersion (i.e., differences in community variation comparing samples within the same site). Average MVDISP index values revealed an increase in the compositional variability of bacterial communities across sample aspects with increasing elevation (Figure 4; Regression *p*-value = 0.042). Variability in bacterial community composition among sample clusters was lowest for samples collected at 700 m (MVDISP = 0.095) and highest at an elevation of 1700 m (MVDISP = 1.50) (Figure 4; PERMDISP *p*-value = 0.002). Almost all of the data from higher elevation sites (> 900 m, except 1500 m, MVDISP = 0.53) were more variable (MVDISP > 0.80) than those from lower elevation sites (*t*-test *p*-value = 0.025).

The ten most dominant families represented 53% of the overall bacterial community and thus are expected to be important drivers of the overall changes in bacterial community composition observed across the site. The family Chthoniobacteraceae was most dominant across the study site (representing 13% of all taxa detected), followed by the family Thermogemmatisporaceae (10%) belonging to the phylum Chloroflexi. Sequences related to the Thermogemmatisporaceae were most abundant at higher altitude, representing 14% of DNA sequence reads over 900 m but just 2% of sequence reads below 900 m (*t*-test *p*-value < 0.001; see Figure S2). The family Koribacteraceae (eighth most dominant overall) and Ellin6513 (10^{th} most dominant overall) also were more abundant at high elevation representing 4.5% and 4.5% of DNA sequence reads detected over 900 m, respectively, but

just 1.8% and 0.8% of sequence reads below 900 m (*t*-test *p*-values < 0.001). In contrast, members of the family Gaiellaceae (ninth most dominant) were more abundant at lower elevations; their average abundance was 1.9% and 6.4% at elevations above and below 900 m, respectively; *t*-test *p*-value < 0.001). At high elevation sites (i.e., above 900 m), members of the family Ellin6513 were more abundant on shadier aspects of the mountain (mean abundance = 5.3%) than on the ridge or sunnier side at high elevation (mean abundance = 3.3%; *t*-test *p*-value < 0.001).

The family Thermogemmatisporaceae includes bacteria assumed to be adapted for growth at high temperature (King and King 2014; Yabe *et al*. 2011) and the ability to use substrates such as carbon monoxide as an energy source (King and King 2014). Since our study revealed a greater abundance of presumed thermotolerant Thermogemmatisporaceae at higher, cooler elevations, we explored the similarity of DNA sequences collected in the present study to known thermophile members of this family. Representative Thermogemmatispora OTU sequences were compared to a variety of published gene sequences and their similarity plotted using a phylogenetic tree (Supplementary Figure S3). The OTUs found in the present study belonged to two distinct subdivisions. The majority were affiliated (≥ 97% sequence similarity) with established clades of the Chloroflexi previously detected in alpine environments (Costello and Schmidt 2006; Zinger *et al*. 2009).

Relative importance of resource versus non-resource environmental factors as determinants of bacterial community richness and composition

We used variance partitioning procedures to confirm that the group of explanatory variables categorised as ‘non-resource environmental variables’ (that is, soil pH, temperature, and moisture) independently explained the greatest amount of observed heterogeneity in bacterial

community richness (Figure 5; 21%). 'Resource variables' (organic C, total N, NO_3-N, NH_4-N, Olsen P, and aboveground plant biomass) did not independently explain any variation in bacterial community richness. In contrast, variability in 'non-resource environmental variables' independently explained just 6% of the observed variation in bacterial community composition, assessed from Bray-Curtis measures of community similarity, compared to 11% explained by 'resource variables'; the shared variance explained by both groups of factors accounted for the greatest component of the observed variation (36%).

To identify individual resource and non-resource factors that contribute or relate most to the variation observed in bacterial community richness and composition across our study sites we used random forest analysis (Figure 6). The only variable to be consistently related to variability in microbial richness was pH. Indeed, the sample site with the greatest richness (SU1, 1300 m, richness = 1644) had a soil pH of 5.9, one of the highest pH values detected among our study sites. On the other hand, the sample with the lowest pH (SH2, 1200 m), of 4.7, had a richness of just 1019, the second lowest value across the study area. Overall, soil pH was more strongly correlated to changes in elevation (as assessed by the square root of the component of variation attributable to these factors in a permutational ANOVA model); a significant statistical interaction was also present between site aspect and elevation (Supplementary Table S2, all $P < 0.001$). Overall, pH was observed to be lower (i.e., closer to neutral) at lower elevation sites located on warmer aspects of the slope (Supplementary Figure S4). The results of random forest analysis confirmed interactions between the effects of resource and non-resource environmental factors in that pH had the strongest effect on the composition of bacterial communities, but in the sites with high pH (> 5.5), concentrations of total carbon were also a significant correlate of bacterial community composition. Besides the impact on whole bacterial communities, we confirmed significant correlations between soil

pH and the relative abundances of dominant family members across the study site. The relative abundance of Thermogemmatisporaceae (the second most abundant family detected, 10%), Koribacteraceae (3.7%), Ellin6513 (3.4%) and Gaiellaceae (3.3%) were each correlated with soil pH (correlation coefficient (Spearman's Rho) = 0.47, 0.60, 0.68 and 0.52, respectively; *p*-values all < 0.001).

DISCUSSION

Across equivalent distances, variation in bacterial community composition across different aspects of a single mountain ridge was far greater than variation detected comparing samples collected at different elevations. Significant variation in the factors which are frequently suggested to be a primary determinant of the biogeography of macroorganisms (Gillman and Wright 2014), i.e., soil temperature and moisture availability, were observed across elevation and aspect gradients. However, variation in bacterial community composition and richness across the mountain microclimate gradient were more closely correlated with soil pH. Together, these findings highlight the complexity of microclimate impacts on soil bacterial communities, and the need to incorporate multiple factors, including site aspect and soil chemical attributes, into assessments of microbial community composition across elevation gradients.

