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Composition and Potential Functions of Rhizobacterial Communities in a Pioneer Plant from Andean Altiplano

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Abstract: Plant microbiota that associate with pioneer plants are essential to their growth and adaptation to harsh conditions found in the Central Volcanic Zone of the Andes. In this sense, the rhizosphere of pioneer species represents a unique opportunity to examine how bacterial communities are recruited and support the growth of plants under abiotic stress conditions, such low nutrient availability, high solar irradiation, water scarcity, soil salinity, etc. In this study, we explored the community composition and potential functions of rhizobacteria obtained from specimens of *Parastrephia quadrangularis* (Meyen) Cabrera, commonly called Tola, grown on the slopes of the Guallatiri, Isluga, and Lascar volcanoes in the Atacama Desert of Chile by using 16S rRNA amplicon sequencing. Sequence analysis showed that the Actinobacteria, Proteobacteria, Acidobacteria, and Bacteroidetes were the most abundant phyla of the rhizobacterial communities examined. A similar diversity, richness, and abundance of OTUs were also observed in rhizosphere samples obtained from different plants. However, most of OTUs were not shared, suggesting that each plant recruits a specific rhizobacterial communities independently of volcanoes slope. Analyses of predicted functional activity indicated that the functions were mostly attributed to chemoheterotrophy and aerobic chemoheterotrophy, followed by nitrogen cycling (nitrate reduction and denitrification), and animal parasites or symbionts. In addition, co-occurrence analysis revealed that complex rhizobacterial interactions occur in *P. quadrangularis* rhizosphere and that members of the *Patulibacteraceae* comprise a keystone taxon. This study extends our understanding on the composition and functions of the rhizobiome, which is pivotal for the adaptability and colonization of pioneer plant to harsh conditions of the Atacama Desert, widely recognized as the driest place on planet Earth.

Keywords: bacterial community; rhizosphere; volcanoes; Andean Altiplano

1. Introduction

Pioneer plants are those that first colonize new or disturbed sites or raw mineral soils, which are created by natural or anthropogenic origin [1]. Microbial interactions at the roots level are crucial for the establishment and colonization of pioneer plant species under extreme conditions [2]. The rhizosphere is the area recognized as the most relevant active zone between roots and soil, whose microbe interactions play a crucial role in nutrient

acquisition, biogeochemical cycling, environmental functions, and carbon sequestration [3]. Root-inhabiting microbes, known as rhizosphere microorganisms or rhizobiome, are specifically adapted to their host and local soil conditions and thereby contribute to the adaptation and survival of their plant hosts to biotic and abiotic stress conditions [4], particularly in extreme environments, such as those found in Alpine ecosystems. In this context, rhizosphere bacteria with nitrogen (N) fixation ability promote the growth of pioneer species by providing a major required N source and by secreting plant growth hormones [4]. Pioneer plant species are also influenced by bacterial functional groups that help in the acquisition of other essential inorganic nutrients, such phosphorus (P), sulfur (S), Potassium (K), and calcium (Ca) [5]. In addition, soil type and plant genotype each impact bacterial root colonization and together establish and maintain core microbiota in the rhizobiome [6]. Interestingly, several studies have observed that the host plant assembles specific bacterial communities, often irrespective of the geographic region [7,8].

The Andean Altiplano is considered as a pristine and arid ecoregion within in the Central Volcanic Zone (CVZ) of the Andes [9] and is shared by southern Peru, western Bolivia, and northern Argentina and Chile [10]. This region is characterized by extreme weather and geographic conditions, including high altitude areas with scarce precipitation and elevated evaporation rates, thermal fluctuations, and solar radiation [11], which leads in the presence of diverse and unique highland ecosystems [12]. The CVZ is composed by several stratovolcanoes with high fumarolic activity, resulting in mineral deposition in surrounding soils [13]. Among them, the Guallatiri (6071 m above sea level [MASL]), Isluga (5550 MASL), and Lascar volcanoes (5000 MASL) are considered as the most active in the Chilean Altiplano [14].

Rocky volcanic slopes are complex extreme habitats for plant colonization due to the incidence of solar radiation and the coarse soil nature with low water retention ability [15]. Pioneer and later-successional plants growing in the Altiplano have an adaptative relationship with patterns of water and temperature stress [16]. Therefore, selective pressure and coevolution of bacterial communities provides a unique opportunity to examine bacteria role in plants fitness and survival in nutrient-depleted soils under extreme conditions [17].

Plants belonging to the family Asteraceae are pioneer plants and are widely distributed in arid and semiarid regions of the world, including the Altiplano. Members of genus *Parastrephia* are commonly present in Altiplano shrubland vegetation and are capable of growing in oligotrophic and scarce nutrient environments at altitudes from 3500 to 5000 MASL, evidencing a high adaptability and tolerance to drought stress [16].

Over the last few decades, researchers have examined the microbial ecology of the Altiplano pioneer plants and have expressed interest in their biotechnological potential [18–22]. We hypothesized that Altiplano plant species alone, rather than sampling sites, determines the composition of associated bacterial communities, resulting in plant species specificity for shaping the rhizobiome. Therefore, in this study, we explored the composition of rhizobacterial community associated with *Parastrephia quadrangularis* (Meyen) Cabrera grown on the slope of the Guallatiri, Isluga, and Lascar volcanoes by using 16S rRNA Amplicon Sequencing. In addition, we also evaluated the putative functions of that rhizobacterial community that are likely involved in nutrient acquisition and stress tolerance by *P. quadrangularis* growing in Chilean Andean Altiplano conditions.

