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Azorella Cushion Plants and Aridity are Important Drivers of Soil Microbial Communities in Andean Ecosystems

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ABSTRACT

Cushion plants are specialized keystone species of alpine environments that can have a positive effect on ecosystem structure and function. However, we know relatively little about how cushion plants regulate the diversity and composition of soil microbial communities, major drivers of soil processes and ecosystem functioning. Identifying what factors drive the diversity and composition of soil microbial communities in high-elevation ecosystems is also fundamental to predict how global changes will affect their conservation and the services and functions they provide. Thus, we sampled four sites along the southern Andes following the vegetation belt of *Azorella* cushion species. The field sites spread along a latitudinal gradient and had contrasting levels of aridity, UV-B radiation, mean temperature and soil properties. Overall, *Azorella*, as well as aridity and UV-B radiation, were the major drivers of the distribution, composition and diversity of soil microbial communities in the studied ecosystems of the Chilean Andes. UV-B radiation affected particularly soil fungi, while soil properties such as pH, total C and N content, essential predictors of microbial diversity globally, had a much lower effect on the composition of soil microbial communities. Understanding the factors driving the structure and composition of microbial communities, particularly the role of cushion plants and the feedbacks between plant, climate

Author contributions SRE and CA planned and designed the research. SRE, CA, LAC, AG and FIP performed field work. MM did laboratory analysis. SRE, CA, MD-B and JAM analyzed data. AG, PM and FIP contributed to the interpretation. SRE wrote the first version of the manuscript with key inputs from CA, MD-B and JAM. All authors discussed and contributed to the last version of the manuscript.

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and soil is of uttermost importance for the preservation of the functionality of high-elevation ecosystems threatened by climate change.

Key words: Andes; Atacama; Chile; High mountains; Microbial network; Torres del Paine; UV-B.

HIGHLIGHTS

- *Azorella* and aridity are the main drivers of soil microbial community composition in Andean ecosystems.
- Different clusters of soil microbes associate with distinct levels of aridity and UV-B radiation.
- The effect of *Azorella* on structuring soil microbes is smaller in the most stressful sites.

INTRODUCTION

Mountains cover around 25% of the world surface, harbor major forests and high biodiversity, provide up to 80% of the world's freshwater supply, enable the production of hydropower and support cultural, religious and recreational services (Körner 2002; Donhauser and Frey 2018). Alpine ecosystems are largely characterized by extreme environmental conditions, such as low temperature and high irradiance, but also by a high diversity of habitats. As a result of this and of their inaccessibility, alpine ecosystems also represent some of the last wilderness on Earth (Körner 2002), but they are severely threatened by global warming (for example, Pauli and others 2012; Steinbauer and others 2018).

Soil microbes are crucial for soil development and nutrient cycling in alpine ecosystems, modulating not only plant establishment and succession but also greenhouse gas balance at global scale (Donhauser and Frey 2018). Soil pH, organic matter, aridity and vegetation are the most important predictors of soil microbial communities from local to global scales (for example, Fierer and Jackson 2006; Kivlin and others 2011; Tedersoo and others 2014; Maestre and others 2015; Alfaro and others 2017; Delgado-Baquerizo and others 2019). Moreover, in alpine ecosystems, soil microbial diversity and abundance are also conditioned by soil heterogeneity, the presence of rocky shallow oligotrophic soils with low water retention capacity, snow cover, large fluctuations in temperature and moisture, and high irradiance, specifically UV-B radiation (Donhauser and Frey 2018 and references therein).

These harsh environmental conditions are particularly important in bare soil and rocks, which occupy large areas of alpine zones, but the presence of plants provides new habitats for soil microbes, contributing to their spatial heterogeneity and increasing beta diversity (Zhang and others 2015; Geremia and others 2016; Siles and Margesin 2016, 2017; Chen and others 2016; Fernández-Gómez and others 2019; but see Lazzaro and others 2015).

Plants respond to the harsh alpine environment with a high degree of specialization, among which the cushion life form is one of the most characteristic adaptations (Aubert and others 2014). Cushion plants have a low, compact structure with a hemispherical, or mat-like, much-branched canopy (Raunkiaer 1934). The compact cushion form serves as protection from strong winds and constitutes a heat and litter trap, increasing organic matter and soil nutrient content under the canopy (Körner 2002; Chen and others 2014). The ameliorated conditions inside and under the cushion can facilitate the establishment and growth of other plant species, thus promoting plant diversity (for example, Cavieres and others 2002; Arroyo and others 2003; Badano and others 2006; Antonsson and others 2009; Yang and others 2010; Cavieres and others 2014; Schöb and others 2013, 2014) and the abundance of soil fauna and arthropods (Korner 2002; Molina-Montenegro and others 2006; Molenda and others 2012; Minor and others 2016). Consequently, cushion plants are keystone nurse species in alpine environments with a great impact on ecosystem diversity and functioning.

Cushion plants can be also a selective force for soil microbial communities, offsetting the influence of abiotic factors in community assembly (Roy and others 2013). Some assessed have studied the effect of cushion plants on soil microbes (Roy and others 2013; Rehakova and others 2015; Chang and others 2018; Wang and others 2020), including arbuscular mycorrhizal fungi (Casanova-Katny and others 2011), but more research is needed due to the large soil heterogeneity of alpine zones and the phylogenetic diversity of cushion-forming species (Boucher and others 2016). These studies have also rendered contrasting results. *Silene acaulis*, a widespread cushion species, has a stronger effect on soil bacteria than fungal communities along a gradient of soil types and elevation (Roy and others 2013). This cushion species minimizes the effect of bedrock type and soil properties on soil bacteria resulting in similar communities under *S. acaulis* in different soil types (Roy and others 2013). However, the effect on fungal communities was more

complex and dependent on cushion plant genotype (Roy and others 2018). On the Himalayas, opposing results have been found for different cushion species. *Thylacospermum ceaspitosum* reduces the diversity of culturable bacteria in cold, arid Trans-Himalayan habitats (Rehakova and others 2015), whereas *Arenaria polytrichoides* and *Chionocharis hookeri* have a positive effect on rhizospheric bacterial communities in the Sino-Himalayan region (Chang and others 2018). Also, a recent study with *T. ceaspitosum* showed that this species might be more important for structuring soil fungal communities than for bacterial communities (Wang and others 2020). Finally, *Azorella madreporica* in the Central Andes increases the abundance of arbuscular mycorrhizal fungal spores under its canopy up to six times, with a consequent positive effect on the mycorrhization of beneficiary plants (Casanova-Katny and others 2011), but its effect on other soil microbes is unknown. In fact, the genus *Azorella*, in the Andes, contains some of the biggest and most impressive alpine cushion plants (Pugnaire and others 2020) and many studies have shown a positive effect of *Azorella* species on plant diversity (for example Arroyo and others 2003; Badano and others 2006; Cavieres and others 2007), as well as an increase in soil nutrient content under its canopy (Mihoc and others 2016), thus acting as a nurse species. Despite this, the effect of *Azorella* cushions on the diversity and community composition of soil microbial communities in the Andes remains unexplored.

We collected samples in the elevation belt of *Azorella* in Chile, in four field sites (Lauca, Atacama, Farellones and Torres del Paine) stretching along a 3800 km latitudinal gradient in the Andes to assess the role of different abiotic conditions, the presence of *Azorella* and their possible interactions in driving the abundance, diversity and composition of soil bacterial and fungal communities. We hypothesized that (a) *Azorella* cushion plants will have a significant effect on structuring soil microbial communities, which will be stronger in sites with harsher conditions (Roy and others 2013; Cavieres and others 2014); (b) aridity and UV-B radiation will be the main abiotic drivers of soil microbial community composition (Johnson and others 2002; Maestre and others 2015); and (c) soil microbial communities will be more specialized and less diverse in sites with higher aridity and UV-B radiation (Johnson and others 2002; Maestre and others 2015).

MATERIALS AND METHODS

Sampling

We selected four sites—Lauca, Atacama, Farellones and Torres del Paine—following a latitudinal gradient from 18°S to 51°S along the Chilean Andes (Table 1). Soil samples were collected in the austral summer between December 2015 and January 2016 in the vegetation belt dominated by cushion-forming species of the *Azorella* genus (*A. compacta* in Lauca and Atacama, *A. madreporica* in Farellones and *A. monantha* in Torres del Paine). Climatic data for each site (mean annual temperature [Tmean], mean minimum temperature [Tmin], mean maximum temperature [Tmax], annual precipitation [P]) were obtained from WorldClim version 2 (http://www.worldclim.org). Data for potential evapotranspiration (ETP) were obtained from "Red Agrometereológica de Chile" (https://agrometeorologia.cl/) and were used to calculate the Aridity Index as $1 - (P/ETP)$. Data for UV-B radiation were obtained from "Dirección Meteorológica de Chile" (Sánchez-Cuevas 2009).

