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Grass dominance drives rhizospheric bacterial communities in a desertic shrub and grassy steppe highland

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ABSTRACT

The rhizosphere is a dynamic root-soil interface characterized by interactions between soil microorganisms and roots. These interactions can be potential drivers of the structure of the plant and bacterial communities in desertic shrub and grassy steppe highlands. We analyzed the relationships of rhizospheric bacterial density and occurrence (presence/absence) with dominance degree of grasses and soil properties in Argentina's Puna ecosystem. Rhizospheric bacterial density was low and showed a strong relationship with the dominance degree of grasses without any significant influence from the soil or other vegetation variables. In addition, we determined rhizospheric bacterial occurrence with PCR-DGGE analysis of the 16S rRNA genes. Actinobacteria, Firmicutes and Proteobacteria were the predominant bacterial groups associated to the rhizosphere of grasses. In Puna highlands, the rhizospheric bacterial community appear driven by the dominance degree of grasses with little influence from other biotic or abiotic factors. We suggest that tight plant-bacterial interactions have evolved in these harsh environments that promote some level of grass dominance and maintain the diversity of the rhizospheric bacterial communities.

1. Introduction

Soil is a heterogeneous environment, harbouring a wide variety of micro-habitats with different environmental conditions in which bacteria are heterogeneously distributed (Ranjard and Richaume, 2001). The rhizospheric soil is a dynamic root-soil interface characterized by interactions between soil microorganisms and roots in which the plants may facilitate some bacterial groups thus ultimately producing specific bacterial communities (Hawkes et al., 2007). In the rhizosphere, availability of most soil nutrients is controlled by interactions between plant roots and microbial soil communities (Marschner and Rengel, 2007).

Highland environments present harsh conditions for bacterial and plant development (Körner, 1999). Also in arid highlands ecosystems, such as the Argentinean Puna, the soils are poor in nutrients and plant nutritional demand may exceed soil nutrient availabilities. Rhizospheric microbial interactions may be key to enable sufficient access to soil nutrient pools and affect plant growth (Dhillon and Zak, 1993; Körner, 1999). We lack information about these interactions and how they could be important in determining the dominance status of some plant species in these ecosystems.

Different physico-chemical and biological features of the soil may determine bacterial diversity. Abiotic factors are determinants on the bacterial community structure in grasslands (Regan et al., 2014) and arid ecosystems (Dhillon and Zak, 1993). Also, plant species can modify the associated community of microorganisms, mainly by the composition, quality, and quantity of root exudates (Marschner et al., 2001; de Graaff et al., 2010), and thereby influence rhizosphere bacterial community composition and structure.

Despite the importance of interactions between plants and soil bacteria as drivers of plant community structure (Reynolds et al., 2003), little is known about the relationship between rhizospheric bacterial communities and vegetation structure in highland ecosystems (Lugo et al., 2008; King et al., 2012; Yuan et al., 2014). We hypothesize that rhizospheric bacterial communities are highly influenced by grasses, which can predict the bacterial density and occurrence (presence/absence). The aim of this work was to determine the importance of the dominance degree of grasses on rhizospheric bacterial commu-

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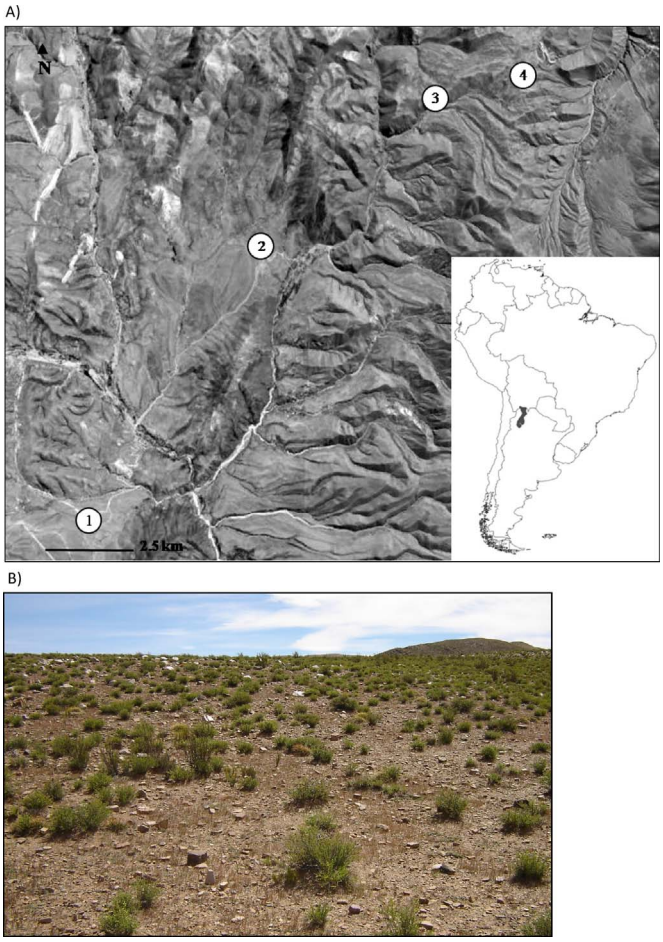