Consistent with several other studies (Fierer *et al*. 2010; Shen *et al*. 2013), and in contradiction of our *Elevation Hypothesis* (Elevation H_1), we detected no significant decline in bacterial taxon richness with altitude. These findings contribute to a wealth of evidence indicating that bacterial communities exhibit fundamentally different responses to elevation than macro-organisms (Fierer and Jackson 2006; Lauber *et al*. 2009); gradients in the richness of both plant and animal communities are frequently observed with elevation (Aubry

et al. 2005; Carneiro *et al*. 2013). Critically, and in support of our *Aspect Hypothesis* (Aspect H_2), we confirmed that, when comparing samples across equivalent distances, bacterial community attributes were more impacted by variation in sampling site aspect than elevation. That is to say that bacterial communities at the same elevation but separated by distances of just 100 m across aspects, were far more divergent, on average, than communities separated by elevational distances of up to 900 m. The cause behind the relatively strong impact of aspect compared to elevation is unclear. Although significant differences of over 2 °C were detected among adjacent slopes, equivalent to a ~300 m increase in altitude (Rorison *et al*. 1986), soil bacterial community composition and richness was nevertheless more tightly correlated with soil pH, leading to our third hypothesis (Environment H_3) being accepted.

Ours is not the first study to reveal the strong influence of soil pH on bacterial community composition (Fierer and Jackson 2006; Griffiths *et al*. 2011; Lauber *et al*. 2009), including across elevation gradients (Bryant *et al*. 2008). Soil pH has been identified as being a key predictive variable of bacterial composition in both soil (Fierer and Jackson 2006; Griffiths *et al*. 2011) and aquatic environments (Fierer *et al*. 2007). Changes in bacterial community composition may also be driven by additional soil factors that are, all-be-it indirectly, linked to soil pH. For example, pH can mediate the activity of extracellular enzymes involved in litter decomposition (Griffith *et al*. 1995), thereby impacting soil carbon storage and transformation rates (Kemmitt *et al*. 2006). Soil pH is also a strong mediator of nitrogen mineralisation, which can occur across a wide pH range (Fu *et al*. 1987), but decreases under more acidic conditions. Elevational gradients in soil pH are commonly reported and are frequently attributed to increased rates of mechanical weathering that occurs up to elevations of ~2,000 m in the Southern Alps, beyond which persistent permafrost obviates the freeze-thaw cracking process (Hales and Roering 2016). Increased weathering causes soil

acidification as base cations such as calcium, sodium, phosphate and magnesium are leached from the soil. Although significant gradients in soil temperature, moisture and resource availability were noted across our study site, our results contribute to a growing body of evidence that pH, possibly affected by different rates of weathering across the study site, is a dominant driver of bacterial diversity (Lear *et al*. 2009; Shen *et al*. 2013; Zhang *et al*. 2013).

A hypothesis commonly used by microbiologists is that 'everything is everywhere – the environment selects' (Baas-Becking 1934). However, the relative importance of environmental conditions, such as pH, for microbial community composition is scale-dependent. Many authors suggest that non-symbiotic populations of bacteria exhibit global distributions (Barberan *et al*. 2015; Brown *et al*. 2012). Nevertheless, any limitation to their movement will give rise to distance decay patterns in bacterial community composition. Therefore, even if bacteria are universally distributed, spatial variability in their community composition will reflect spatial patterns of their key environmental drivers. Where β-diversity patterns in microbial communities observed across space are not found to be related to variation on environmental attributes, they are hypothesised to result from dispersal limitation (Martiny *et al*. 2011). The decrease in similarity of bacterial communities with elevation observed in this study is likely to result from a combination of stochasticity in bacterial dispersal and increasing environmental differences among samples at increasing distances; further experimental work is required to tease apart the relative importance of these effects.

It is noteworthy that members of the family Thermogemmatisporaceae were dominant in the community across our alpine study site. These spore-forming bacteria have been recovered from geothermal soils and biofilms (King and King 2014; Yabe *et al*. 2011) and also from soils surrounding natural gas vents (de Miera *et al*. 2014). Strains cultured from these

environments are thermophilic, exhibiting growth optima > 50 ^{o}C (Yabe *et al*. 2011). Why do members of this apparently thermophilic family dominate the composition of communities across this alpine elevation gradient? Strong, negative correlation of their abundance with soil pH suggests that mechanisms of environmental selection operate among these communities. Interestingly, the abundance of Thermogemmatisporaceae was greatest in soils of lower pH, closer to the pH optima reported for members of this family (Yabe *et al*. 2011). However, direct evidence of cellular metabolic activity (e.g., using stable isotope probing or transcriptome-based methodologies) would be required to determine if these bacteria are indeed active within the soil of the study site. Another explanation is that not all organisms related to Thermogemmatisporaceae are thermophilic. Indeed, DNA sequences identified in the present study were similar to those found in other cool environments, including acidic alpine tundra soils of the Northern Hemisphere (Costello and Schmidt 2006; Zinger *et al*. 2009), indicating the widespread distribution of these taxa in alpine zones. However, microorganisms capable of forming resistant endospores are frequently found in environments that do not appear to support their metabolic activity. It is therefore conceivable that thermotolerant bacteria, such as members of the family Thermogemmatisporaceae, may be more resistant to long-range atmospheric transport and the extreme UV, low moisture and low nutrient conditions it provides. This notion is supported by studies that have identified resistant, but viable, spores from thermophilic organisms, particularly members of the Geobacteraceae, in samples from the upper troposphere (deLeon-Rodriguez *et al*. 2013) as well as within cold oceanic currents (Hubert *et al*. 2009; Muller *et al*. 2014). It is possible that these bacteria adhere to the 'Geobacillus paradox' which, owing to resistant properties and longevity, enable quiescent cells to achieve surprisingly high population densities in environments that appear to be poorly suited for their survival (Zeigler 2014), such as soils at high elevation.

CONCLUSION

We found greater differences in both the richness and composition of bacterial communities between samples taken on different mountain aspects, than between samples taken at equivalent distances at different mountain elevations. The findings of the present study lead us to conclude that to appropriately use elevation and aspect gradients as a natural laboratory for assessing the likely impacts of climate change on bacterial communities, we must account for local variation in abiotic conditions because, at least in this case, bacterial composition and richness were more closely related to local abiotic factors (soil pH) than to variation in temperature or resource factors. Further work is needed to better understand the mechanisms underlying microbial diversity patterns and the relative importance of both evolutionary and ecological processes as determinants of bacterial community structure and composition. In particular, the effect on bacterial communities of elevational gradients in pH and other soil factors must be quantified using gradients in elevation and aspect as proxies for climatic variability when predicting community responses to climate change.