2. Materials and Methods

2.1. Sampling Sites

The sampling was carried out in Chile on the slopes of the Guallatiri volcano (18°29′2.98″ S, 69°8′24.36″ W), located in Arica Parinacota Region, the Isluga volcano (19°12′12.20″ S, 68°46′17.53″ W), located in the Tarapacá Region, and the Lascar volcano (23°21′12.00″ S, 67°48′40.64″ W), located in the Antofagasta Region. All sites are characterized by low rates of annual precipitation (<250 mm year^{-1}) and extreme temperature variation (from −10 to 35 °C). The vegetation coverage in each volcanic slope was 60%, and the main plant species type is Altiplano grassland, including members of *Festuca* spp.

and *Stipa* spp. The rhizosphere soils samples of *P. quadrangularis* (Meyen) were collected in December 2018 from three random locations around of the slopes of each volcano. In each site, three quadrants (5 × 5 m per quadrant) were established as three rhizosphere cores. The sampling locations and characteristics of each site are described in Figure 1. In each quadrant, three plant with their rhizosphere (soil closely bound to the roots) were randomly collected and mixed to form a composite soil rhizosphere sample. During sampling, the rhizosphere soil from each plant was carefully removed by excavating the plants roots zone to a depth of 0–20 cm using a cleaned spade and placing the composite soil samples into sterile polyethylene sterile bags. Soil samples were refrigerated at 4 °C, immediately transported to the laboratory, and stored at −80 °C until used for DNA extraction. For determination of chemical properties of rhizosphere soil in each site, soil subsamples from the three composited sampled plants were mixed, and a unique sample (three composited sample mixed) analyzed.
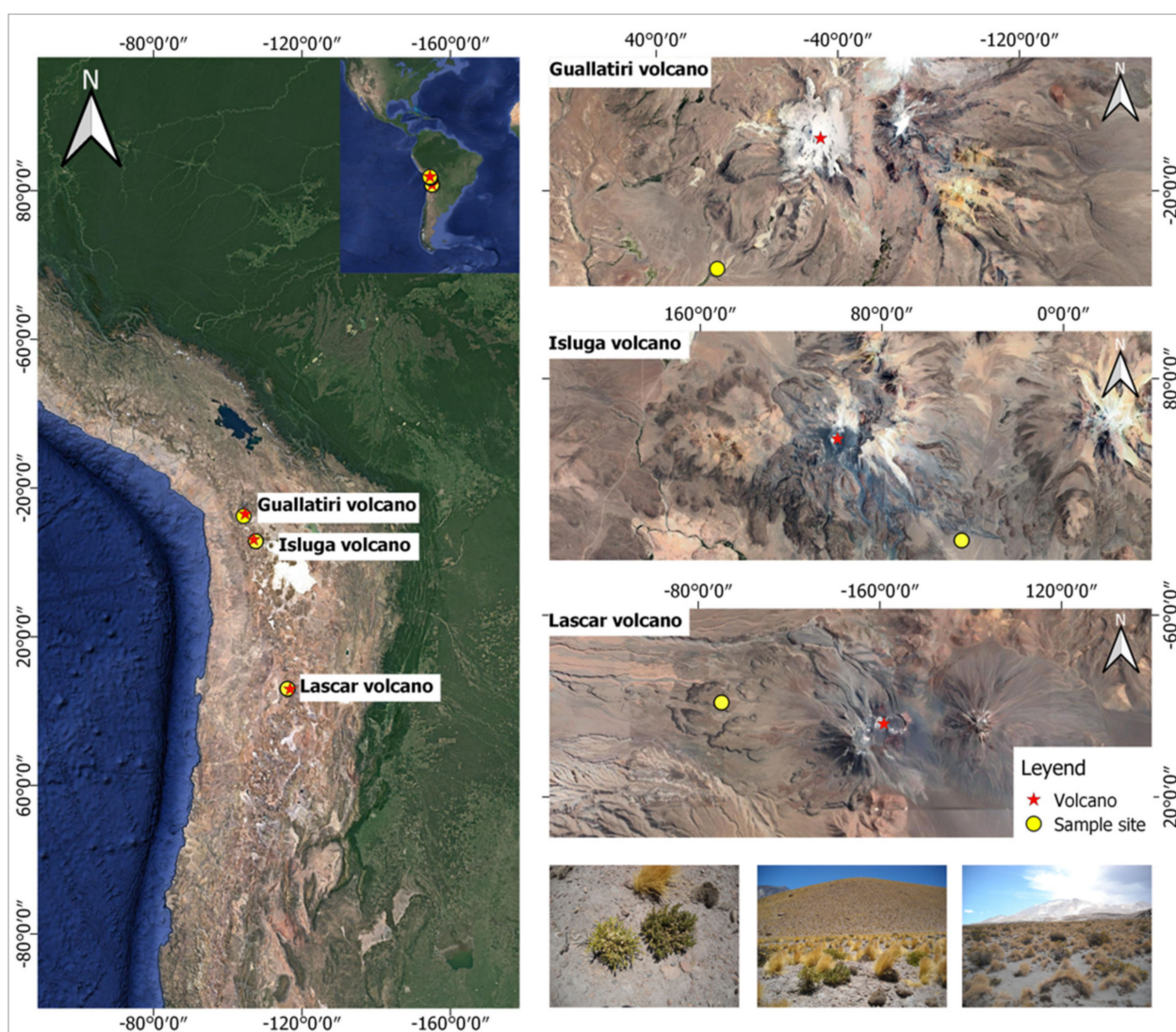

Figure 1. Map of Central Volcanic Zone of the Chilean Altiplano showing the location of sample sites. Map was taken using Google Maps (Map data: Google, Maxar Technologies), volcanoes and sampling sites are shown with red and yellow markers, respectively.

Soil pH was determined in 1:2.5 soil: deionized water slurry. Inorganic N was extracted with 2 M KCl and NO_3^--N determined by the Devarda alloy distillation method [23]. Organic matter content was estimated by using the wet digestion method [24]. Available P

(P_{Olsen}) was extracted using 0.5 M Na-bicarbonate and measured by using the molybdate method [25] (Table 1).

Table 1. Characteristics of sampling sites and total bacterial community of rhizosphere soils from *P. quadrangularis* plants collected from Guallatiri, Isluga, and Lascar volcanoes.