Fig. 1. A Location of studied sites in Puna Argentina. Images obtained in Google earth location. B. Physiognomy of studied sites. Grassy steppe and shrub steppe, include Cyperaceae, Cactaceae and small shrub interrupted by large areas of exposed soil.

nity densities and occurrence. Furthermore, we also assessed if rhizospheric bacterial communities were influenced by soil properties in native arid highland ecosystems.

2. Materials and methods

2.1. Sampling area and design

The study area was located in the Puna, an arid highland (3400–4500 masl) region of Argentina between Iturbe (Jujuy province) and Iruya (Salta province). The cold and drought characterize this ecoregion; there is only summer rainfall, ranging from 41 to 88 mm, and mean annual temperature ranges from 8.5 to 9.5 °C (Ruthsatz, 1977; Cabrera and Willink, 1980). The soil is generally considered poor in organic matter and often sandy and rocky, leading to the formation of plant communities with characteristics of a shrub steppe and grassy steppe interrupted by large areas of exposed soil (Fig. 1a, b) (Cabrera and Willink, 1980; Vorano and Vargas Gil, 2002). In autumn, four sampling sites were selected; altitude, geographic location and physicochemical soil properties are detailed in Table 1. At each site we sampled four plots (25 m^2) with similar physiognomy and slope. In each plot, five individuals of dominant and subordinate (intermediate and rare grasses) Poaceae were collected, including their rhizospheric soil. Grass species were considered dominant when cover was > 75% of total grasses cover, intermediate when covering was between 74 and 26% and rare when covering was < of 25% of total grasses covering (Table S1). Furthermore, other species of grasses were identified but we did not find enough individuals to have proper replication of the dominance degree, therefore this species could not be considered in the analysis.

2.2. Density and identification of rhizospheric bacteria

Rhizospheric bacterial density was determined as CFU g^{-1} of dry weight of soil from five individual grasses belonging to different dominance degree at each sample site. Rhizospheric soil (0.5 g) was homogenized in 10 ml of 0.85% (wt/v) saline and Tween 20. The aliquots (100 µl) were then spread on R2A medium for heterotrophic organisms (Reasoner and Geldreich, 1985) and then incubated at 20 °C for 3–5 days. The number of colony-forming units was determined for each soil sample by triplicate.

Additionally, rhizospheric bacteria of dominant and rare grasses was determined by PCR-DGGE approach. Microbial suspensions were used for extraction of whole DNA by the CTAB method (Ellis et al., 1999) with modifications (Lugo et al., 2008). The quality and quantity of DNA suspensions were then evaluated by electrophoresis on a 0.8% agarose gel followed by staining with ethidium bromide. To amplify 16S rRNA gene fingerprints suitable for denaturing gradient gel electrophoresis (DGGE) analysis, primers 357F-GC (Escherichia coli 16S rDNA positions 341–357f) and 518R (E coli16S rDNA positions 518–534) (Muyzer et al., 1993) were used to amplify the V3 region of the 16S rDNA. Amplification conditions, methodology and running conditions for DGGE was described in Ferrero et al. (2010). DGGE was conducted using a D-Code system (Bio-Rad Laboratories, Inc., Hercules, CA). In each DGGE gel were run samples belonging to the different plots of site; showing the total bands obtain in a complete lane. All bands for each lane from the DGGE gels were carefully excised, and further amplified and sequenced (Ferrero et al., 2010). Most of the bands could be amplified and sequenced; and further considered when calculating the occurrence of bacterial groups. Sequencing was performed directly on PCR products with the 341F primer in Macrogen Inc. (Korea). The partial sequences were then aligned with the reference 16S rRNA gene sequence using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) (Altschul et al., 1997).