FUNDING

This work was supported by funds from an RSNZ Rutherford Discovery Fellowship [LCR1201 to B.J.A.]; MBIE Crown Research Institute core funding from Biodiversity in Production Landscapes [RPA EBD-1 to B.J.A.]; the Miss E L Hellaby Indigenous Grasslands Research Trust [to J.W.]; and an University of Auckland FRDF Grant [No. 3703205 to G.L.].

ACKNOWLEDGEMENTS.

We thank Branch Creek Station for permission to sample on their land, Ray Anderson for assistance with logistics and access to the hill sites; and Cardrona Alpine Resort and staff for help accessing the field site. Soil samples, environmental (i.e. climate and soil physicochemistry) and plant biomass data were collected by BJA and Dr Ralf Ohlemüller as part of the Cardrona Thermal Refugia study. For assistance in the field and with sample processing we thank Ella Hayman, Kent Collins, Jono Glassey, Tosca Mannall, Liam Koedyk, Xiaoqing Li, Elisabeth Hüllbusch, Gretchen Brownstein, Moana Meyer and Dane Tuialii. BJA provided the study design and funded all field work including sample and covariate data collection. All authors commented and contributed to the final manuscript. We thank Peter Tsai for assistance with bioinformatic analysis of the data.

REFERENCES

Allen AP, Brown JH, Gillooly JF. Global Biodiversity, Biochemical Kinetics, and the Energetic-Equivalence Rule. *Science* 2002;**297**: 1545-8.

Altschul SF, Gish W, Miller W *et al.* Basic local alignment search tool. *Journal of molecular biology* 1990;**215**: 403-10.

Anderson MJ. A new method for non ‐ parametric multivariate analysis of variance. *Austral ecology* 2001;**26**: 32-46.

Aubry S, Magnon F, Bonnet V *et al.* Multi-scale altitudinal patterns in species richness of land snail communities in south-eastern France. *J Biogeog* 2005;**32**: 985-98.

Baas-Becking LGM. *Geobiologie of inleiding tot de milieukunde*. The Hague, The Netherlands: WP Van Stockum & Zoon, 1934.

Barberan A, Ladau J, Leff JW *et al.* Continental-scale distributions of dust-associated bacteria and fungi. *P Natl Acad Sci USA* 2015;**112**: 5756-61.

Bennie J, Huntley B, Wiltshire A *et al.* Slope, aspect and climate: Spatially explicit and implicit models of topographic microclimate in chalk grassland. *Ecological Modelling* 2008;**216**: 47-59.

Bertrand R, Lenoir J, Piedallu C *et al.* Changes in plant community composition lag behind climate warming in lowland forests. *Nature* 2011;**479**: 517-20.

Borcard D, Gillert F, Legendre P. *Numerical Ecology with R*. Use R! Series. New York: Springer, 2011.

Brown MV, Lauro FM, DeMaere MZ *et al.* Global biogeography of SAR11 marine bacteria. *Molecular systems biology* 2012;**8**: 595.

Bryant JA, Lamanna C, Morlon H *et al.* Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. *P Natl Acad Sci USA* 2008;**105**: 11505-11.

Calleja-Cervantes ME, Menéndez S, Fernández-González AJ *et al.* Changes in soil nutrient content and bacterial community after 12 years of organic amendment application to a vineyard. *European Journal of Soil Science* 2015;**66**: 802-12.
Caporaso JG, Kuczynski J, Stombaugh J *et al.* QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010;**7**: 335-6.
Carneiro E, Mielke OHH, Casagrande MM *et al.* Skipper Richness (Hesperiidae) Along Elevational Gradients in Brazilian Atlantic Forest. *Neotropical Entomology* 2013;**43**: 27-38.
Carter BJ, Ciolkosz EJ. Slope gradient and aspect effects on soils developed from sandstone in Pennsylvania. *Geoderma* 1991;**49**: 199-213.
Costello EK, Schmidt SK. Microbial diversity in alpine tundra wet meadow soil: novel Chloroflexi from a cold, water-saturated environment. *Environmental microbiology* 2006;**8**: 1471-86.
Davies ZG, Wilson RJ, Coles S *et al.* Changing habitat associations of a thermally constrained species, the silver‐spotted skipper butterfly, in response to climate warming. *Journal of Animal Ecology* 2006;**75**: 247-56.
de Miera LES, Arroyo P, Calabuig ED *et al.* High-throughput sequencing of 16S RNA genes of soil bacterial communities from a naturally occurring CO_2 gas vent. *International Journal of Greenhouse Gas Control* 2014;**29**: 176-84.
deLeon-Rodriguez N, Lathern TL, Rodriguez-R LM *et al.* Microbiome of the upper troposphere: species composition and prevalence, effects of tropical storms, and atmospheric implications. *P Natl Acad Sci USA* 2013;**110**: 2575-80.
Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 2013;**10**: 996-8.
Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. *P Natl Acad Sci USA* 2006;**103**: 626-31.
Fierer N, McCain CM, Meir P *et al.* Microbes do not follow the elevational diversity patterns of plants and animals. *Ecology* 2010;**92**: 797-804.
Fierer N, Morse JL, Berthrong ST *et al.* Environmental controls on the landscape-scale biogeography of stream bacterial communities. *Ecology* 2007;**88**: 2162-73.
Fierer N, Schimel JP, Holden PA. Influence of drying-rewetting frequency on soil bacterial community structure. *Microbial ecology* 2003;**45**: 63-71.
Fu MH, Xu XC, Tabatabai MA. Effect of pH on nitrogen mineralization in crop-residue-treated soils. *Biol Fert Soil* 1987;**5**: 115-9.
Gilliam FS, Galloway JE, Sarmiento JS. Variation with slope aspect in effects of temperature on nitrogen mineralization and nitrification in mineral soil of mixed hardwood forests. *Canadian Journal of Forest Research* 2015;**45**: 958-62.
Gillman LN, Wright SD. Species richness and evolutionary speed: the influence of temperature, water and area. *J Biogeog* 2014;**41**: 39-51.
Griffith MB, Perry SA, Perry WB. Leaf litter processing and exoenzyme production on leaves in streams of different pH. *Soil Biol Biochem* 1995;**102**: 460-6.
Griffiths RI, Thomson BC, James P *et al.* The bacterial biogeography of British soils. *Environ Microbiol* 2011;**13**: 1642-54.
Grundmann GL. Spatial scales of soil bacterial diversity--the size of a clone. *FEMS microbiology ecology* 2004;**48**: 119-27.
Hales TC, Roering JJ. Climate-controlled variations in scree production, Southern Alps, New Zealand. *Geology* 2016;**88**: 701-4.
Huber E, Wanek W, Gottfried M *et al.* Shift in soil–plant nitrogen dynamics of an alpine–nival ecotone. *Plant and Soil* 2007;**301**: 65-76.