Samples	Guallatiri	Isluga	Lascar
Sampling point			
Location coordinates	18°29′2.98″ S 69°8′24.36″ W	19°12′12.20″ S 68°46′17.53″ W	23°21′12.00″ S 67°48′40.64″ W
Altitude (MASL) [a]	4386	3983	4353
Chemical properties			
N (mg kg $^{-1}$)	10	10	12
P (mg kg $^{-1}$)	23	37	22
K (mg kg $^{-1}$)	145	207	217
Organic matter (g kg $^{-1}$)	1.48	1.62	1.82
pH$_{H2O}$	5.59	4.77	5.93
CEC (cmol+ kg $^{-1}$) ¥	2.05	2.26	3.47
Sum of bases	1.91	1.98	3.44
Cu (mg kg $^{-1}$)	0.80	0.29	2.97
Zn (mg kg $^{-1}$)	0.30	0.35	0.70
S (mg kg $^{-1}$)	13	98	44
Richness			
Sobs [†]	1325 ± 510 [A]	1137 ± 437 [A]	830 ± 13 [A]
ACE [‡]	1478 ± 630 [A]	1281 ± 503 [A]	912 ± 10 [A]
Chao1	1463 ± 643 [A]	1249 ± 518 [A]	889 ± 13 [A]
Alpha diversity			
Coverage (%)	98.73 ± 0.98 [A]	98.94 ± 0.66 [A]	99.35 ± 0 [A]
Shannon	6.09 ± 0.42 [A]	5.86 ± 0.44 [A]	5.31 ± 0.02 [A]
Simpson	0.007 ± 0.005 [A]	0.009 ± 0.005 [A]	0.014 ± 0.002 [A]

[a] Altitude in meters above sea level (MASL). ¥ Calculated as (Al × 100)/CEC. where CEC = cation exchange capacity = Σ (K. Ca. Mg. Na. and Al. † Sobs: number of OTUs observed at 97% similarity. ‡ ACE: abundance-based coverage estimate. * The similar letter denote no significant differences ($p \leq 0.05$) by ANOVA followed by Tukey's post-hoc test.

2.2. DNA Extraction, Library Preparation and 16S rRNA Amplicon Sequencing

Total genomic DNA was extracted from all rhizosphere samples. In order to improve total DNA extraction, triplicate samples (~5 g) were taken from each rhizosphere samples and sonicated at 120 kHz in 20 mL of sodium phosphate buffer 0.1 M, pH 8 [26]. The obtained pellets were processed with DNeasy® PowerSoil DNA isolation kits (QIAGEN, Carlsbad, CA, USA) using the PowerLyzer® 24 homogenizer (QIAGEN Carlsbad, CA, USA) bead-beating protocol, according to the manufacturer's instructions. The quality and quantity of DNA extracts were measured using a Qubit4™ (Thermo Fisher Scientific, Waltham, MA, USA) and using broad range DNA, RNA, and protein assay kits to assure the presence of only DNA after extraction. The V4 hypervariable region of the 16S rRNA gene was amplified, for bacteria and archaea, by using primer set 515F (5′-GTG CCA GCM GCC GCG GTA A-3′) and 806R (5′-GGA CTA CHV GGG TWT CTA AT-3′). This primer set was chosen due to its species richness and diversity coverages [27]. The primers included the specific Illumina adapters, and the dual indexing method was used for the amplicon library construction [28].

All amplification products examined on 1.5% agarose gels fragments were purified using the Agencourt AMPure XP system (Beckman Coulter, Brea, CA, USA), following the manufacturer's instructions. A second PCR was used to apply dual indexes and Illumina sequencing adapters Nextera XT Index Primers (Illumina, San Diego, CA, USA), using 8 cycles PCR (16S Metagenomic Sequencing Library Preparation, Illumina). The amplicon libraries were purified using Agencourt AMPure XP system (Beckman Coulter, Inc., Indianapolis, IN, USA), and the quality control was performed on a Typestation™

4150 platform (Agilent Technologies, Santa Clara, CA, USA). Amplicons were pooled and paired—end sequenced, to a read length of 300 bp, on the Illumina MiSeq™ platform (Illumina, Inc.) with the support of the University of Minnesota Genomics Center (UMGC, Minneapolis, MN, USA).

2.3. Bioinformatic Processing of the Sequences and Statistical Analysis of the Data

The sequencing data sets were analyzed using mothur program ver. 1.34.0 (https://www.mothur.org, accessed on 4 September 2021). Raw sequence reads were trimmed and processed using SHI7 to obtain high quality data (QC > 35) [29–31]. The trimmed sequenced were aligned into operational taxonomic unit (OTUs), chimeric sequences were removed with UCHIME [29], and sequences with >97% similarity were clustered with an open-reference de novo approach to reduce misclustering errors and to preserve all sample sequences [32]. Non-related sequence reads (e.g., chloroplast and mitochondria) were removed via QIIME [33], and data was rarefied to 18,000 reads for biodiversity analysis. Taxonomic assignment was done using the Greengenes database and NINJA-OPS [34]. Richness (OTUs observed, abundance-based coverage estimate, Chao1, and Jacknife) and diversity (coverage, Shannon index, Simpson index, and q-stat) values were also calculated also the mothur program. The taxonomic distribution of bacterial communities was graphed in R using the "ggplot2" package (https://www.r-project.org/, accessed on 4 September 2021). Shared OTUs among rhizobacterial communities across samples were observed via VennDiagram in R. The prediction of functional traits was performed using the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database and scripts [35].

Network analysis of rhizobacterial community was constructed based on Spearman correlation matrix via WGCNA package [36]. Co-occurrence network properties (numbers of nodes and edges) were calculated by using *igraph* package. Calculations of closeness centrality and betweenness centrality for each node, as well as the image network, was built as described by Zhang et al. [37]. In addition, the occurrence of putative keystone taxa was determined as described by Berry and Widder [38]. Gephi software was used to visualize the co-occurrence network [39].

3. Results

3.1. Chemical Properties of Rhizosphere Soils

The soil chemical properties of the composited samples are summarized in Table 1. The total N contents in all sampled sites were similar, whereas P and K values were greater in soils from Lascar than those from the Guallatiri and Isluga volcano sites. As expected, the organic matter contents of all rhizosphere samples were extremely low (OM < 2%). Low pH values were observed in soils from the Isluga volcano (pH 4.7), whereas pH values near 6.0 were observed in the rhizosphere samples from the Guallatiri and Lascar volcanos. Moderate cation exchange capacity (CEC), from 2.05 to 3.47 $cmol_{(+)}$ kg^{-1}, were observed in all samples. A higher content of Cu and Zn were observed in the rhizosphere soils from the Lascar volcano in comparison with soils from the other volcano sites analyzed, with mean values of 2.97 and 0.70 mg kg^{-1}, respectively.