In each location, soil samples (top 5 cm) were collected under five *Azorella* cushions and in adjacent open spaces (bare soil) that were at least 2 m apart from any plant. Samples to estimate soil water content (SWC) and soil organic matter (SOM) were collected in two separate 15-ml cap tubes, sealed with parafilm and weighed in the laboratory immediately after sampling. Samples for biogeochemical characterization were collected, air-dried and stored in 15-ml cap tubes until processing. Samples for SWC measurement from Torres del Paine (Paine hereafter) were damaged during transport and could not be processed. Samples for DNA extraction were collected using spades and corers that were ethanol-disinfected between each sampling, stored in UV-sterilized paper bags, dried using silica gel and stored at −80 °C until processing.

Soil Analyses

Gravimetric SWC (g/g soil) was determined by mass loss after drying at 105 °C for 72 h. SOM (g/g soil) was determined in the same soil samples by mass loss after ignition at 450 °C for 20 h. Soil pH was determined by mixing 5 g of soil in 20 mL of distilled water with 0.01 mg of dissolved $CaCl_2$ using a glass electrode. Soil carbon (C) and nitrogen (N) content were estimated in a Carlo Erba NA 2500 elemental analyzer (Lakewood, NJ, USA). All biogeochemical analyses were carried out at the

Table 1. Details of the Sites Sampled in the Chilean Andes: Region, Geographical Coordinates, Elevation, Main Climatic Variables and Mean UV-B Radiation.

Site	Region	Latitude	Longitude	Elevation (masl)	Tmean (°C)	Tmax (°C)	Tmin (°C)	P (mm)	Aridity 1-(P/PET)	UV (mW/m²)
Lauca	XV (Arica-Parinacota)	-18.16389	-69.46944	4050	1.3	12.4	-12.6	300	0.79	342.1
Atacama	II (Antofagasta)	-22.41389	-68.03444	4400	3.6	15.4	-10.9	44	0.97	301.7
Farellones	RM (R. Metropolitana)	-33.33333	-70.26666	3200	1.2	14.4	-9.6	471	0.65	249.2
Paine	XII (Punta Arenas)	-51.09889	-72.71500	870	3.3	13.2	-5.6	700	0.25	139.2

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DNA Extraction and Quantitative PCR

DNA was extracted from 200 mg of dry soil using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA). DNA concentration was estimated using a NanoDrop 2000c (Thermo Scientific, Wilmington, USA) and samples were stored at -80 °C. The abundance of archaea, bacteria and fungi was determined via quantitative PCR, using an Eco Real-Time PCR System (Illumina, San Diego, California, USA) and KAPA SYBR® FAST qPCR Master Mix Universal (Kapa Biosystems, Boston, Massachusetts, USA. Bacterial and archaeal 16S rRNA gene fragments were amplified and quantified, using 0.5uM of 338F-518R and, 931F-M1100R primer sets, respectively (Einen and others 2008). Fungal 18S rRNA gene fragment was quantified using 1.25uM of each primer FR1-FF390 (Prevost-Boure and others 2011). Standard curves were prepared using a serial dilution of a purified PCR product and amplified from a DNA mix as template. Two independent qPCRs were performed for each sample. The specificity of PCR product was evaluated by running products on a 1% (w/v) agarose gel. The standard curves had R^2 values of 0.997, 0.992 and 0.996, and the amplification efficiency estimated to 101%, 92% and 101% for bacteria, archaea and fungi, respectively. Microbial abundance was expressed as copies number per gram of soil.

Barcoded Amplicon Sequencing and Bioinformatics

The structure of microbial communities was assessed by high-throughput sequencing of the 16S rRNA gene for archaea/bacteria and internal transcribed spacer marker gene (ITS) for fungi. 16S rRNA and ITS amplicon sequencing was done using Earth Microbiome Project standard protocols (https://earthmicrobiome.org/protocols-and-standards/) at the facilities of Genyo (Granada, Spain). Briefly, amplicon PCR was performed in triplicate on the V4 region of the 16S rRNA gene using the primer pair 515f-806r and on the ITS1 region with the primer pair ITS1f-ITS2 (Walters and others 2016). Bar-coded PCR products were quantified using a Qubit dsDNA (Thermo Fisher Scientific) instrument and pooled in equal concentrations. The multiplexed DNA library was purified using Agencourt AMPure XP beads (Beckman Coulter). Amplicon quality and size

were checked with a High Sensitivity DNA Assay (Bioanalyzer 2100, Agilent) and sequenced in an Illumina MiSeq sequencing platform using the Illumina reagent kit V3 (600 cycles) generating 300-bp pair-end reads. A PCR negative control and a commercial mock community sample (ZymoBIOMICS Microbial Community Standard, Zymo Research, Irvine, CA, US) were also sequenced as controls.

Initial rRNA 16S and ITS sequence processing and diversity analyses were conducted in Microbiome Helper version 0.3. Microbiome Helper is a virtual box image of the platform QIIME 1.9.1 (Caporaso and others 2010) with additional software and databases (Comeau and others 2017). Raw 16S rRNA pair-end reads were quality checked with FastQC version 0.11.5 (Andrews 2010). Stitching of pair-end reads was accomplished with PEAR version 0.9.10 (Zhang and others 2014). FASTX-Toolkit version 0.014 (Gordon and others 2009) was used to filter out reads that had a quality score of less than 35 over at least 90% of the bases. Following read filtering, potentially chimeric sequences were screen out using VSEARCH version 1.11.1 (Rognes and others 2016). OTU picking was carried out with the QIIME open-reference method using SortMeRNA version 2.0 (Kopylova and others 2012) for the reference-based step against the SILVA 128 database (Quast and others 2013) and SUMACLUST version 1.0 (Mercier and others 2013) for the de novo clustering step (using a cutoff of 97% sequence similarity). Processing of ITS reads was accomplished with the same pipeline described before for the rRNA 16S reads with some modifications. Taxonomy was assigned with mothur version 1.39.5 (Schloss and others 2009) against the fungal ITS UNITE database version 7 dynamic (Kõjalg and others 2005).

For both 16S rRNA and ITS OTU tables, a dynamic cutoff (as opposed to removing just singletons) was employed to filter out OTUs having less than 0.1% of the total number of sequences, as previously recommended (Comeau and others 2017). The final OTU tables, containing 4,784,951 and 1,406,247 read counts for 16S rRNA and ITS, respectively, were normalized by subsampling (or rarefying) to 41,673 and 17,822 reads per sample. A total of 7442 OTUs were detected, of which 5990 OTUs were prokaryotes (25 corresponded to Archaea), and 1452 OTUs were fungi. Rarefaction curves showed that sampling was adequate for both prokaryotes and fungi.

The raw reads have been deposited in Sequence Read Archive (SRA) of the NCBI and are available under the BioProject ID PRJNA679783.

Data Analysis

Partial least square (PLS) regression was used to analyze prokaryotic and fungal beta diversity, that is, differences between sites and microhabitats, and the relationship with abiotic variables (di Rienzo and others 2017). Individual PLS were used separately for all reads of prokaryotes and fungi, but it was not possible to build a PLS for all 7442 OTUs identified for both groups. Microbial communities have a highly skewed distribution with few dominant species that are expected to be prevalent across contrasting environments, and constitute the largest fraction of the microbial biomass. Thus, we reduced the number of OTUs for further PLS sequential analyses, retaining OTUs that accounted for 90% and 70% of the total reads for each group. In all cases, PLS results were almost identical (Figure S1), so we used 331 fungal OTUs (90% of the total fungal abundance) and 806 prokaryotic OTUs (70% of total prokaryotic OTUs, composed by 99% bacteria and 1% archaea) to build a joint PLS for the whole microbial community. GLMs were applied to test for significant differences between sites, microhabitats and their interaction using the information summarized by the two first PLS axes.

Co-occurrence networks were used to identify the existence of modules of bacterial and fungal phylotypes (OTU level) that appear together more than expected by chance. Networks built independently for bacteria or fungi rendered similar results (not shown); therefore, we used networks including both types of microorganisms. To build co-occurrence networks, pairwise Spearman's rank correlations (ρ) were calculated between the dominant 806 prokaryotic and 331 fungal OTUs (see above; PLS analysis). Co-occurrence was considered robust if the Spearman's correlation coefficient (ρ) was > 0.65 and $p < 0.01$. We calculated the relative abundance of ecological modules within our correlation network as the averaged standardized relative abundance of taxa within each module. Networks were constructed and visualized using the 'igraph' package for R, and cluster_optimal() was used to identify network modules (Csardi and others 2006).