Hubert C, Loy A, Nickel M *et al.* A constant flux of diverse thermophilic bacteria into the cold arctic seabed. *Microbiology* 2009;**325**: 1541-4.
Hunckler RV, Schaetzl RJ. Spodosol development as affected by geomorphic aspect, Baraga County, Michigan. *Soil Science Society of America Journal* 1997;**61**: 1105-15.
Illumina. 16S Metagenomic Sequencing Library Preparation. 2013. Availale at: http://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.ilmn.
Kalra YP, Maynard DG. *Methods manual for forest soil and plant analysis* volume 319, 1991.
Kemmitt SJ, Wright D, Goulding KWT *et al.* pH regulation of carbon and nitrogen dynamics in two agricultural soils. *Soil Biol Biochem* 2006;**38**: 898-911.
Kessler M. Altitudinal zonation of Andean cryptogam communities. *Journal of Biogeography* 2000;**27**: 275-82.
King CE, King GM. Description of *Thermogemmatispora carboxidivorans* sp. nov., a carbon-monoxide-oxidizing member of the class *Ktedonobacteria* isolated from a geothermally heated biofilm, and analysis of carbon monoxide oxidation by members of the class *Ktedonobacteria*. *International Journal of Systematic and Evolutionary Microbiology* 2014;**64**: 1244-51.
Klindworth A, Pruesse E, Schweer T *et al.* Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic acids research* 2013;**41**: e1-e1.
Knapp AK, Fay PA, Blair JM *et al.* Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science* 2002;**298**: 2202-5.
Lauber CL, Hamady M, Knight R *et al.* Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol* 2009;**75**: 5111-20.
Lauber CL, Strickland MS, Bradford MA *et al.* The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry* 2008;**40**: 2407-15.
Lear G, Niyogi D, Harding J *et al.* Biofilm bacterial community structure in streams affected by acid mine drainage. *Appl Environ Microbiol* 2009;**75**: 3455-60.
Liaw A, Wiener M. Classification and Regression by randomForest. R news. 2002; 2 (3): 18–22.
Lomolino MV. Elevation gradients of species-density: historical and prospective views. *Global Ecology and Biogeography* 2001;**10**: 3-13.
Martiny JBH, Eisen JA, Penn K *et al.* Drivers of bacterial beta-diversity depend on spatial scale. *P Natl Acad Sci USA* 2011;**108**: 7850-4.
Mason OU, Scott NM, Gonzalez A *et al.* Metagenomics reveals sediment microbial community response to Deepwater Horizon oil spill. *Isme Journal* 2014;**8**: 1464-75.
McArdle BH, Anderson MJ. Fitting multivariate models to community data: a comment on distance‐based redundancy analysis. *Ecology* 2001;**82**: 290-7.
McCain CM, Grytnes JA. Elevational gradients in species richness. *eLS* 2010, DOI 10.1002/9780470015902.a0022548.
McDonald D, Price MN, oodrich J *et al.* An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary anaylses of Bacteria and Archaea. *ISME J* 2012;**6**: 610-8.
Miller JO, Galbraith JM, Daniels WL. Soil organic carbon content in frigid southern Appalachian mountain soils. *Soil Science Society of America Journal* 2004;**68**: 194-203.

Morris SJ. Spatial distribution of fungal and bacterial biomass in southern Ohio hardwood forest soils: fine scale variability and microscale patterns. *Soil Biology and Biochemistry* 1999;**31**: 1375-86.
Muller AL, de Rezende JR, Hubert CRJ *et al.* Endospores of thermophilic bacteria as tracers of microbial dispersal by ocean currents. *ISME J* 2014;**8**: 1153-65.
Oksanen J, Blanchet FG, Kindt R *et al.* Package 'vegan'. *Community ecology package, version* 2015: 2.-1.
Peres-Neto P, Legendre P, Dray S *et al.* Variation partioning of species data matrices: estimation and comparison of fractions. *Ecology* 2006;**87**: 2614-25.
Rahbek C. The relationship among area, elevation, and regional species richness in neotropical birds. *American Naturalist* 1997; **149:** 875-902.
Rahbek C. The role of spatial scale and the perception of large-scale species-richness patterns. *Ecology letters* 2004;**8**: 224-39.
Ramirez KS, Leff JW, Barberan A *et al.* Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. *Proc R Soc B* 2014;**281**: 20141988.
Rayment GE, Lyons DJ. *Soil chemical methods: Australasia* volume 3: CSIRO publishing, 2011.
Rinnan R, Rousk J, Yergeau E *et al.* Temperature adaptation of soil bacterial communities along an Antarctic climate gradient: predicting responses to climate warming. *Global Change Biology* 2009;**15**: 2615-25.
Rorison IH, Sutton F, Hunt R. Local climate, topography and plant growth in Lathkill Dale NNR. I. A twelve-year summary of solar radiation and temperature. *Plant, Cell and Environment* 1986;**9**: 49-56.
Sariyildiz T, Anderson JM, Kucuk M. Effects of tree species and topography on soil chemistry, litter quality, and decomposition in Northeast Turkey. *Soil Biology and Biochemistry* 2005;**37**: 1695-706.
Schaetzl RJ, Anderson S. *Soils: Genesis and Geomorphology*. Soils: Genesis and Geomorphology, DOI 10.1017/cbo9780511815560. Cambridge: Cambridge Univ Press, 2005.
Shen C, Ni Y, Liang W *et al.* Distinct soil bacterial communities along a small-scale elevational gradient in alpine tundra. *Frontiers in microbiology* 2015;**6**: 582.
Shen C, Xiong J, Zhang H *et al.* Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. *Soil Biology and Biochemistry* 2013;**57**: 204-11.
Sidari M, Ronzello G, Vecchio G *et al.* Influence of slope aspects on soil chemical and biochemical properties in a Pinus laricio forest ecosystem of Aspromonte (Southern Italy). *European Journal of Soil Biology* 2008; **44**: 364-72.
Singh D, Lee-Cruz L, Kim W-S *et al.* Strong elevational trends in soil bacterial community composition on Mt. Halla, South Korea. *Soil Biology and Biochemistry* 2014;**68**: 140-9.
Singh D, Takahashi K, Kim M *et al.* A hump-backed trend in bacterial diversity with elevation on Mount Fuji, Japan. *Microbial ecology* 2012;**63**: 429-37.
Stephenson NL. Climatic control of vegetation distribution: The role of water balance. *Amer Nat* 1990;**135**: 649-70.
Stevens GC. The elevational gradient in altitudinal range: an extension of Rapoport's latitudinal rule to altitude. *American Naturalist* 1992; **140:** 893-911.
Thomas CD, Bodsworth EJ, Wilson RJ *et al.* Ecological and evolutionary processes at expanding range margins. *Nature* 2001; **411**: 577-81.

