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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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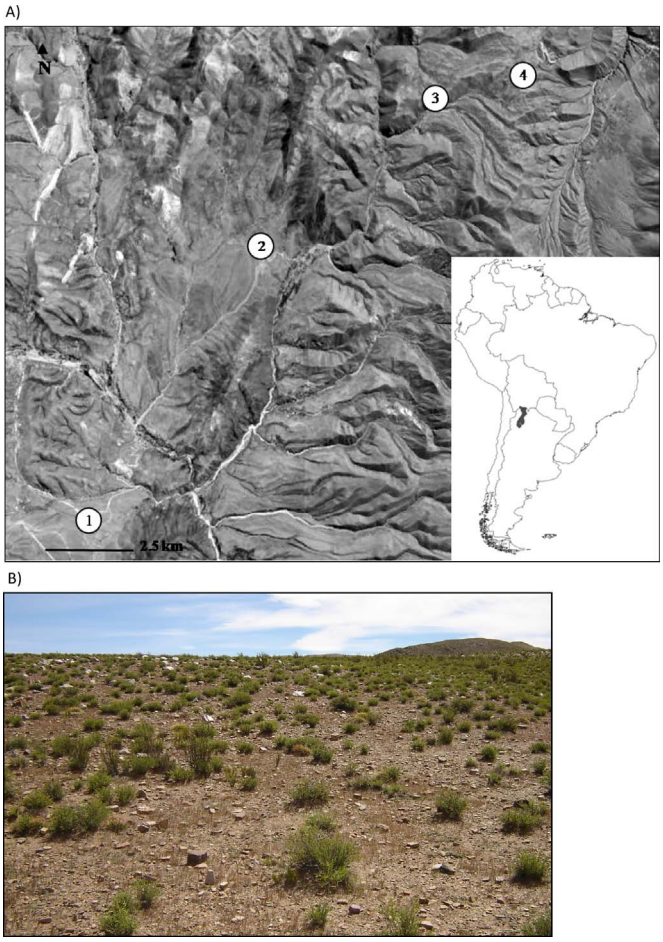

Fig. 1. A Location of studied sites in Puna Argentina. Images obtained in Google earth location. B. Physiognomy of studied sites. Grassy steppe and shrub steppe, include Cyperaceae, Cactaceae and small shrub interrupted by large areas of exposed soil.

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