We conducted a random forest analysis (Breiman 2001) to identify the main predictors of the identified network modules. The potential predictors included in the analysis were abiotic variables (mean temperature, mean maximum and mean

minimum temperatures, mean annual precipitation, aridity, UV-B radiation, soil C, soil N, soil C:N and pH) and *Azorella* presence/absence. Random forest is a novel machine learning algorithm that extends standard classification and regression tree (CART) methods by creating a collection of classification trees with binary divisions (Wei and others 2010). Unlike traditional CART analyses, the fit of each tree is assessed using randomly selected cases (1/3 of the data), which are withheld during its construction (out-of-bag [OOB] cases). The importance of each predictor variable is determined by evaluating the decrease in prediction accuracy (that is, increase in the mean square error [MSE] between observations and OOB predictions) when data for that predictor are randomly permuted. This decrease is averaged over all trees to produce a final measure of importance (van Elsas and others 2012). The accuracy importance measure was computed for each tree and averaged over the forest (5000 trees). These analyses were conducted using the *randomForest* package (Liaw and Wiener 2002) of the R statistical software, version 3.0.2 (http://cran.r-project.org/). The significance of the model and the cross-validated R^2 were assessed with 5000 permutations of the response variable (node in the co-occurrence networks) using the *A3* R package for R (Fortmann-Roe 2015). Similarly, the significance of the importance of each predictor on module occurrence was assessed by using the *rfPermute* package for R (Archer 2013).

The Relative Interaction Index (RII, Armas and others 2004) was used to further assess the effect of *Azorella* on SWC, SOM, soil pH, soil C, soil N, soil C:N and abundance of microorganisms based on total DNA concentration and qPCR values. RII was calculated as (value under cushion − value in bare soil)/(value under cushion + value in bare soil). RII values range from −1 (largest negative effect of the cushion compared to bare soil) to + 1 (largest positive effect) where 0 indicates the lack of a net effect of the cushion compared to bare soil. Using the data collected under five *Azorella* cushions and in adjacent open spaces (bare soil), we calculated 95% confidence intervals to determine whether the obtained RII values were significantly different from 0.

RESULTS

General Characterization

Overall, bacterial communities were dominated by Proteobacteria, Actinobacteria, Verrucomicrobia, Acidobacteria and Bacteroidetes, in decreasing order of relative abundance (Figure 1). These phyla totaled more than 80% of reads in all sites. Proteobacteria were dominant in all sites with 21–31% of total reads, except in Paine where the most abundant phyla were Actinobacteria (32%) and Verrucomicrobia (18%). Soil fungal communities were dominated by Ascomycota, with values ranging from 60% of reads in Farellones to 85% in Lauca (Figure 1). Basidiomycota was the second most abundant phylum, varying from 10% of reads in Lauca to 31% in Atacama. Zygomycota represented 15–21% of total reads in Paine and Farellones, but had a relative lower abundance in the more arid sites, Lauca (4.5%) and Atacama (0.7%). Glomeromycota had a much lower relative abundance, with the highest value in the most mesic site, Paine (Figure 1).

Drivers of Soil Microbial Communities

Multivariate analysis of soil microbial communities using PLS clearly separated the four studied sites following a gradient defined by aridity, UV-B and mean minimum and maximum temperature (Figure 2, Table 2, Figure S1). Within each site, the presence of *Azorella* cushions was associated with differences in soil properties and contributed to further separation of the soil microbial communities (Figure 2, Table 2, Figure S1). This differentiation between microhabitats was more obvious in the two mesic sites, Farellones and Paine (Figure 2, Figure S1).

Correlation networks were used to identify ecological clusters of bacteria and fungi strongly co-occurring with each other (Delgado-Baquerizo and others 2018), and random forest modeling was used to evaluate their ecological preferences (Figure 3). Ten network modules, formed by microbial OTUs strongly co-occurring with each other, were identified and the four major predictors of their relative abundance were aridity and UV-B (10 modules), mean temperature (7) and *Azorella* cushions (7, Figure 3A). Using the most important predictor for each model, the ten network modules were classified into seven ecological clusters according to their environmental preferences (Figure 3B, Figure S2). However, several covarying environmental factors were significant for all modules (Figure 3A). For example, *Azorella* presence or absence was the main predictor of the composition of modules 2 and 3, respectively, but climatic factors, UV-B, soil C and soil C:N were also significant predictors for these modules (Figure 3A). Overall, the most important predictors for the relative abundance of ecological clusters were
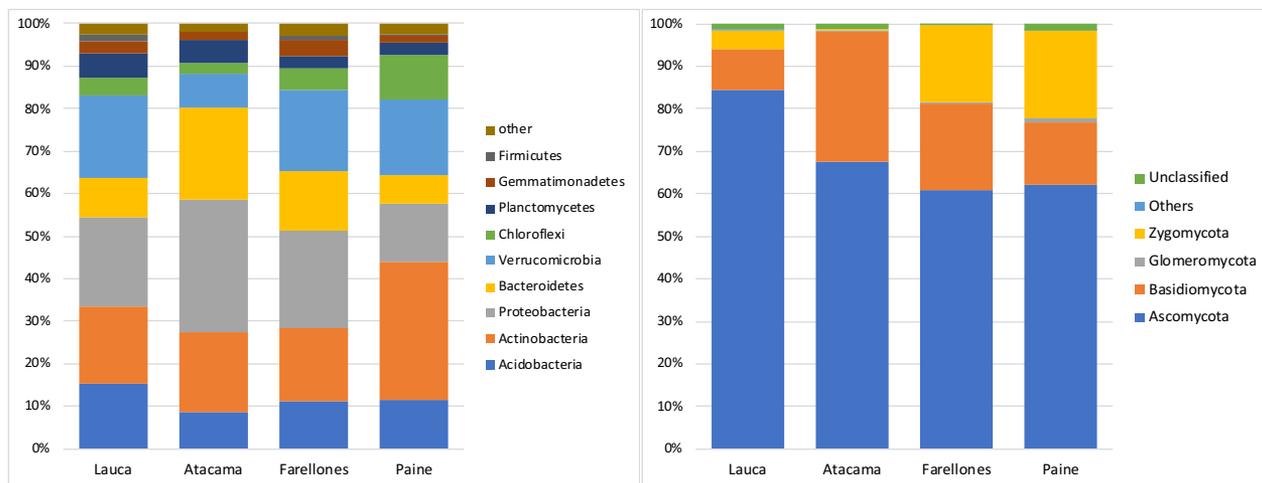
Figure 1. Overall relative abundances of the main bacterial and fungal phyla in each studied site.
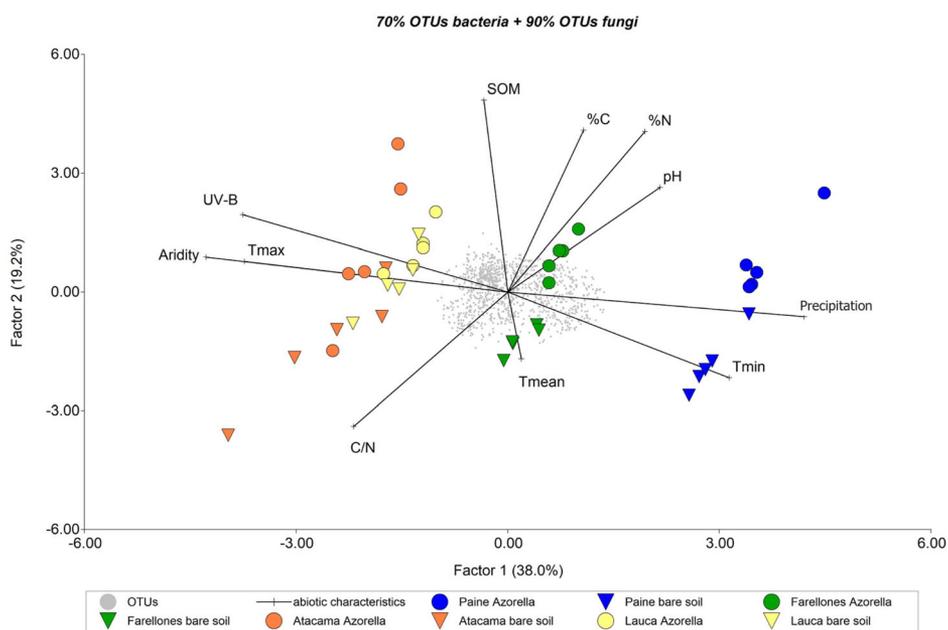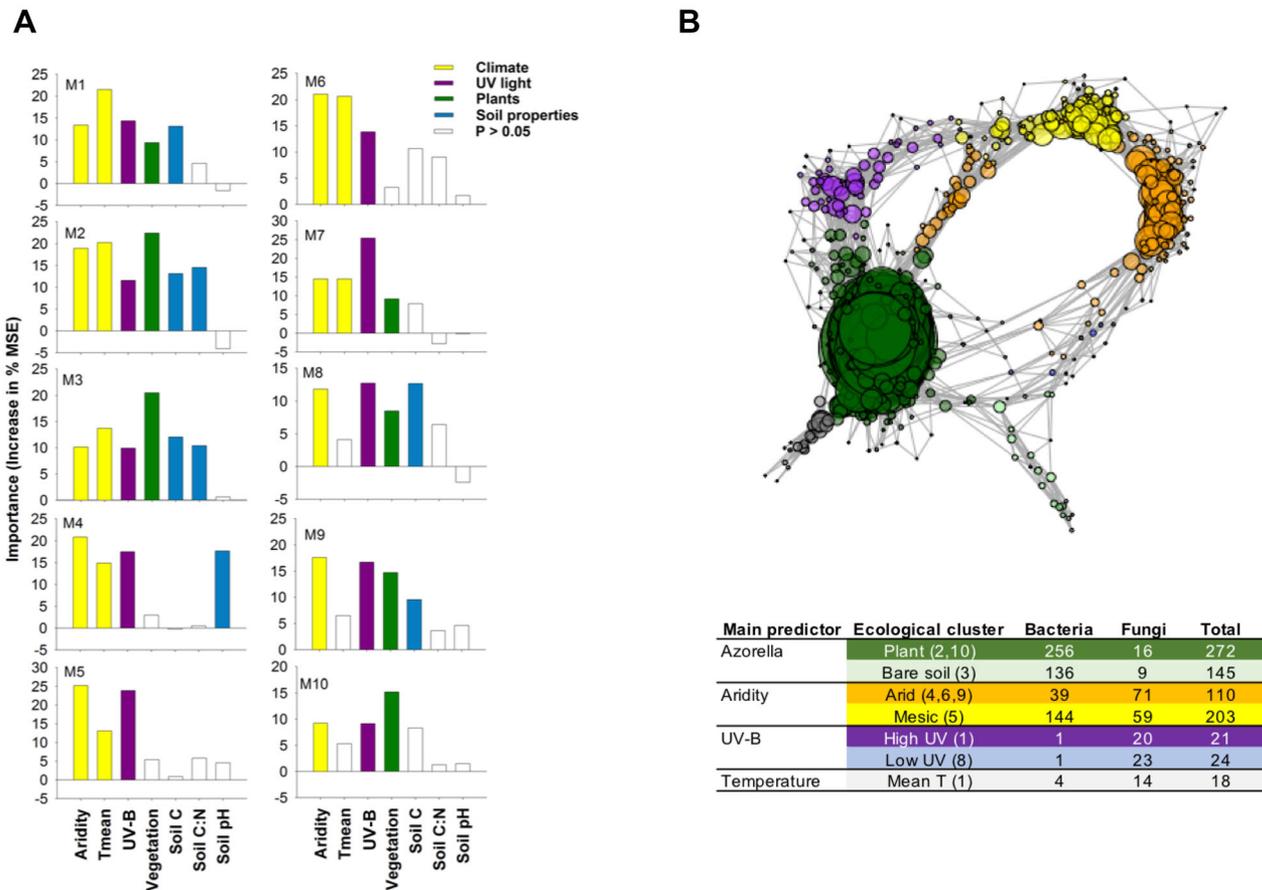