3.2. Alpha Diversity and Taxonomic Assignments of Rhizobacterial Community

Sequence data analysis revealed a similar coverage percentage among rhizosphere samples from three volcanic locations analyzed (Table 1). The total number of OTUs observed (3292), at the 97% similarity level, was lower in plant rhizosphere samples from the Lascar volcano (830), compared with plants obtained from the Isluga (1137) and Guallatiri (1325) volcano sites. A similar trend was observed for the abundance-based coverage estimate (ACE) and the Chao1 index, which ranged from 889 to 1463 OTUs per samples. Similarly, the Shannon and Simpsons indexes varied from 5.31 to 6.09 and from 0.007 and 0.014, respectively (Table 1). There was no significant difference in Alpha diversity indexes (ANOVA, *p* > 0.05) among the rhizosphere bacterial communities from plants obtained from the three sampled locations.

The taxonomic assignment of 16S rRNA sequences of the rhizosphere samples revealed that Actinobacteria (38.4 to 44.6%), followed by Proteobacteria (22.2 to 32.1%), Acidobacteria (6.3 to 11.4%), and Bacteroidetes (4.6 to 6.5%) were the most abundant phyla in all sequenced samples (Figure 2a). In addition, members of Planctomycetes (2.6 to 6.5%), Chloroflexi (1.3 to 5%), and Verricumicrobia (0.9 to 3.7%) were also observed as abundant phyla in rhizosphere of plant from the Guallatiri and Isluga sites (Figure 2a). Based on the relative abundances of minor taxa, broad taxonomic diversity among samples was found (Figure 2b). A greater presence of bacterial groups associated to native plants was observed in the Guallatiri rhizosphere samples compared to those present in the Lascar and Isluga samples. The rare taxa associated to minor relative abundance were dominated by other members of the phyla Armatimonadetes (0.2 to 0.8%) and Nitrospirae (0.1 to 0.3), with a small percentage associated with unclassified WPS-2 and AD3.
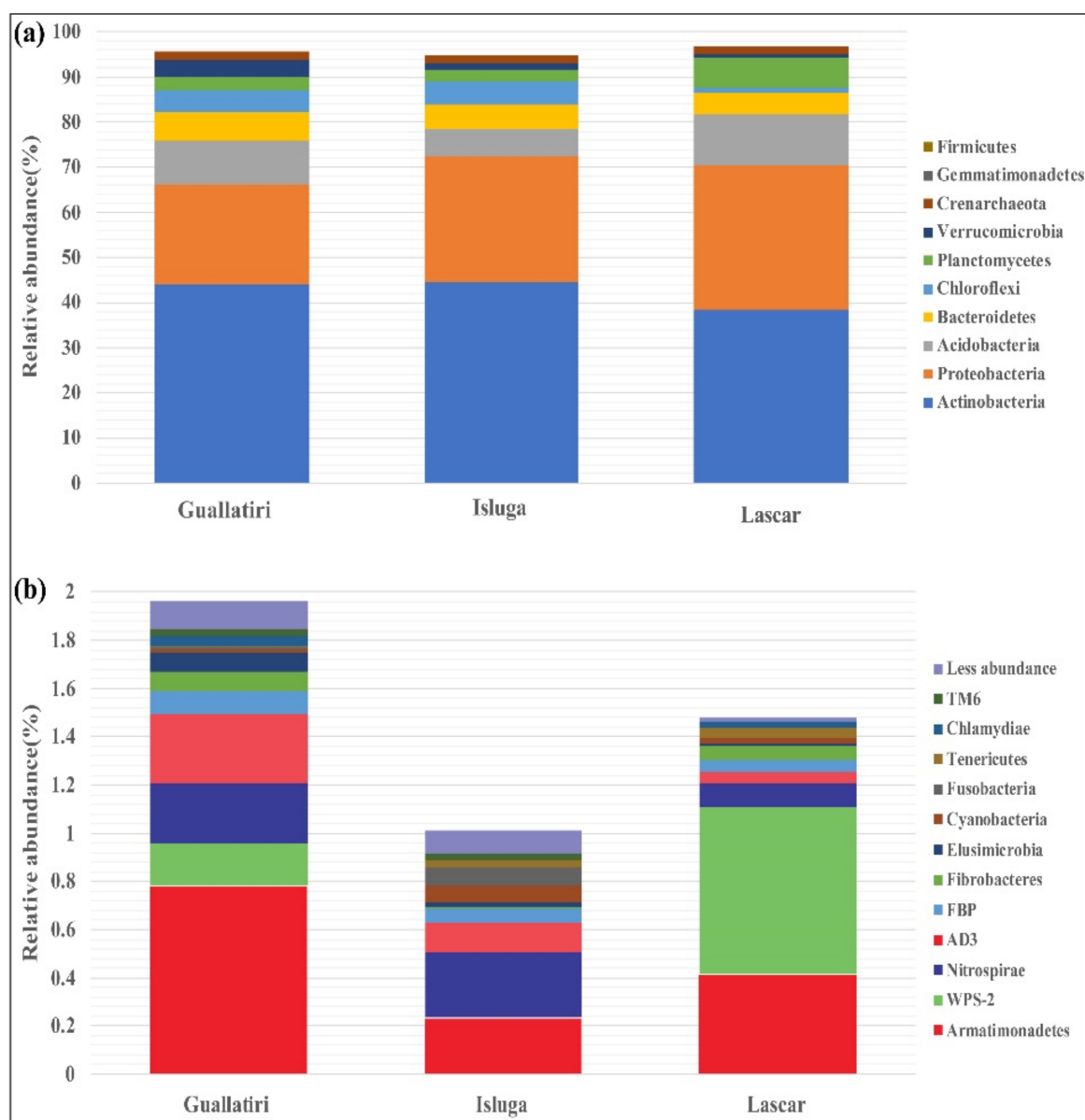

Figure 2. Mean relative abundances of major (**a**) and minor (**b**) phylum-level taxa of total rhizobacterial communities from *P. quadragularis* grown on the slopes of the Guallatiri, Isluga, and Lascar volcanoes.