Thompson JA, Kolka RK. Soil carbon storage estimation in a forested watershed using quantitative soil-landscape modeling. *Soil Science Society of America Journal* 2005;**69**: 1086-93.
Titshall L, O'Connor T, Morris C. Effect of long-term exclusion of fire and herbivory on the soils and vegetation of sour grassland. *African Journal of Range and Forage Science* 2000;**17**: 70-80.
van der Heijden MG, Bardgett RD, van Straalen NM. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters* 2008;**11**: 296-310.
Vos M, Wolf AB, Jennings SJ *et al.* Micro-scale determinants of bacterial diversity in soil. *FEMS microbiology reviews* 2013;**37**: 936-54.
Wang J, Soininen J, Zhang Y *et al.* Contrasting patterns in elevational diversity between microorganisms and macroorganisms. *Journal of Biogeography* 2011;**38**: 595-603.
Wang Q, Garrity GM, Tiedje JM *et al.* Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 2007;**73**: 5261-7.
Weiss SB, Weiss AD. Landscape-level phenology of a threatened butterfly: a GIS-based modeling approach. *Ecosystems* 1998;**1**: 299-309.
Weltzin JF, Loik ME, Schwinning S *et al.* Assessing the response of terrestrial ecosystems to potential changes in precipitation. *Bioscience* 2003;**53**: 941-52.
Yabe S, Aiba Y, Sakai Y *et al. Thermogemmatispora onikobensis* gen. nov., sp. nov. and *Thermogemmatispora foliorum* sp. nov., isolated from fallen leaves on geothermal soils, and description of *Thermogemmatisporaceae* fam. nov. and *Thermogemmatisporales* ord. nov. within the class *Ktedonobacteria*. *International Journal of Systematic and Evolutionary Microbiology* 2011;**64**: 903-10.
Zeglin LH, Bottomley PJ, Jumpponen A *et al.* Altered precipitation regime affects the function and composition of soil microbial communities on multiple time scales. *Ecology* 2013;**94**: 2334-45.
Zeigler DR. The *Geobacillus* paradox: why is a thermophilic bacterial genus so prevalent on a mesophilic planet? *Microbiology* 2014;**160**: 1-11.
Zhang B, Liang C, He HB *et al.* Variations in soil microbial communities and residues along an altitude gradient on the northern slope of Changbai Mountain, China. *PloS one* 2013;**8**: e66184.
Zhang Q, Zhou W, Liang G *et al.* Distribution of soil nutrients, extracellular enzyme activities and microbial communities across particle-size fractions in a long-term fertilizer experiment. *Applied Soil Ecology* 2015;**94**: 59-71.
Zinger L, Shahnavaz B, Baptist F *et al.* Microbial diversity in alpine tundra soils correlates with snow cover dynamics. *ISME J* 2009;**3**: 850-9.