Figure 2. PLS regression for the dominant OTUs of bacteria and fungi in all samples. For both groups, sites were significantly separated along axis 1, while a significant interaction between site (Lauca, Atacama, Farellones and Paine) and microhabitat (beneath *Azorella* and bare soil) was found along axis 2. This significant site:microhabitat interaction is further explored in Table 2. Colors represent sites: orange—Lauca; yellow—Atacama; green—Farellones; blue—Paine. Symbols represent microhabitat: circles—under *Azorella*; inverted triangles—bare soil.

Table 2. Results of GLM for the Effect of Site and Microhabitat on Separating Samples along Axes 1 and 2 of the PLS Regression.

Axis 1					Axis 2			
Source	numDF	denDF	*F*-value	*p*-value	numDF	denDF	*F*-value	*p*-value
Site	3	32	356.11	<0.0001	3	32	15.7	<0.0001
Microhabitat	1	32	14.76	0.0005	1	32	39.01	<0.0001
Site: microhabitat	3	32	1.11	0.3585	3	32	4.76	0.0075

A

B

Main predictor	Ecological cluster	Bacteria	Fungi	Total
Azorella	Plant (2,10)	256	16	272
	Bare soil (3)	136	9	145
Aridity	Arid (4,6,9)	39	71	110
	Mesic (5)	144	59	203
UV-B	High UV (1)	1	20	21
	Low UV (8)	1	23	24
Temperature	Mean T (1)	4	14	18

Figure 3. A Importance of the main predictors on the relative abundance of the ten modules identified in the co-occurrence network of soil microbial communities after random forest analysis. **B** Co-occurrence network of soil microbial communities with colors representing the seven ecological clusters defined by random forest analysis. Dark green: Presence of *Azorella*. Light green: Absence of *Azorella*. Orange: Aridity. Yellow: Mesic ecosystems. Purple: High UV-B. Blue: Low UV-B. Gray: Tmean. Nodes are sized relative to the number of interactions. The table shows the main predictors, ecological clusters, the original modules contained in each ecological cluster (in brackets) and the number of bacterial and fungal OTUs in each ecological cluster.

the presence of *Azorella* and aridity. The relative abundance of 52% of the total OTUs was defined by the presence (34%) or absence (18%) of *Azorella* cushions (Figure 3B). Aridity was associated with the relative abundance of 39% OTUs (11% with dry ecosystems and 28% with mesic ecosystems) (Figure 3B). The number of interactions among co-occurrent taxa was higher in the ecological cluster associated with *Azorella* cushions (Figure 3b).

Bacteria and fungi were affected differently by these main predictors. Over 95% of the OTUs defined by the presence or absence of *Azorella* plants were bacteria, but UV-B radiation and mean temperature affected primarily fungal taxa (Figure 3b). Aridity affected both microbial groups, but a higher relative proportion of bacterial OTUs was associated

with mesic sites, while a higher proportion of fungi was associated with dry sites (figure 3b).

The presence of *Azorella* predicted a higher relative abundance of Deltaproteobacteria, Gammaproteobacteria, Rhizobiales (Alphaproteobacteria), *Blastocatellia* (Acidobacteria), Verrucomicrobia and Tectomicrobia (Table S1). The fungi *Mortierella alpina* was also associated with the presence of *Azorella*. On the contrary, Actinobacteria and the archaea Thaumarchaeota were associated with bare soil (Table S1).

Actinobacteria, such as Gaiellales and Solirubrobacterales, were far more abundant in mesic sites, while Proteobacteria, mainly Alphaproteobacteria, *Mehtylobacteriaceae, Acetobacteraceae* or *Sphingomonodaceae*, were more abundant in arid sites (Table S1). The presence of *Thelebolus*

globosus (Leotiomycetes) and the yeast *Naganishia friedmannii* (Basidiomycota) was also associated with aridity.

UV-B radiation affected primarily fungal taxa since only two bacterial OTUs were found in the modules defined mainly by UV-B (Figure 3b, Table S1). These two bacterial taxa were an Actinobacteria, class Thermoleophilia, order Gaiellales for low UV-B (<250 mW/m^2) and an Acidobacteria, family *Holophagae, subgroup 10, ABS-19,* for high UV-B (>300 mW/m^2). On the other hand, high UV-B was associated only with fungi from the Ascomycota, mainly Chaetothyriales like *Cladophialophora* sp. Contrastingly, low UV-B radiation predicted the abundance of a higher number of fungal taxa, such as *Cistella* sp. and *Peziza* sp. (Ascomycota), *Clavaria*, *Ceratobasidium* (Agaricomycetes) or *Mortierella pseudozygospora.*

Leotiomycetes (mainly order Heliotiales), Agaricomycetes (Agaricales and Cantharellales) and Morterierellomycotina were associated with more mesic conditions—either mesic sites, presence of plants or low UV-B radiation—whereas the Ascomycota Dothideomycetes (mainly Pleosporales) and Eurotiomycetes (mainly order Chaetothyriales) were defined either by aridity or high UV-B radiation. Mean temperature affected fewer microbial taxa: Heliotales and Plesoporales were the main fungal groups defined by mean temperature (Table S1).