At the family level, greater relative abundances of taxa were associated with members of the *Pseudonocardiaceae* (6.4 to 8.5%), followed by *Acetobacteraceae* (2.6 to 15.4%), *Geodermatophilaceae* (3.9% to 10.2%), and *Sphingomonadaceae* (3.5 to 4.1%). Interestingly, a higher dominance of members of the *Acetobacteraceae* and *Geodermatophilaceae* families were observed in samples obtained from the Lascar volcano, with abundances around of 10 % and 15%, respectively (Figure 3a). At the genus level, the major relative abundances were attributed to *Modestobacter* (2.6 to 5.5%), followed by *Segetibacter* (1.6 to 2.7%), *Actinomycetospora* (1.2 to 2.8%), and *Kaistobacter* (1.5 to 2.3%) genera (Figure 3b).
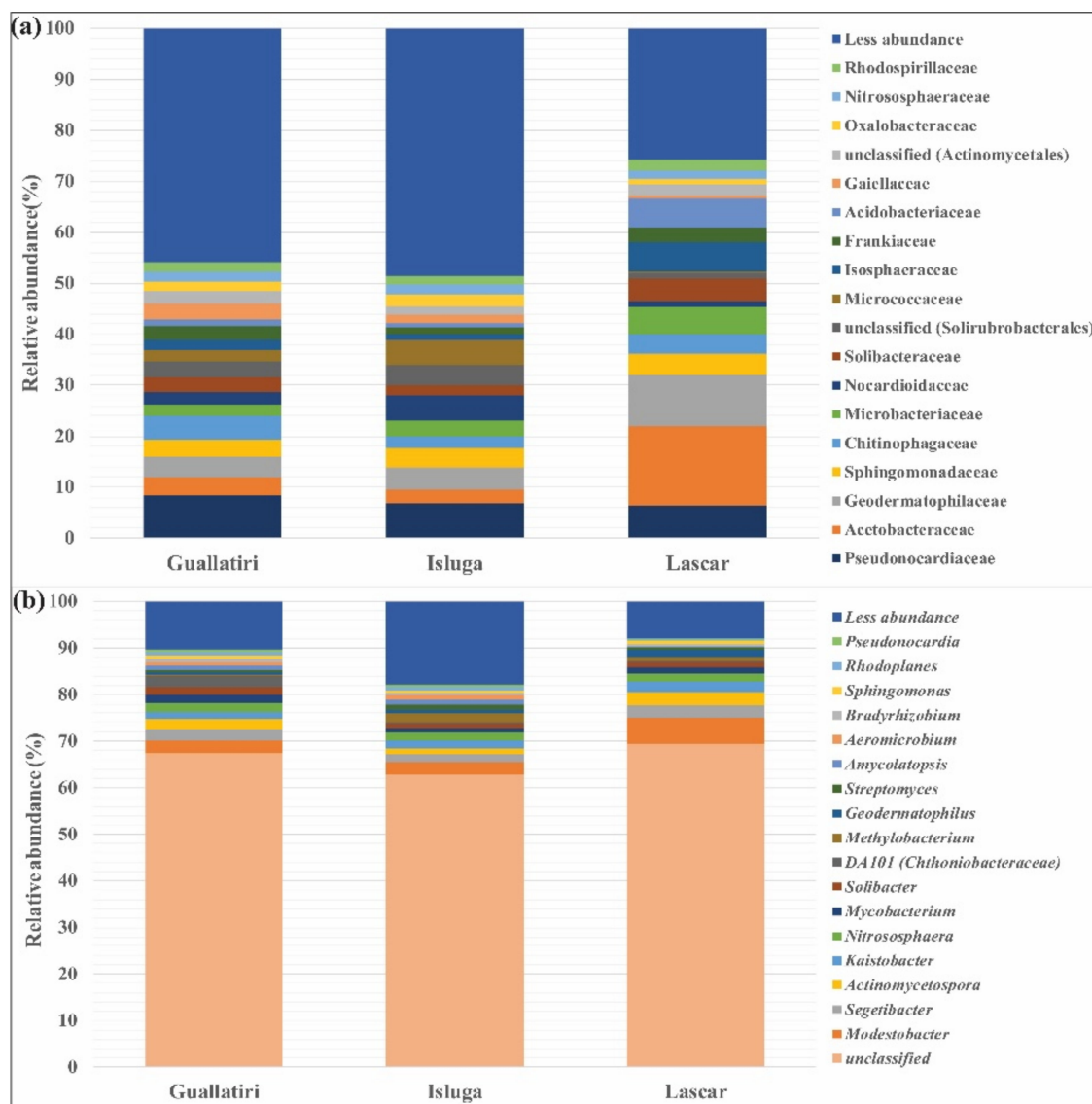

Figure 3. Mean relative abundances of family (**a**) and genus (**b**) level taxa in total rhizobacterial communities from *P. quadragularis* grown at the Guallatiri, Isluga, and Lascar volcano sites.

3.3. Shared OTUs and Predicted Functions of Rhizobacterial Community Members

Beta diversity analysis showed a clear difference in the rhizobacterial community structure across diverse Altiplano ecosystems (Figure 4a), with samples from the Isluga (1366) and Guallatari (1252) sites having the greatest number of unique OTUs. Moreover, only 13% of OTUs (596) were shared in the rhizobacterial communities of plants among three locations analyzed.

The main shared OTUs found among all rhizosphere samples are summarized in Table 2. The OTUs assigned to the Actinobacteria, Bacteroidetes, Proteobacteria, Crenar-

chaeota, Acidobacteria, and Verrumicrobia phyla were the most shared across all rhizosphere samples, with higher or equal proportions at 1%. Among these, members of the genera *Modestobacter*, *Segetibacter,* and *Actinomycetospora* had great relative abundances in the rhizosphere samples, with values ranging from 2.6 to 5.5%, 1.6 to 2.7%, and 1.2 to 2.8%, respectively.