2.3. Statistical analyses

Rhizospheric bacterial density was analyzed in relation to dominance degree of grasses as a randomized complete block design (block: sites, n = 4) with analysis of variance (ANOVA) and differences between means determined using Tukey HSD tests ($P \leq 0.05$). Assumptions of normality, homogeneity of variance, and additivity were tested. With all the soil and vegetation variables, we performed variable selection analyses via stepwise and all subset approaches (Murtaugh, 2009) prior to multiple linear regression with bacterial density as the response variable to determine if relationships existed with other potentially influential variables. To further explore the importance of grass dominance and soil variables on rhizospheric bacterial communities we performed two multivariate statistical analyses despite the unbalanced nature of our data (Table S2). First, to visualize differences in rhizospheric bacterial community we performed a non-metric multidimensional scaling (NMDS). Finally, we also performed a canonical redundancy analysis (RDA) (Legendre and Anderson, 1999) to explore how key soil variables could influence the rhizospheric bacterial community. All statistical analyses were conducted in R (R Core Team, 2016).

3. Results and discussion

In Puna, the degree of dominance of grass species was the most important factor determining rhizospheric bacterial density based on stepwise regression. Grasses with intermediate dominance had the greatest rhizospheric bacterial density compared to the grasses with rare or dominant degrees (Fig. 2). The degree of dominance by grass

Table 1
Characterization of the sampling sites. Data are means ± standard error. References: CEC = cation exchange capacity, ESP = exchangeable sodium percentage, BS = Bases Saturation.

Characteristic[a]	Study site			
	1	2	3	4
Site				
Altitude (masl)	3449	3571	3779	3956
Coordinates	23° 00′ 3.5″S; 65° 22′ 1.3″W	22° 55′50.7″S; 65° 19′9.7″W	22° 53′ 35.1″S; 65° 16′ 20.8″W	22° 53′16.8″S; 65° 14′ 58.6″W
Solar exposure(°)	165 ± 0	170 ± 0	160 ± 0	180 ± 0
Plant cover (%)	91 ± 0.3	61.25 ± 1.25	60 ± 1.51	52.5 ± 1.31
Soil				
Water saturation (g/kg)	213 ± 3.7	240.8 ± 10.1	348.5 ± 5.8	354 ± 7
pH (1:2.5 KCl)	6.14 ± 0.05	6.58 ± 0.03	5.88 ± 0.05	5.91 ± 0.03
pH (1:2.5 agua)	6.76 ± 0.04	7.27 ± 0.04	6.52 ± 0.07	6.46 ± 0.04
Electric conductivity (mS/cm)	0.05 ± 0.01	0.21 ± 0.01	0.07 ± 0.01	0.09 ± 0.01
C_{ox} (g/kg)	5.1 ± 0.2	6.5 ± 0.2	16.3 ± 0.6	15 ± 1.1
N (g/kg)	0.6 ± 0.02	0.8 ± 0.02	1.6 ± 0.05	1.7 ± 0.1
C/N	8.54 ± 0.17	8.37 ± 0.09	10.42 ± 0.16	8.94 ± 0.23
Organic matter (g/kg)	8.7 ± 0.3	11.2 ± 0.3	28.1 ± 1	25.8 ± 1.8
Total P (mg/kg)	285 ± 4.1	347 ± 3.4	459.5 ± 14.89	375.88 ± 10.72
Available P (mg/kg)	34.3 ± 0.66	12.43 ± 0.55	57.9 ± 3.55	13.18 ± 0.86
Ca^{2+} ($cmol_c$/kg)	3.36 ± 0.04	9.43 ± 0.57	4.33 ± 0.09	4.57 ± 0.28
Mg^{2+} ($cmol_c$/kg)	0.69 ± 0.02	2.99 ± 0.2	0.8 ± 0.03	1.31 ± 0.11
Na^{+} ($cmol_c$/kg)	0.37 ± 0.03	0.52 ± 0.04	0.38 ± 0.04	0.43 ± 0.06
K^{+} ($cmol_c$/kg)	0.55 ± 0.02	0.75 ± 0.02	0.96 ± 0.04	0.95 ± 0.02
Total cations ($cmol_c$/kg)	4.97 ± 0.07	13.69 ± 0.78	6.47 ± 0.11	7.26 ± 0.41
CEC ($cmol_c$/kg)	5.25 ± 0.06	13,52 ± 0.84	7.99 ± 0.15	8.6 ± 0.64
BS (%)	94.8 ± 1.11	101,6 ± 0.83	81.06 ± 1.36	85.23 ± 1.2
ESP (%)	7.13 ± 0.56	3,82 ± 0.2	4.83 ± 0.47	5.04 ± 0.61