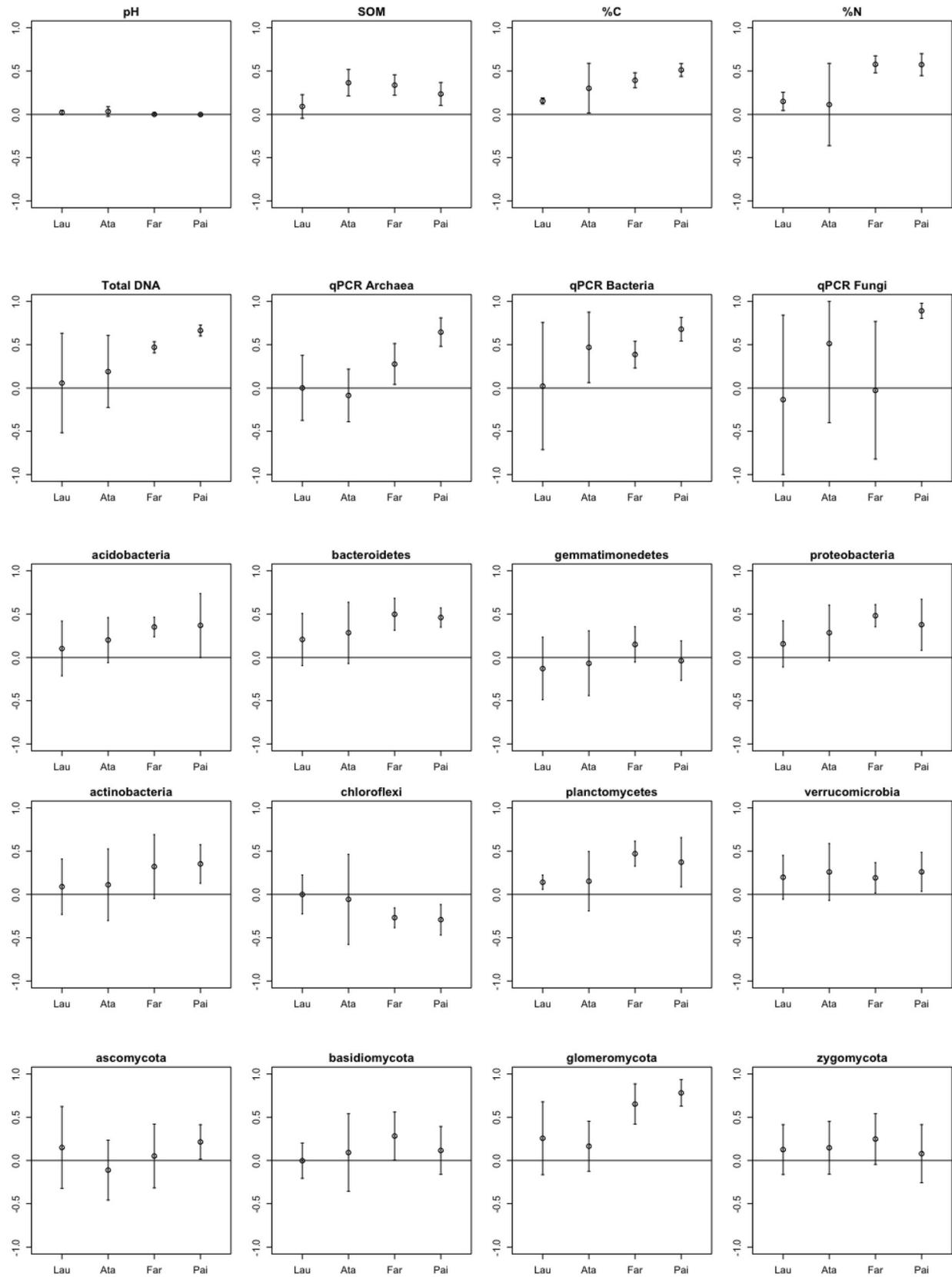## Effect of *Azorella* Cushion Plants on Soil Properties and Microbial Diversity

Azorella cushion plants had a general significant positive effect on SOM, C, N and SWC in all sites, while the effect of *Azorella* on soil total DNA and overall abundance of soil microbes (as determined by qPCR) was more important in Farellones and Paine, the more mesic sites, than in the harsher locations (Figure 4, Figure S3.1). *Azorella* had a significant positive effect on the diversity of six main bacterial phyla (Acidobacteria, Actinobacteria, Bacteroidetes, Planctomycetes, Proteobacteria and Verrucomicrobia) in the more mesic sites (Figure 4). A significant positive effect of *Azorella* was also found on the diversity of Glomeromycota in the two more mesic sites (Figure 4) and marginally for Ascomycota in Paine and Basidomycota in Farellones (Figure 4). Similar patterns, with a larger positive effect in the mesic sites, were found for the richness and abundance of bacterial and fungal phyla (Figure S3.2, S3.3). However, *Azorella* cushions also had a site-dependent negative effect on the abundance of Proteobacteria in arid Atacama; and of Actinobacteria, Bacteroidetes, Gemmatimonadetes and Ascomycota in the mesic sites (Figure S3.2, S3.3).

DISCUSSION

Our data provide strong evidence that *Azorella* cushion plants, aridity and UV-B radiation are the most important ecological predictors of the distribution of soil bacteria, archaea and fungi in the studied Andean ecosystems. By contrast, soil properties such as pH, C or N content, often referred to as the most important drivers of soil microbial communities (for example, Tedersoo and others 2014; Fierer 2017), had a smaller effect on the overall composition of microbial communities. Our field sites in the Chilean Andes were primarily separated by aridity and UV-B radiation levels, defining a gradient from more extreme conditions in Atacama to less extreme conditions in Torres del Paine. Surprisingly, soil properties accounted more for the differences between microhabitats within each site than among sites, which could also explain their lower contribution as predictors of the soil microbial communities. For example, soil pH, which has been considered a primary predictor of the diversity and composition of soil microbial communities at local (Alfaro and others 2017; Mandakovic and others 2018) and global scales (Fierer and others 2009; Fierer 2017), was not a major factor affecting soil microbial communities in this study. The relatively small differences in soil pH values between sites, in spite of the different soil types sampled, might minimize the often-reported association between pH and microbes, and reduce its importance as a predictor of soil microbial community composition in the studied ecosystems.

Our results confirm the key role of *Azorella* cushions as main determinants of soil microbial communities' composition and diversity in these extreme ecosystems, supporting the importance of cushion plants for microbial community composition, adding to the work conducted in the Alps (Roy and others 2013, 2018) and Himalayas (Rehakova and others 2015; Chang and others 2018) with other cushion species. Cushion plants were not only positively associated with soil microbial abundance, richness and diversity (as shown by the RII analysis) but also led to the development of specific plant-associated microbial communities. In contrast to a recent report for the cushion species, *Thylacospermum ceaspitosum* (Wang and others 2020), the presence of *Azorella* plants was mainly associated with bacterial taxa, likely due to the quicker response and faster growth of bacterial

communities in response to improved conditions under plants (Goberna and others 2014). *Azorella* cushions were the main predictors of the distribution of a large number of Acidobacteria, Proteobacteria, Verrucomicrobia and Tectomicrobia taxa and also had a clear effect on the richness of the fungal phyla Basidiomycota, Zygomycota and Glomeromycota. A number of Blastocatellia also appeared to be driven by either the presence or absence of *Azorella* cushions. Most isolated Blastocatellia are adapted to nutrient limitation and drought, showing a broad tolerance range for temperature (Huber and others 2017). Differences in Blastocatellia abundance have also been found between bulk soil and the rhizosphere of other perennial plants in high-elevation grasslands in Atacama (Fernández-Gómez and others 2019). *Azorella* cushions also increased the richness and abundance of symbiotic arbuscular mycorrhizal fungi (Glomeromycota) in the mesic sites, which might contribute to the facilitative effect of *A. madreporica* cushions (Casanova-Katny and others 2011; Montesinos-Navarro and others 2019).