Table 2. Relative abundance (%) and taxonomic affiliation of the 20 most shared operational taxonomic units (OTUs) across rhizobacterial communities associated of *P. quadrangularis* collected from Guallatiri, Isluga, and Lascar volcanoes.

Genus	Closest Relative Taxonomic Affiliation	Guallatiri (%)	Isluga (%)	Lascar (%)
Modestobacter	Actinobacteria; Actinobacteria; Actinomycetales; Geodermatophilaceae	2.6	2.7	5.5
Segetibacter	Bacteroidetes; Saprospirae; Saprospirales; Chitinophagaceae	2.5	1.6	2.7
Actinomycetospora	Actinobacteria; Actinobacteria; Actinomycetales; Pseudonocardiaceae	2.2	1.2	2.8
Kaistobacter	Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae	1.53	1.71	2.28
Nitrososphaera	Crenarchaeota; Thaumarchaeota; Nitrososphaerales; Nitrososphaeraceae	1.96	1.79	1.74
Mycobacterium	Actinobacteria; Actinobacteria; Actinomycetales; Mycobacteriaceae	1.71	0.87	1.31
Solibacter	Acidobacteria; Solibacteres; Solibacterales; Solibacteraceae	1.49	1.06	0.99
DA101	Verrucomicrobia; Spartobacteria; Chthoniobacterales; Chthoniobacteraceae	2.52	0.32	0.45
Methylobacterium	Proteobacteria; Alphaproteobacteria; Rhizobiales; Methylobacteriaceae;	0.45	1.73	0.74
Geodermatophilus	Actinobacteria; Actinobacteria; Actinomycetales; Geodermatophilaceae	0.22	0.77	1.52
Streptomyces	Actinobacteria; Actinobacteria; Actinomycetales; Streptomycetaceae	0.62	1.20	0.49
Amycolatopsis	Actinobacteria; Actinobacteria; Actinomycetales; Streptomycetaceae	0.95	0.87	0.35
Aeromicrobium	Actinobacteria; Actinobacteria; Actinomycetales; Nocardioidaceae	0.63	1.15	0.11
Bradyrhizobium	Proteobacteria; Alphaproteobacteria; Rhizobiales; Bradyrhizobiaceae	0.96	0.47	0.43
Sphingomonas	Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae	0.64	0.53	0.64
Rhodoplanes	Proteobacteria; Alphaproteobacteria; Rhizobiales; Hyphomicrobiaceae	0.70	0.59	0.41
Pseudonocardia	Actinobacteria; Actinobacteria; Actinomycetales; Pseudonocardiaceae	0.60	0.64	0.21
Kribbella	Actinobacteria; Actinobacteria; Actinomycetales; Nocardioidaceae	0.36	0.87	0.17
Burkholderia	Proteobacteria; Betaproteobacteria; Burkholderiales; Burkholderiaceae	0.24	0.19	0.95
Devosia	Proteobacteria; Alphaproteobacteria; Rhizobiales; Hyphomicrobiaceae	0.24	0.53	0.28

The predicted bacterial functional traits among taxa in each rhizosphere samples are shown in Figure 4b. Of these categories, Chemoheterotrophy (28.9 to 32.5%) and aerobic chemoheterotrophy (25.2 to 27.3%) were the most abundant presumptive metabolic functions of bacterial community across all samples. In contrast, a minor proportion of OTUs were attributed with functions related to nitrification, aerobic ammonia oxidation, ureolysis, methylotrophy, and animal parasites or symbionts, which together accounted for 44.7% of the total relative abundance in all rhizosphere samples. Moreover, the predicted bacterial functional groups did not vary significantly across the samples evaluated (Figure 4b).
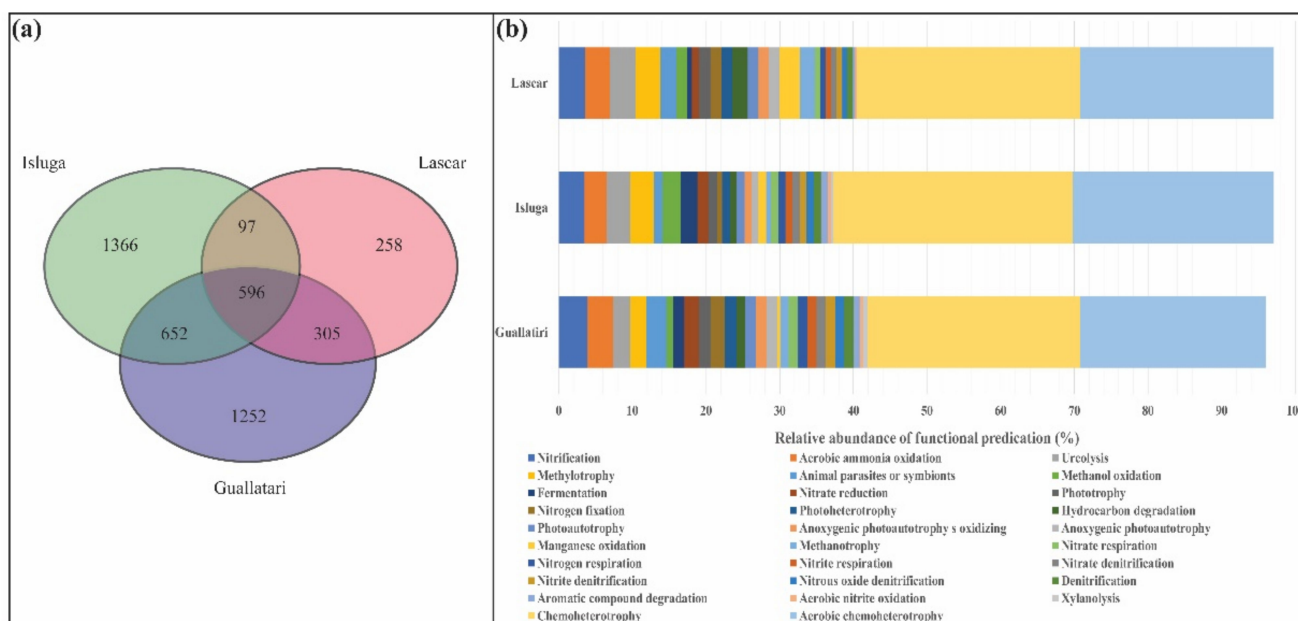
Figure 4. (**a**) Shared operational taxonomic units (OTUs) among bacterial communities in the rhizosphere of *P. quadragularis* grown at the Guallatiri, Isluga, and Lascar volcano sites. (**b**) Mean relative abundances of microbial functional groups in the rhizobacterial communities of the *P. quadragularis* grown at the the Guallatiri, Isluga, and Lascar volcano sites.