[a] The analyses were as follows: Walkley-Black method for C oxidable; Kjeldahl method for N (include organic nitrogen and NH_4^+ exchangeable); Acid digestion for total P; Bray-Kurtz method for available P; cations were extracted with CH_3COONH_4; Ca^{2+} and Mg^{2+} were analyzed by atomic absorption spectrometry and K^+ and Na^+ with atomic emission spectrometry.

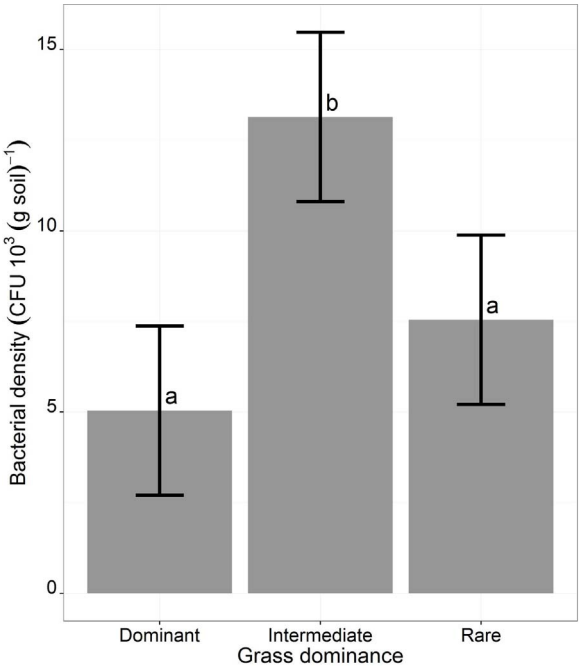

Fig. 2. Density of rhizosphere soil bacterial communities along three dominance levels of Poaceae in native Puna highlands, Argentina. Values are means (n = 4) with 95% confidence intervals and different letters indicate statistically significant differences between means (Tukey HSD tests with $P \leq 0.05$).

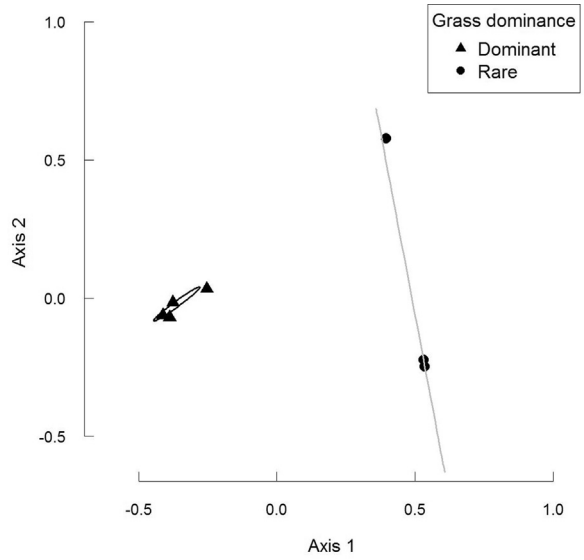

Fig. 3. Non-parametric multidimensional scaling (NMDS) plot of rhizospheric bacteria identified via PCR-DGGE found in two Poaceae dominance levels (Dominant and Rare) in soils in native Puna highlands, Argentina. The Jaccard dissimilarity index was used and derived from presence and absence data derived from the PCR-DGGE. This ordination was performed to visualize the similarity in the rhizospheric bacterial community between the different Poaceae dominance levels. Each point symbolizes a single bacterial community and ellipses represent 95% confidence intervals around the dominance level centroids. Non-overlapping ellipses may be considered significantly different and may be used to visually estimate the degree of similarity (overlap) or difference (no overlap) in the bacterial community. Some jitter was added to overlying points.