Azorella cushions were positively associated with SWC, %C and %N. The improvement of soil conditions by nurse plants, and its effect on soil microorganisms, is well described in the literature and largely explained by the amelioration of microclimatic conditions and the accumulation of litter (for example, Pugnaire and others 2004; Cavieres and others 2007; Goberna and others 2014; Mihoc and others 2016; Rodríguez-Echeverría and others 2013, 2016). These conditions created by nurse species result in increases in soil C and water content, thus promoting microbial growth and, therefore, microbial abundance (Goberna and others 2014; Hortal and others 2015; Rodríguez-Echeverría and others 2016; Lozano and others 2017). We expected this positive effect to be more important in the harsher sites where abiotic conditions may be more limiting for microbial growth. However, this was not confirmed by the results, since the effect of *Azorella* on soil microbes was clearer and more important in the two mesic sites, Farellones and Paine. This result might be partially explained by both arid sites sharing the same

Azorella species, *A. compacta*, which could have a lower facilitation effect on soil microbes than the other two *Azorella* species (Pugnaire and others 2020). However, the larger overlap between *Azorella* and bare soil samples in the most arid site, Atacama, suggests that plant identity is not solely responsible for the lower effect of *Azorella* on soil microbial communities with arid conditions. Therefore, our data might indicate the existence of an aridity threshold above which plants cannot significantly affect microbial communities, and thus, they do not change significantly between microhabitats. A similar unexpected pattern was previously found in the association of plants with the dominant cushion *Thylacospermum caespitosum* in an elevational gradient in a dry Himalayas ecosystem. Under the harsher conditions, cushions did not provide a better growth microhabitat (Dvorský and others 2013). The lower nutrient content and the little thermal amelioration provided by the cushions in such extreme environmental conditions, together with a smaller production of root exudates, can partially explain this lack of effect of cushions on other plants and on soil microbial communities. Accordingly, we found minor differences in N content between bare soil and cushions in Lauca and Atacama in comparison with the two mesic sites. Also, a lower microbial diversity in the rhizosphere of four dominant native species than in bulk soil has also been reported in high-elevation Atacama tussock grasses (Fernández-Gómez and others 2019). This could be explained by the strong filter imposed by the extreme environmental conditions in the more arid sites which might result in soil microbial communities well adapted to stress that cannot thrive in the ameliorated conditions beneath the cushions (Maestre and others 2015; Neilson and others 2017).

Although an increase in aridity can decrease bacterial and fungal abundance and diversity (Azua-Bustos and others 2012; Maestre and others 2015; Neilson and others 2017), there is a lack of correlation between precipitation and the diversity of soil prokaryotic communities along broad climatic gradients (Angel and others 2010). This might be explained because soil microbe responses to aridity are not directly related to water shortage, but mainly driven by reductions in soil organic C associated with aridity (Maestre and others 2015; Neilson and others 2017). Still, prokaryotic diversity was limited by the extremely high levels of aridity in Atacama (Skujins 1984).

Notably, Proteobacteria were clearly associated with aridity, being the most abundant and diverse

phylum in Atacama, in contrast to other studies in the Atacama hyperarid region (Maza and others 2019), while Actinobacteria were dominant in Paine, the most humid site in our gradient. This result contrasts with previous reports showing a higher abundance of Actinobacteria in soils from arid sites (Neilson and others 2012; see also Mandakovic and others 2018 for the Atacama desert), suggesting that this pattern might not always apply to high-elevation systems. Our results are, however, in agreement with a global meta-analysis, showing that Actinobacteria is the dominant phylum in soils of the Southern Hemisphere (Delgado-Baquerizo and others 2018). Regarding soil fungi, Ascomycota was the dominant group in all sites, with a trend of increasing abundance with aridity. Mean annual precipitation has been previously described as the main predictor of fungal diversity (Tedersoo and others 2014), but a clear effect of aridity on fungal abundance or diversity was not found in this study.

Remarkably, the yeast *Naganishia friedmannii* (Basidiomycota) which was found associated with aridity in our study has been reported as the dominant taxa in eukaryotic communities in volcanoes over an altitude of 6000 m in Atacama (Schmidt and others 2017). The microfungi *Thelebolus globosus,* associated with aridity, as well as the yeasts *Guehomyces pullulans* and *Mrakia frigida* which were driven mainly by mean temperature, have also been previously found in Antarctic and high-elevation soils (Margesin and others 2002; Furbino and others 2014; Cavello and others 2017).

Interestingly, despite the high UV-B radiation and aridity levels, bare soils in Lauca presented the highest values of total soil DNA concentration, diversity and abundance of the three studied microbial groups. It is generally accepted that exposure to high levels of solar UV-B radiation can have a negative effect on microbial biomass and activity (Björn 2002; Johnson and others 2002; Bornman and others 2015), but microorganisms' susceptibility to UV-B varies greatly; thus, this result deserves further investigation. UV-B radiation was a particularly important predictor for fungal taxa, suggesting a higher sensitivity of fungi than bacteria to UV-B radiation (Duguay and Klironomos 2000; Björn 2002), although a recent report suggests that UV-B influences bacterial communities globally (Delgado-Baquerizo and others 2019). Besides its mutagenic effect, UV-B radiation also acts as a photomorphogenic clue in fungi, inducing sporulation in many species (Paul and Gwynn-Jones 2003) which could explain, at least partially, our results.

Taken together, our results suggest that *Azorella*, along with aridity and UV-B radiation, are the major drivers of the distribution, composition and diversity of soil microbial communities in the studied ecosystems of the Chilean Andes. These high-elevation ecosystems are unique, vulnerable habitats that might hold a yet undescribed exclusive diversity of soil microbes. This work contributes to understanding the factors driving the composition of microbial communities, particularly the role of cushion plants, which is of utmost importance for preserving the functionality of high-elevation ecosystems threatened by climate change.

REFERENCES

Alfaro FD, Manzano M, Marquet PA, Gaxiola A. 2017. Microbial communities in soil chronosequences with distinct parent material: the effect of soil pH and litter quality. Journal of Ecology 105: 1709-1722.

Andrews S. 2010. FastQC: a quality-control tool for high-throughput sequence data. Babraham Institute, Cambridge, United Kingdom.

Angel R, Soares MIM, Ungar ED, Gillor O. 2010. Biogeography of soil archaea and bacteria along a steep precipitation gradient. The ISME Journal 4: 553–63.

Antonsson H, Bjork RG, Molau U. 2009. Nurse plant effect of the cushion plant *Silene acaulis* (L.) Jacq. in an alpine environment in the sub-arctic Scandes, Sweden. Plant Ecology and Diversity 2: 17–25.

Archer E. 2013. Estimate permutation p-values for importance metrics. R package version 1.5.2

Arroyo MTK, Cavieres LE, Peñaloza A, Arroyo-Kalin MA. 2003. Positive associations between the cushion plant *Azorella monantha* (Apiaceae) and alpine plant species in the Chilean Patagonian Andes. Plant Ecology 169: 121-129.

Armas C, Ordiales R, Pugnaire FI. 2004. Measuring plant interactions: a new comparative index. Ecology 85: 2682–2686.

Aubert S, Boucher F, Lavergne S, Renaud J, Choler P. 2014. 1914–2014: a revised worldwide catalogue of cushion plants 100 years after Hauri and Schröter. Alpine Botany 124:59–70.

Azua-Bustos A, Urrejola C, Vicuña R. 2012. Life at the dry edge: Microorganisms of the Atacama Desert. FEBS Letters 586: 2939–2945.

Badano, EI, Jones, CG, Cavieres, LA, Wright, JP. 2006. Assessing impacts of ecosystem engineers on community organization: a general approach illustrated by effects of a high-Andean cushion plant. Oikos 115: 369-385.

Bertness MD, Callaway RM. 1994. Positive interactions in communities. Trends in Ecology and Evolution 9: 191–193.

Björn LO. 2002. Effects of Ultraviolet-B Radiation on Terrestrial Organisms and Ecosystems with Special Reference to the Arctic. In: Hessen DO, editor. UV Radiation and Arctic Ecosystems. Springer: Berlin, Heidelberg. pp 93–121.

Bornman JF, Barnes PW, Robinson SA, Ballaré CL, Flint SD, Caldwell MM. 2015. Solar ultraviolet radiation and ozone depletion-driven climate change: effects on terrestrial ecosystems. Photochemical & Photobiological Sciences 14: 88–107.

Boucher FC, Lavergne S, Basile M, Choler P, Aubert S. 2016. Evolution and biogeography of the cushion life form in angiosperms. Perspectives in Plant Ecology, Evolution and Systematics 20: 22-31.