3.4. Co-Occurrence Networks and Putative Keystone Taxa across Rhizobacterial Community Members

A network analysis was conducted in order to determine co-occurrence patterns and putative keystone taxa of bacterial communities across the rhizosphere samples. Results in Figure 5 show the occurrence of 1487 nodes (e.g., OTUs) and 1547 edges, indicative of the connectivity between rhizomicrobiota communities. The network properties diameter and transitivity were 31.98 and 0.639, respectively. Members of the *Patulibacteraceae* family where identified as the main putative keystone taxa. However, the abundance of this keystone taxon was only 0.02% and was not detected in rhizosphere from Isluga volcano.
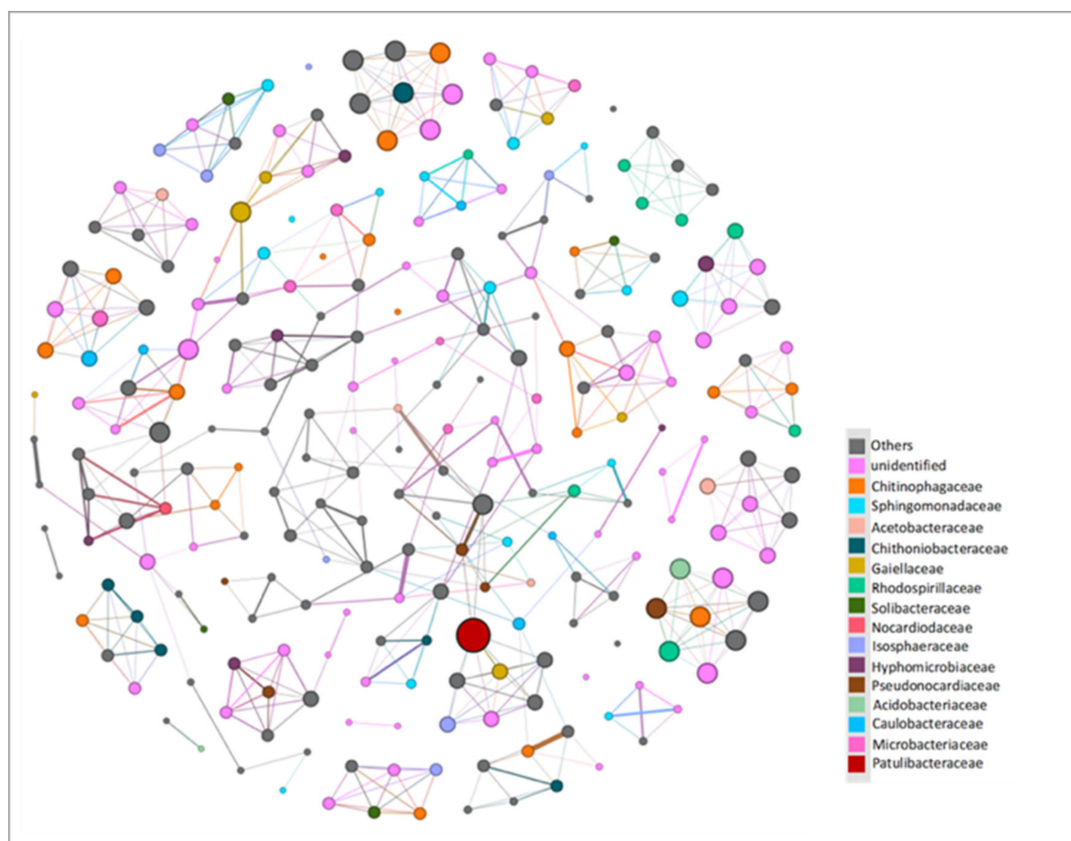
Figure 5. Co-occurrence network of the main OTUs based on correlation analysis. Nodes correspond to microbial OTUs and edges to the microbial associations. A connection stands for a strong (Spearman's ρ > 0.6) and highly significant (*p* < 0.001) correlation. The size of each node is proportional to the number of degrees.

4. Discussion

Rhizosphere microbiota plays a crucial role in plant fitness and development, as well their adaptation and tolerance to environmental stresses. Plant–microbe interactions among one of the key components that support pioneer plants grown under extreme environments [40]. Studies focusing on the rhizobiome of pioneer plants growing in extreme arid regions and dry environments has increased due to current climate crisis [41]. Thus, natural vegetation able to colonize harsh oligotrophic habitats, particularly highlands with extreme variation of temperature, dehydration, and solar irradiation, offer a great opportunity to understand how plants select specific rhizobacteria to assure their survival. Advances in plant microbiome from desert areas might be key to improving soil fertility and plant stress tolerance and crop productivity, particularly in soil exposed to accelerated desertification processes. For these reasons, we chose to examine *Parastrephia* plants that are known to be resistant to cold, high-radiation, salinity, and drought, as well as those considered as common inhabitants of the Altiplano ecoregions [42].