species played an important role in explaining bacterial density since we did not identify any significant soil or other vegetation variables determining changes in bacterial density after extensive variable selection approaches or a multiple linear regression analysis with variables considered potentially important (Adjusted-$R^2 = 0.02$, $P = 0.396$). Likely grasses with intermediate dominance were not associated with specific bacterial communities compared to the dominant or rare grasses, therefore higher bacterial densities could be found in the rhizosphere of intermediate grasses. Similarly to the bacterial density, rhizospheric bacterial occurrence differed according to the degree of dominance of the grass species (Fig. 3). In addition the RDA analyses did not show any significant effect of soil variables on the community of bacteria (Fig. S1). There are records of differential effects of plants species on the rhizospheric bacterial community composition

Table 2
Assignment of bacterial taxonomic groups to band sequences extracted from a DGGE gel based one180 bp and the closest relatives match of known phylogenetic affiliation, considering different altitude and grasses species and dominance degree.

Altitude (masl)	Grass species/Dominace degree	DGGE band	Phylogenetic group	Closest relatives (acc.number)	Similarity (%)
3449	*Bouteloua simplex* Lag/ Dominant	1Bs1	Actinobacteria	*Gordonia malaquae* type strain IMMIB WWCC-22 (AM406674)	97
		2Bs1	Alphaproteobacteria	*Sphingomonas* sp. BSL8 (DQ885954)	98
		3Bs1	Alphaproteobacteria	Uncultured Rhizobium/Agrobacterium DGGE band B4-4 (AY758581)	97
3449	*Microchloa indica* (L. f.) P. Beauv./ Rare	1Mi1	Betaproteobacteria	*Burkholderia* sp. E2 (AB265148)	96
		2Mi1	Actinobacteria	Uncultured actinobacterium clone TWAC-7(DQ662893)	100
		3Mi1	Firmicutes	*Bacillus* sp. HZDC17 (EF190361)	100
		4Mi1	Firmicutes	*Bacillus* sp. '*Bacillus* M2' (EF409309)	99
3571	*Microchloa indica* (L. f.) P. Beauv./ Dominant	1Mi2	Betaproteobacteria	*Burkholderia* sp. KAR48 (EF451678)	96
		2Mi2	Alphaproteobacteria	Uncultured Rhizobium/Agrobacterium DGGE band B4-4 (AY758581)	97
		3Mi2	Alphaproteobacteria	*Sphingomonas* sp. BSL8 (DQ885954)	98
		4Mi2	Proteobacteria	*Cupriavidus* sp. amp18 clone 2 (DQ530650)	89
		5Mi2	Actinobacteria	*Gordonia malaquae* type strain IMMIB WWCC-22 (AM406674)	97
3571	*Bouteloua simplex* Lag./ Rare	1Bs2	Firmicutes	Uncultured Bacillaceae bacterium (EF019629)	100
		2Bs2	Firmicutes	Uncultured Firmicutes bacterium (EF018849)	100
		3Bs2	Actinobacteria	Uncultured actinobacterium clone LKAC-34 (DQ675158)	98
		4Bs2	Firmicutes	*Bacillus* sp. '*Bacillus* M2' (EF409309)	99
		5Bs2	Actinobacteria	Uncultured actinobacterium clone TWAC-40 (DQ662890)	100
3779	*Microchloa indica* (L. f.) P. Beauv./ Dominant	1Mi3	Betaproteobacteria	*Burkholderia* sp. KAR48 (EF451678)	96
		2Mi3	Alphaproteobacteria	Uncultured Rhizobium/Agrobacterium DGGE band B4-4 (AY758581)	97
		3Mi3	Alphaproteobacteria	*Sphingomonas* sp. BSL8 (DQ885954)	98
		4Mi3	Proteobacteria	*Cupriavidus* sp. amp18 clone 2 (DQ530650)	89
		5Mi3	Actinobacteria	*Gordonia malaquae* type strain IMMIB WWCC-22 (AM406674)	97
		6Mi3	–	Uncultured bacterium clone G (AF543365)	–
3779	*Bouteloua simplex* Lag./ Rare	1Bs3	Betaproteobacteria	*Burkholderia* sp. E2 (AB265148)	96
		2Bs3	Actinobacteria	Uncultured actinobacterium clone TWAC-7 (DQ662893)	100
		3Bs3	Firmicutes	*Bacillus* sp. HZDC17 (EF190361)	100
		4Bs3	Firmicutes	*Bacillus* sp. '*Bacillus* M2' (EF409309)	99
3956	*Nassella nidulans* (Mez) Barkworth/ Dominant	1Nn1	Firmicutes	*Bacillus* sp. JZHS21 (DQ658962)	95
		2Nn1	Actinobacteria	*Gordonia malaquae* type strain IMMIB WWCC-22 (AM406674)	97
		3Nn1	–	Uncultured bacterium clone BFA_072 (EF443776)	–
		4Nn1	Alphaproteobacteria	Uncultured Rhizobium/Agrobacterium DGGE band B4-4 (AY758581)	97
		5Nn1	Actinobacteria	Uncultured actinobacterium clone LKAC-51 (DQ675176)	98