Breiman L. 2001. Random forest. Machine Learning 45: 5.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI and others. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7: 335–336.

Casanova-Katny MA, Torres-Mellado GA, Palfner G, Cavieres LA. 2011. The best for the guest: high Andean nurse cushions of *Azorella madreporica* enhance arbuscular mycorrhizal status in associated plant species. Mycorrhiza 21: 613–22

Cavello I, Albanesi A, Fratebianchi D, Garmedia G, Vero S, Cavalitto S. 2017. Pectinolytic yeasts from cold environments: novel findings of *Guehomyces pullulans, Cystofilobasidium infirmominiatum* and *Cryptococcus adeliensis* producing pectinases. Extremophiles 21: 319.

Cavieres L, Arroyo MTK, Peñaloza A, Molina-Montenegro M, Torres C. 2002. Nurse effect of *Bolax gummifera* cushion plants in the alpine vegetation of the Chilean Patagonian Andes. Journal of Vegetation Science 13: 547–554

Cavieres LA, Badano EI, Sierra-Almeida A, Molina-Montenegro MA. 2007. Microclimatic modifications of cushion plants and their consequences for seedling survival of native and non-native herbaceous species in the high Andes of central Chile. Arctic, Antarctic, and Alpine Research 39: 229-236.

Cavieres LA, Brooker RW, Butterfield BJ, Cook BJ, Kikvidze Z, Lortie CJ, Michalet R, Pugnaire FI, Schöb C, Xiao S, et al. 2014. Facilitative plant interactions and climate simultaneously drive alpine plant diversity. Ecology Letters 17: 193–202.

Chang S, Chen J, Su J, Yang Y, Sun H. 2018. Seasonal comparison of bacterial communities in rhizosphere of alpine cushion plants in the Himalayan Hengduan Mountains. Plant Diversity 40: 209-216.

Chen J, Yang Y, Stocklin J, Cavieres LA, Peng D, Li Z, et al. 2014. Soil nutrient availability determines the facilitative effects of cushion plants on other plant species at high elevations in the south-eastern Himalayas. Plant Ecology & Diversity 8: 199-210.

Chen Y, Ding J, Peng Y, Li F, Yang G, Liu L, Qin S, Fang K, Yang Y. 2016. Patterns and drivers of soil microbial communities in Tibetan alpine and global terrestrial ecosystems. Journal of Biogeography 43: 2027-2039.

Comeau AM, Douglas GM, Langille MGI. 2017. Microbiome Helper: a Custom and Streamlined Workflow for Microbiome Research. MSystems 2: e00127-16.

Csardi G, Nepusz T. 2006. The igraph software package for complex network research. InterJournal, Complex Systems 1695.

Delgado-Baquerizo M, Oliverio AM, Brewer TE, Benavente-González A, Eldridge DJ, Bardgett RD, Maestre FT, Singh BK, Fierer N. 2018. A global atlas of the dominant bacteria found in soil. Science 359: 320–325.

Delgado-Baquerizo M, Eldridge DJ. 2019. Cross-biome drivers of soil bacterial alpha diversity on a worldwide scale. Ecosystems, 22, 1220–1231.

di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. 2017. InfoStat versión 2017. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar

Donhauser J, Frey B. 2018. Alpine soil microbial ecology in a changing world. FEMS Microbiology Ecology 94: fiy099.

Duguay KJ, Klironomos JN. 2000. Direct and indirect effects of enhanced UV-B radiation on the decomposing and competitive abilities of saprobic fungi. Applied Soil Ecology 14: 157–164.

Dvorský M, Doležal J, Kopecký M, Chlumská Z, Janatková K, Altman J, de Bello F, Řeháková K. 2013. Testing the stress-gradient hypothesis at the roof of the world: effects of the cushion plant *Thylacospermum caespitosum* on species assemblages. PLoS One 8: e53514.

Einen J, Thorseth IH, Øvreas L. 2008. Enumeration of Archaea and Bacteria in seafloor basalt using real-time quantitative PCR and fluorescence microscopy. FEMS Microbiology Letters 282: 182–187.

Fernández-Gómez B, Maldonado J, Mandakovic D, Gaete A, Gutiérrez RA, Maass A, Cambiazo V, González M. 2019. Bacterial communities associated to Chilean altiplanic native plants from the Andean grassland soils. Scientific Reports 9: 1042.

Fierer N, Jackson RB. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Science of the United States of America 103: 626-631.

Fierer N, Strickland MS, Liptzin D, Bradford MA, Cleveland CC. 2009. Global patterns in belowground communities. Ecology Letters 12: 1238–49.

Fierer N. 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nature Reviews Microbiology 15: 579–590.

Fortmann-Roe S. 2015. Consistent and clear reporting of results from diverse modeling techniques: The A3 Method. Journal of Statistical Software 66: 1-23.

Furbino LE, Godinho VM, Santiago IF, Pellizari FM, Alves TM, Zani CL, Junior PA, Romanha AJ, Carvalho AG, Gil LH, Rosa CA, Minnis AM, Rosa LH. 2014. Diversity patterns, ecology and biological activities of fungal communities associated with the endemic macroalgae across the Antarctic peninsula. Microbial Ecology 67: 775-87.

Geremia RA, Puşcaş M, Zinger L, Bonneville J, Choler P. 2016. Contrasting microbial biogeographical patterns between anthropogenic subalpine grasslands and natural alpine grasslands. New Phytologist 209: 1196-1207.

Goberna M, Navarro-Cano JA, Valiente-Banuet A, García C, Verdú M. 2014. Abiotic stress tolerance and competition-related traits underlie phylogenetic clustering in soil bacterial communities. Ecology Letters 17: 1191–1201.

Gordon A. 2009. FASTX-Toolkit: FASTQ/A short-reads pre-processing tools. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. http://hannonlab.cshl.edu/fastx_toolkit/

Hortal S, Bastida F, Moreno JL, Armas C, García C, Pugnaire FI. 2015. Benefactor and allelopathic shrub species have different effects on the soil microbial community along an environmental severity gradient. Soil Biology and Biochemistry 88: 48-57.

Huber KJ, Geppert A M, Gross U, Luckner M, Wanner G, Cooper P, Abakah J, Janssen I, Overmann J. 2017. *Aridibacter nitratireducens* sp nov., a member of the family Blastocatellaceae, class Blastocatellia, isolated from an African soil. International Journal of Systematic and Evolutionary Microbiology 67: 4487-4493

Johnson D, Campbell CD, Lee JA, Callaghan TV, Gwynn-Jones, D. 2002. Arctic microorganisms respond more to elevated UV-B radiation than CO2. Nature 416: 82–83.

Jost, L. (2006). Entropy and diversity. Oikos, 113, 110– 116.

Kivlin SN, Hawkes CV, Treseder, KK. 2011. Global diversity and distribution of arbuscular mycorrhizal fungi. Soil Biology & Biochemistry 43: 2294–2303.

Kõjalg U, Larsson KH, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vrålstad T, Ursing BM. 2005. UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytologist 166: 1063-1068

Kopylova E, Noé L, Touzet H. 2012. SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. Bioinformatics 28: 3211–3217.

Körner C. 2002. Mountain biodiversity, its causes and function: an overview. In: Körner C. & Spehn E, eds. Mountain biodiversity: a global assessment. Parthenon Publishing.

Lazzaro A, Hilfiker D, Zeyer J. 2015. Structures of microbial communities in alpine soils: seasonal and elevational effects. Frontiers in Microbiology 6: 1330.

Liaw A, Wiener M. 2002. Classification and regression by RandomForest. R News, 2/3: 18

Lozano, Y. M., C. Armas, S. Hortal, F. Casanoves, F. I. Pugnaire, 2017. Disentangling above-belowground facilitation drivers in arid environments: the role of soil microorganisms, soil properties and microhabitat. New Phytologist, 216: 1236-1246.

Maestre FT, Delgado-Baquerizo M, Jeffries TC, Eldridge DJ, Ochoa V. 2015. Increasing aridity reduces soil microbial diversity and abundance in global drylands. Proceedings of the National Academy of Science of the United States of America 112: 15684–15689.

Mandakovic, D, Rojas C, Maldonado J, Latorre M, Travisany D, Delage E, Bihouée A, and others 2018. Structure and co-occurrence patterns in microbial communities under acute environmental stress reveal ecological factors fostering resilience. Scientific Reports, 8: 5875.

Margesin R, Zacke G, Schinner F. 2002. Characterization of heterotrophic micro-organisms in alpine glacier cryoconite. Arctic, Antarctic and Alpine Research 34: 88– 93.