In this study, we examined the composition and functions of rhizobacterial community associated with *P. quadrangularis* growing on the slopes of the Guallatiri, Isluga, and Lascar volcanoes. DNA metabarcoding analysis of 16S rRNA gene libraries (V4 region) from rhizosphere samples revealed great microbial richness (revealed by number of OTUs) present in association with plant roots. Our results showed that the rhizospheres of all analyzed samples had a large relative abundance of members of the phyla Actinobacteria, followed by the Proteobacteria. Members of Actinobacteria and Proteobacteria phyla are commonly reported in the rhizobiome of plants grown Chilean Altiplano, including native plant such as *Atriplex* sp., *Stipa* sp., *Baccharis scandens*, *Solanum chilense*, *Calamagrostis crispa*,

Nassella nardoides, *Jarava frigida*, and *Pycnophyllum bryoides* [43–45]. Coincidently, members of Proteobacteria have been observed to be dominant in the endospheres of *Distichlis spicate* and *Pluchea absinthioides,* both known pioneer plants of the Atacama Desert (Chile) [46].

At the family and genus level, members of *Pseudonocardiacea* and *Modestobacter* were observed as dominant groups in all analyzed samples, respectively. In this sense, the presence of members of the family *Pseudonocardiaceae* in rhizosphere samples is often observed in extreme biomes, such as arid soils and stone surfaces [47,48]. Similarly, roots of the halophyte *Salicornia europaea* (Linn.) were dominant by actinobacterial phylotypes phelotypes with high dominance by the *Modestobacter* genus [49]. Members of this genera are also described as a crucial component of the Atacama Desert soils [50,51].

Chemoheterotrophy and aerobic chemoheterotrophy were the most abundant putative metabolic functions of bacterial community members associated with the *P. quadrangularis* rhizosphere across all samples. Functions related to nitrification, aerobic ammonia oxidation, and ureolysis were also found, but in more minor proportions. While several studies have been focused on unraveling the microbiome in Altiplano ecosystems, the functional studies of bacterial communities present in soils and plants are still very limited, mainly due to the ability to obtain samples and their nature. A recent genomic study of soil bacteria revealed a high proportion of xerotolerant, halotolerant, and radioresistant microbes, with predicted functions associated to nitrate and sulfate pathways in the hyperarid core of the Atacama Desert [52]. In this sense, whole genome sequencing analysis of *Modestobacter altitudinis* sp. strains recently isolated from a highlands of the Atacama Desert soils, showed genes that are associated with stress response, including osmotic stress, resistant to UV radiation, temperature, and carbon starvation [50]. These stress tolerance abilities would offer higher competitiveness to this bacterial group to survive under extreme conditions. *Modestobacter* is also recognized as a rare taxon playing a role in soil formation, degradation processes and nutrient cycling in Atacama highland soils [53]. In this sense, several bacterial and fungal taxa found in Atacama soil are recognized for their role in plant nutrition and stress protection. Wu et al. [54] observed that the microbial potential for denitrification and associated gene abundance are conserved despite lowered microbial richness across a long hyperaridity gradient in the Atacama Desert. However, the functional predictions based on OTUs requires attention due to limitation of the assigned OTUs and their real predictions traits.

The co-occurrence network of the rhizobacterial communities of *P. quadrangularis* revealed the presence of 1487 nodes and 1547 edges. These values are greater than previously reported in the rhizosphere of desert plant with values ranging from 360 to 443 nodes [55]. Network results also found that members of the family *Patulibacteraceae* (Actinobacteria phylum) represent a keystone taxon among the rhizosphere samples from volcanic slope. Astoga-Eló [56] observed the presence of 970 nodes and 1324 edges based on the co-occurrence analysis obtained from the rhizobacterial community associated to *Cistanthe longiscapa* (Montiaceae), during flowering event in the Atacama Desert. This study also indicated the occurrence of the one unique keystone in the *C. longiscapa* rhizosphere, reveling that the networks are modulated for few taxa. To our knowledge, the occurrence of keystone taxa in the plant and soil microbiomes from the Altiplano of Atacama Desert has not been reported thus far, so comparison to other studies is difficult.

In contrast to our stated hypothesis, and despite the similar functions and taxonomic composition observed, our data shows that that sampling sites, rather than Altiplano plant species, determines the composition of associated bacterial communities. Similar results (obtained using denaturing gradient gel electrophoresis and 454-pyrosequencing approaches) were found in the rhizobacterial community structures associated with native plants species grown in Chilean extreme environments [43]. All of these approaches showed that native plant species not only assembled microbiomes with distinct community structure, but also differ with respect to the composition of their rhizobiome in samples obtained from differing sites and between plant species grown in the same environment. These differences are also observed between endophytic bacterial communities inhabit-

ing roots and leaves of native plants from the Atacama Desert and Patagonia regions of Chile, where there are higher portions of OTUs. [46]. Zhang et al. [37] recently reported a niche differentiation in the microbial community associated with Antarctic vascular plants, suggesting that pioneer plants drive the niche differentiation by selection of distinctive microbial assemblages. Thus, desert pioneer plants selected specifically microbial communities to their needs to grow and survive under extreme conditions, which are determined by specific soil conditions.

5. Conclusions

The Altiplano of the Atacama Desert is a hostile environment determining a selective pressure on the pioneer plants, including the recruitment of specific beneficial bacterial taxa that promote the adaptation of pioneer vegetation to extreme environments. In this study, the analysis of the bacterial communities revealed that Actinobacteria, Proteobacteria, Acidobacteria, and Bacteroidetes are the dominant taxa in the *P. quadrangularis* grown on the slope of the Guallatiri, Isluga, and Lascar volcanoes. The alpha diversity analysis did not reveal significant difference in the diversity, richness, and abundance of OTUs in rhizosphere samples. However, higher proportions of OTUs were unique and not shared across the rhizosphere samples of each volcano. Besides, many similar bacterial functions were found among rhizosphere samples, in which chemoheterotrophy, aerobic chemoheterotrophy, and nitrogen cycling represented the major assignations. The co-occurrence network analysis revealed the complexity of the bacterial associations in *P. quadrangularis* rhizosphere, highlighting the *Patulibacteraceae* family as the keystone taxa. The present findings helpfully improve the current knowledge about the recruitment of specific rhizobacterial communities by pioneer plants inhabiting different volcanoes slopes and the pivotal role of rare taxa in the adaptability and colonization of pioneer plants to the Altiplano extreme conditions.

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