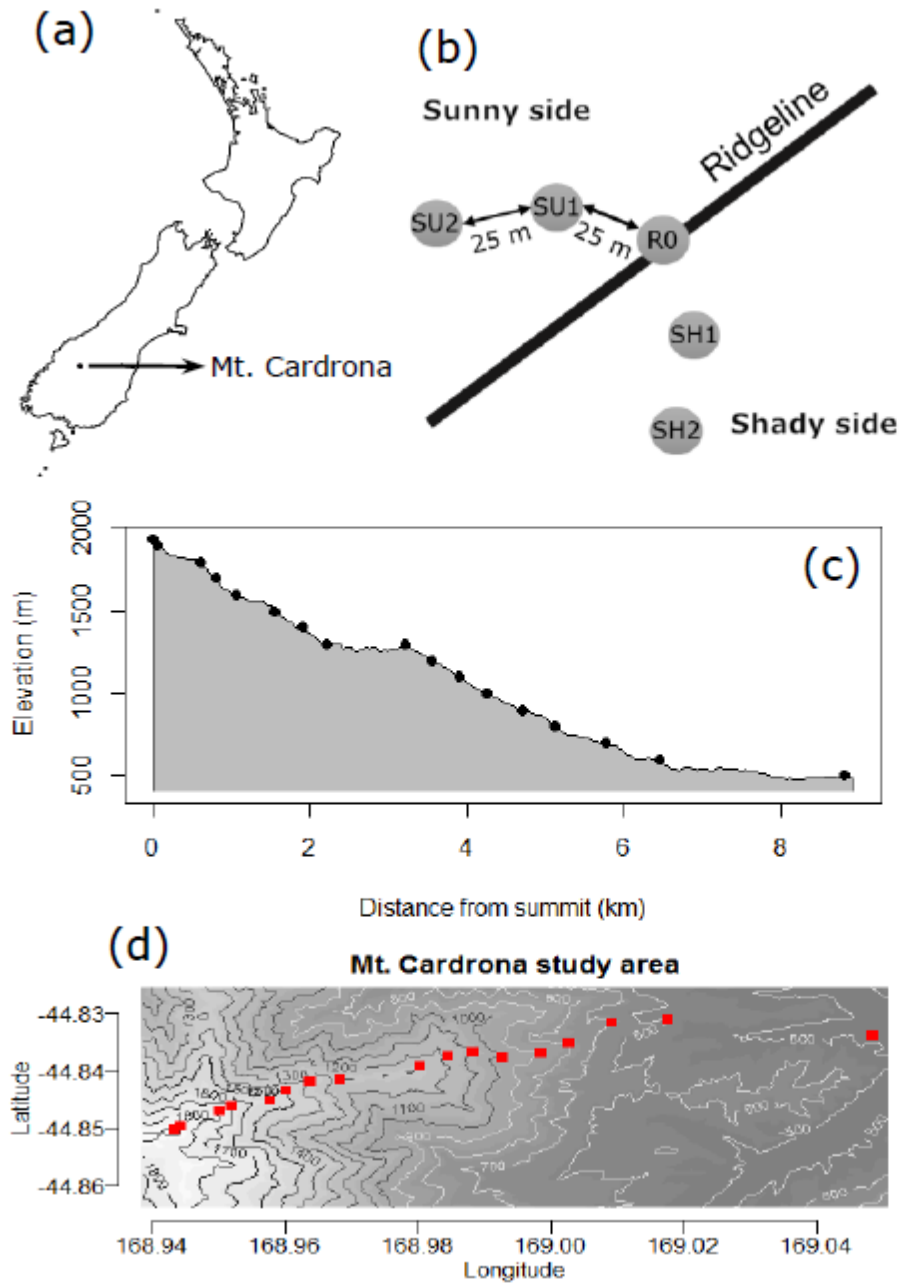

Figure 1. Sample locations on Mt Cardrona. (a) Mt. Cardrona is located in Otago (44.85° S, 168.95° E), New Zealand; (b) at each elevation, samples were collected on both sides, and on the main ridge of mountain at intervals of 25 m (geographic distance); (c) mountain profile showing the distance from the summit of each sampling location along the elevation gradient; (d) samples were collected at elevation intervals of 100 m along either side of the main ridge on north-eastern side of Mt Cardrona.

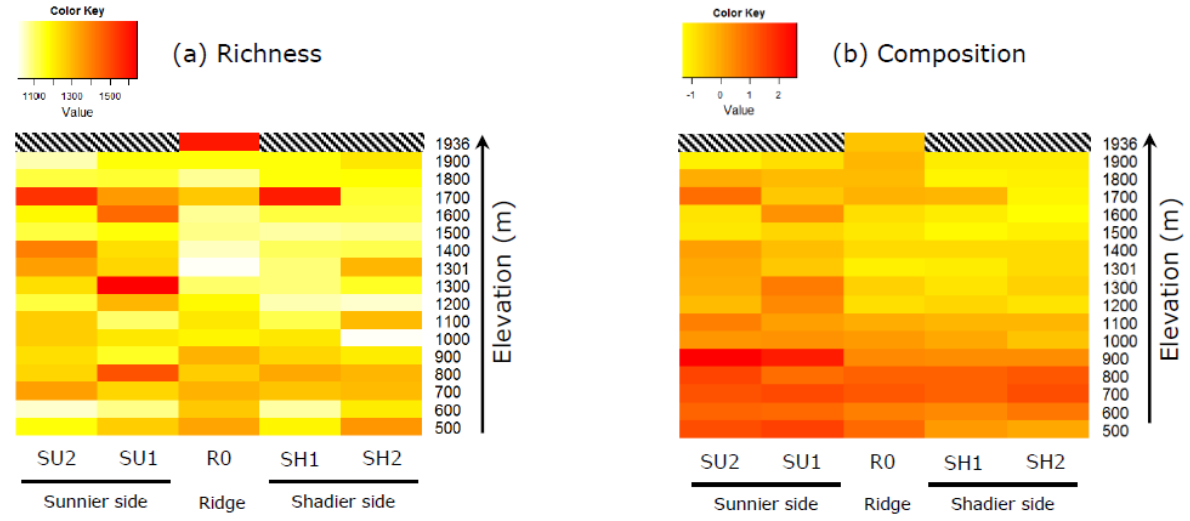

Figure 2. Variation in relative bacterial (a) taxon richness or (b) composition across the study site. Different rows represent data collected from different elevation bands; different columns represent data from different aspects. Sample data are assigned colours (a) across a gradient from red (highest average 97%OTU richness) to yellow (lowest average 97%OTU richness) or (b) across a gradient from red (highest 1D configuration score) to yellow (lowest 1D configuration score) after data reduction by non-metric multidimensional scaling of Bray-Curtis distance data. Sites at the top of the mountain where communities were not sampled are indicated by hatched lines.

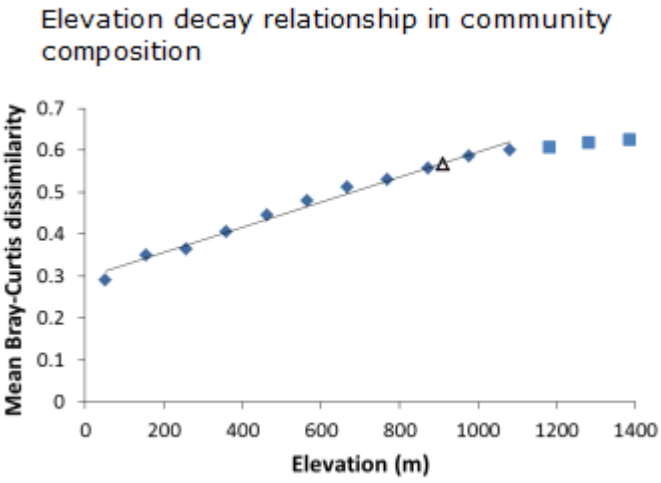

Figure 3. Scatterplot showing average Bray-Curtis dissimilarity (from Figure 2(b)) comparing sample data separated by different elevational distances. In the scatterplot, the linear trendline for the data is $y = 0.0003x + 0.30$ ($R^2 = 0.99$) using the first 11 data points (represented as diamonds). The hollow triangle shows the average distance among all samples collected from different aspects of the mountain, calculated by their mean Bray-Curtis dissimilarity.