(Marschner et al., 2001; Kuske et al., 2002). Therefore, the relationship between the degree of dominance of Puna grasses and rhizospheric bacterial community could be explained considering that plants with the same dominance degree usually occupy the same ecological niche showing a similar behavior even at the level of plant-bacteria interaction. In Puna plant communities, dominance of grasses would be a good predictor of bacterial density as it was recorded for different plant families in other highland ecosystem (King et al., 2012).

Bacterial density in the rhizosphere of the Puna grasses was lower than bacterial records from others arid grasslands (Kuske et al., 2002) and alpine communities (Edwards et al., 2006). The low bacterial densities may be due to the harsh environmental conditions characterizing the Puna. In fact, the bacterial density levels found in the Puna are comparable with densities previously observed in the Puna (Lugo et al., 2008), other harsh ecosystems such as glaciers (Zhang et al., 2010) and deserts (Lester et al., 2007).

All DGGE bands were excised, re-amplified and sequenced. DGGE bands that appeared to be identical in the profiles derived using different plots of site, also produced identical sequences (Fig. S2, Table 2). Most DGGE bands showed high levels of similarity to clones of uncultured bacteria recovered from soil environments or sequences that were closely related to organisms that were isolated from soils. The majority of sequences that were related to previously isolated organisms belonged to the defined phylogenetic groups, α-, β-Proteobacteria, Actinobacteria and Firmicutes (Table 2). Sequences with uncertain affiliations were obtained by DGGE with high similarities (between 97 and 100%) with database sequences of bacterial clones or DGGE bands recovered from soil environments. These results are consistent with bacterial communities in other highlands, alpine and arid ecosystems where these groups were dominant (Rutz and Kieft, 2004; Chowdhury et al., 2009; Yuan et al., 2014; Yasir et al., 2015). Particular characteristics of these groups including spore formation (Driks, 2002) and resistance to low water potential (Hawkes et al., 2007), which could explain the successful of these groups in these hard environments.

In Puna highlands, bacterial communities seem to be governed by the dominance degree of grasses with little influence from other measured biotic or abiotic factors. Plant-bacteria interactions could have a considerable impact on the plant community structure as observed in studies of community dynamics (Reynolds et al., 2003; Bever et al., 2012). The relationships observed in our study allowed us to conclude that grasses might select a specific bacterial community and thus, rhizospheric soil bacteria would depend on the structure of the grass community. These interactions between plant and bacteria likely evolved under harsh environment conditions, thus promoting some level of grass dominance and the maintenance of rhizospheric bacterial diversity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pedobi.2017.04.004.

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