Maza F, Maldonado J, Vásquez-Dean J, Mandakovic D, Gaete A, Cambiazo V, González M. 2019. Soil bacterial communities from the Chilean Andean highlands: Taxonomic composition and culturability. Frontiers in Bioengineering and Biotechnology 5: 10.

Mercier C, Boyer F, Bonin A, Coissac E. 2013. SUMATRA and SUMACLUST: fast and exact comparison and clustering of sequences. http://metabarcoding.org/sumatra/.

Mihoč MAK, Giménez-Benavides L, Pescador DS, Sánchez AM, Cavieres LA, Escudero A. 2016. Soil under nurse plants is always better than outside: a survey on soil amelioration by a complete guild of nurse plants across a long environmental gradient. Plant and Soil 408: 31–41.

Minor MA, Babenko AB, Ermilov SG, Khaustov,AA, Makarova OL. 2016. Effects of cushion plants on high-altitude soil microarthropod communities: cushions increase abundance and diversity of mites (Acari), but not springtails (Collembola). Arctic, Antarctic, and Alpine Research 48: 485-500.

Molenda O, Reid A, Lortie CJ. 2012. The alpine cushion plant *Silene acaulis* as foundation species: a bug's-eye view to facilitation and microclimate. PLoS One 7: e37223

Molina-Montenegro MA, Badano EI, Cavieres LA. 2006. Cushion plants as microclimatic shelters for two ladybird beetles species in alpine zone of central Chile. Arctic, Antarctic, and Alpine Research 38: 224-227.

Montesinos-Navarro A, Valiente-Banuet A, Verdú M. 2019. Mycorrhizal symbiosis increases the benefits of plant facilitative interactions. Ecography, 42: 447-455

Neilson JW, Quade J, Ortiz M, Nelson WA, Legatzki A, Tian F, LaComb M, Betancourt JL, Wing RA, Soderlund CA, Maier RM. 2012. Life at the hyperarid margin: novel bacterial diversity in arid soils of the Atacama Desert, Chile. Extremophiles 16: 553.

Neilson JW, Califf K, Cardona C, Copeland A, van Treuren W, Josephson KL, Knight R, Gilbert JA, Quade J, Caporaso JG, Maier RM. 2017. Significant impacts of increasing aridity on the arid soil microbiome. MSystems 2: e00195-16.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20: 241–248.

Paul ND, Gwynn-Jones D. 2003. Ecological roles of solar UV radiation: Towards an integrated approach. Trends in Ecology and Evolution 18: 48–55.

Pauli H, Gottfried M, Dullinger S, Abdaladze O, Akhalkatsi M, Alonso JLB, Coldea G, Dick J, Erschbamer B, Calzado RF, et al. 2012. Recent Plant Diversity Changes on Europe's Mountain Summits. Science 336: 353–355.

Prevost-Boure N, Christen R, Dequiedt S, Mougel C, Lelievre M, Jolivet C, Shahbazkia HR, Guillou L, Arrouays D, Ranjard L. 2011. Validation and application of a PCR primer set to

quantify fungal communities in the soil environment by real-time quantitative PCR. PLoS ONE 6: e24166.

Pugnaire FI, Armas C, Valladares F. 2004. Soil as a mediator in plant-plant interactions in a semi-arid community. Journal of Vegetation Science 15: 85-92.

Pugnaire FI, Morillo, JA, Armas C, Rodríguez-Echeverría S, Gaxiola A. 2020. Azorella compacta: survival champions in extreme, high-elevation environments. Ecosphere 11:e03031.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acid Research 41: D590-D596.

Raunkiaer C. 1934. The Life Forms of Plants and Statistical Plant Geography. Clarendon press.

Řeháková K, Chroňáková A, Krištufek V, Kuchtová B, Čapková K, Scharfen J, Čapek P, Doležal J. 2015. Bacterial community of cushion plant Thylacospermum ceaspitosum on elevational gradient in the Himalayan cold desert. Frontiers in Microbiology 6: 1–16.

Rodríguez-Echeverría S, Armas C, Pistón N, Hortal S, Pugnaire FI. 2013. A role for belowground biota in plant-plant facilitation. Journal of Ecology 101: 1420-1428.

Rodríguez-Echeverría S, Lozano YM, Bardgett RD. 2016. Influence of soil microbiota in nurse plant systems. Functional Ecology 30: 30–40.

Rognes T, Flouri T, Nichols B, Quince C, Mahé F. 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4: e2584.

Roy J, Albert CH, Ibanez S, Saccone P, Zinger L, Choler P, Clément JC, Lavergne S, Geremia RA. 2013. Microbes on the cliff: alpine cushion plants structure bacterial and fungal communities. Frontiers in Microbiology 4: 64.

Roy J, Bonneville JM, Saccone P, Ibanez S, Albert CH, Boleda M, Gueguen M, Ohlmann M, Rioux D, Clément J-C, Lavergne S, Geremia RA. 2018. Differences in the fungal communities nursed by two genetic groups of the alpine cushion plant, Silene acaulis. Ecology and Evolution 8: 11568–11581.

Sánchez-Cuevas S. 2009. Comportamiento de la radiación ultravioleta en Chile. Dirección Meteorológica de Chile. pp. 57.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied Environmental Microbiology 75: 7537–7541.

Schmidt SK, Vimercati L, Darcy JL, Arán P, Gendron EMS, Solon AJ, Porazinska D, Dorador C. 2017. A Naganishia in high places: functioning populations or dormant cells from the atmosphere? Mycology 8: 153-163.

Schöb, C., C. Armas, F.I. Pugnaire. 2013. Direct and indirect interactions co-determine species composition in nurse plant systems. Oikos 122: 1371–1379.

Schöb C., I. Prieto, C. Armas, F.I. Pugnaire. 2014. Consequences of facilitation: one plant's benefit is another plant's cost. Funtional Ecology 28: 500–508.

Siles JA, Margesin R. 2016. Abundance and diversity of bacterial, archaeal, and fungal communities along an altitudinal gradient in alpine forest soils: what are the driving factors? Microbial Ecology 72: 207-220

Siles JA, Margesin R. 2017. Seasonal soil microbial responses are limited to changes in functionality at two Alpine forest sites differing in altitude and vegetation. Scientific Reports 7: 2204.

Skujins J. 1984. Microbial ecology of desert soils. Advances in Microbial Ecology 7: 49–91.

Steinbauer MJ, Grytnes JA, Jurasinski G, Kulonen A, Lenoir J, Pauli H, et al. 2018. Accelerated increase in plant species richness on mountain summits is linked to warming. Nature 556: 231-234.

Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, Ruiz LV, Vasco-Palacios AM, Thu PQ, Suija A, et al. 2014. Global diversity and geography of soil fungi. Science 346: 1256688.

van Elsas JD, Chiurazzi M, Mallon CA, Elhottovã D, Krištůfek V, Salles JF. 2012. Microbial diversity determines the invasion of soil by a bacterial pathogen. Proceedings of the National Academy of Sciences of the United States of America 24: 1159–1164

Walters W, Hyde ER, Berg-Lyons D, Ackermann G, Humphrey G, Parada A, Gilbert JA, Jansson JK, Caporaso JG, Fuhrman JA, Apprill A, Knight R. 2016. Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys. mSystems 1: 915.

Wang C, Richard M, Ziyang L, Xingpei J, Xiangtai W, Gaosen Z, Lizhe A, Shuyan C, Sa X. 2020. Disentangling large- and small-scale abiotic and biotic factors shaping soil microbial communities in an alpine cushion plant system. Frontiers in Microbiology, 111: 925

Wei CL, Rowe GT, Briones EE, Boetius A, Soltwedel T, Caley MJ, Soliman Y, Huettmann F, Qu F, Yu Z, et al. 2010. Global patterns and predictions of seafloor biomass using random forests. PLoS ONE 5: e15323.

Yang Y, Niu Y, Cavieres LA, Sun H. 2010. Positive associations between the cushion plant Arenaria polytrichoides (Caryophyllaceae) and other alpine plant species increase with altitude in the Sino- Himalayas. Journal of Vegetation Science 21: 1048–1057

Zhang J, Kobert K, Flouri T, Stamatakis A. 2014. PEAR: a fast and accurate Illumina paired-end reAd mergeR. Bioinformatics 30: 614–620.

Zhang Y, Cong J, Lu H, Li G, Xue Y, Deng Y, Li H, Zhou J, Li D. 2015. Soil bacterial elevational pattern. Microbial Biotechnology 8: 739-746.