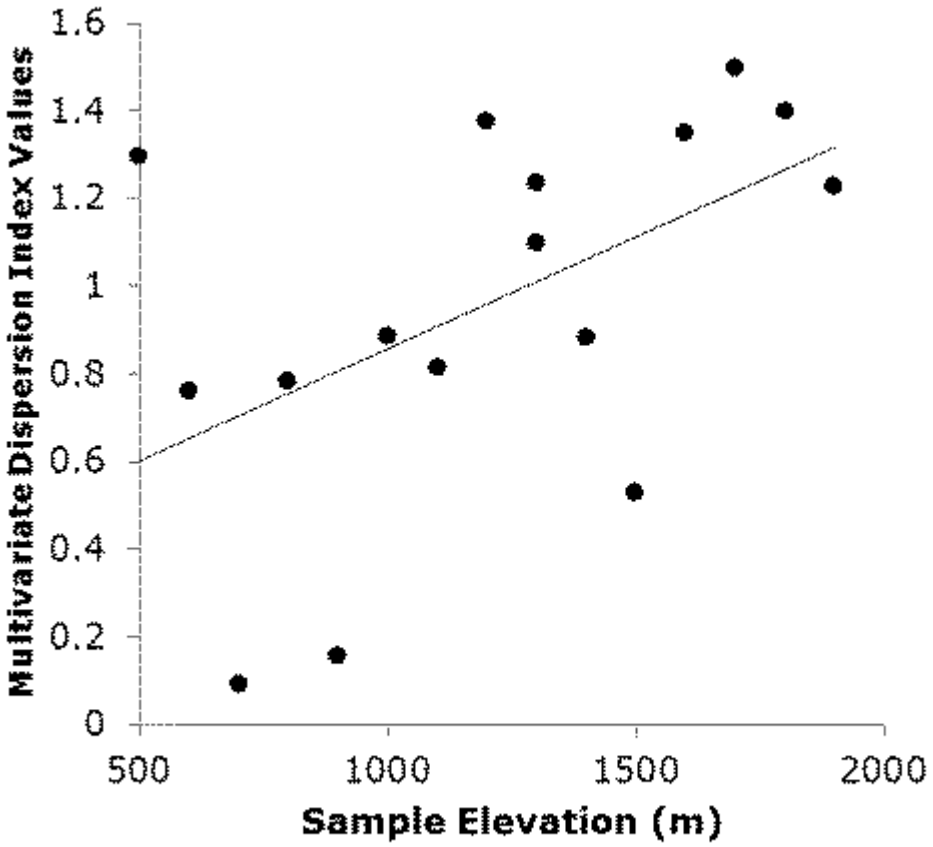

Figure 4. Scatterplot showing the relationship between sample elevation (m) and variability in bacterial community composition among samples collected at different clusters. Variability in bacterial community composition was quantified at each sample elevation using multivariate dispersion (MVDISP) index values, which describe the dispersion of sample data in multivariate space using a Bray Curtis distance (as in Figure S1). Linear trend line for the data is $y = 0.0005x + 0.35$ ($R^2 = 0.26$).

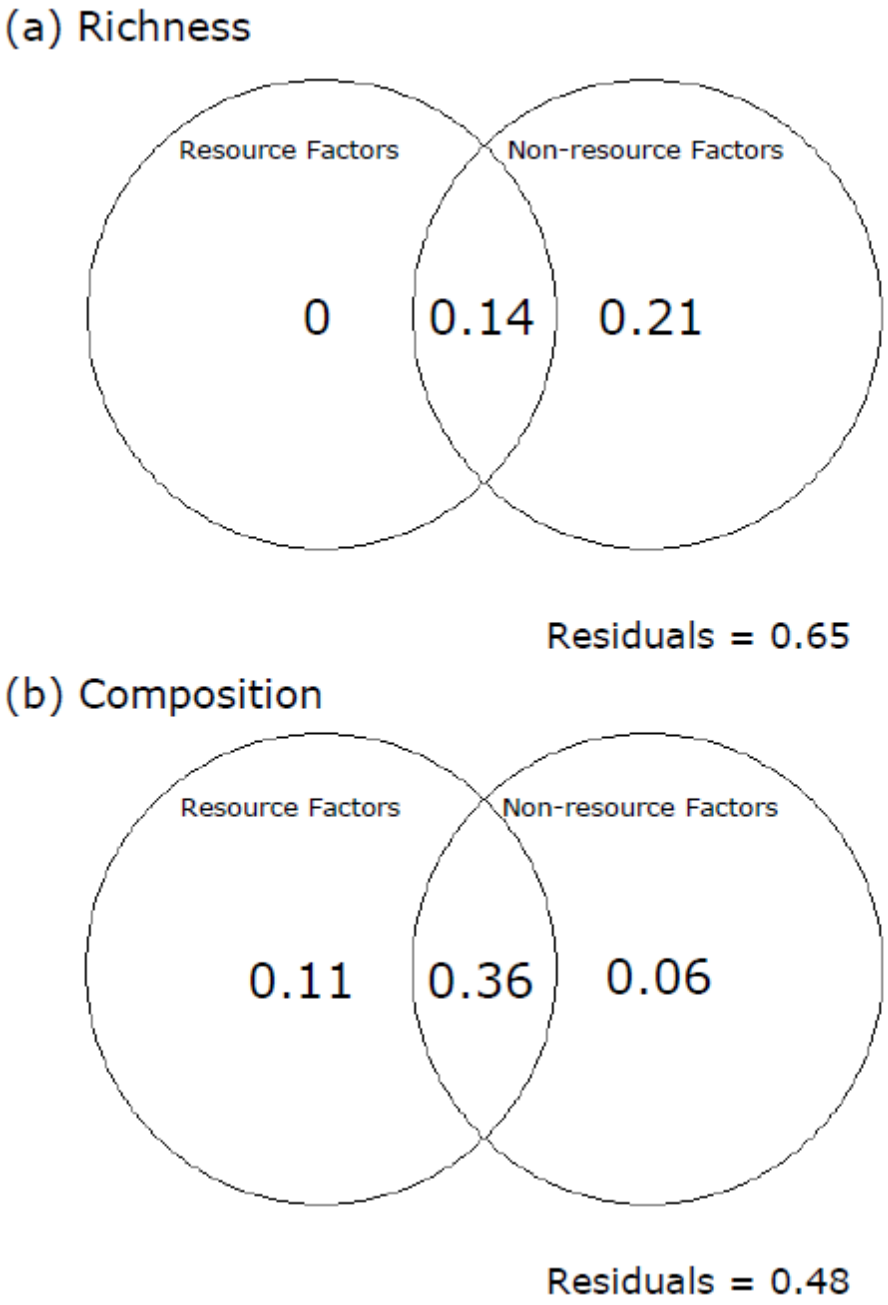

Figure 5. Venn diagrams providing a graphical representation of the variance partitioning of bacterial community (a) richness and (b) composition (community Bray-Curtis similarity) between resource and non-resource and environmental factors.

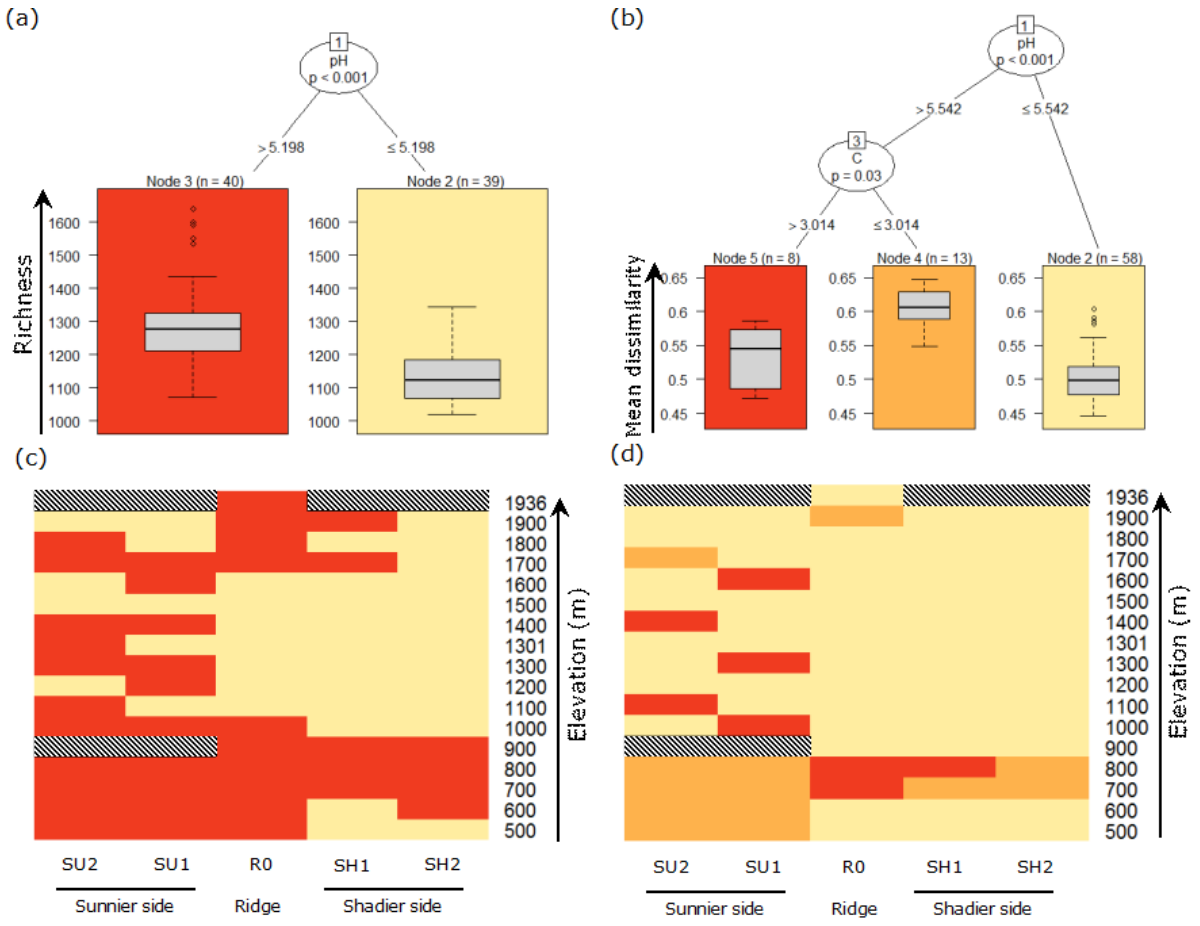

Figure 6. Multivariate regression tree of bacterial community (a) richness and (b) community composition associated with sampling location. The decision tree identifies variance in bacterial richness or compositional similarity caused by threshold values of elevation, resource or non-resource environmental factors. The values attached to each branch mark the criteria used by the regression tree to group samples based on differences in bacterial richness or composition. Corresponding box and whisker plots show richness of bacterial OTUs, or average composition (1D configuration score of non-metric multidimensional scaling plot) associated with each node of the data. (c, d) Graphical representation showing the spatial location of samples associated with each terminal node of the decision tree for bacterial richness and composition data, respectively. Bacterial richness refers to the number of distinct 97% OTUs identified within sample cluster, from the analysis of 27,500 DNA sequences. Sites where sample data were not collected or analysed are indicated by hatched lines.