Microb Ecol (2008) 55:705–713
DOI 10.1007/s00248-007-9313-3

ORIGINAL ARTICLE

Arbuscular Mycorrhizal Fungi and Rhizospheric Bacteria Diversity Along an Altitudinal Gradient in South American Puna Grassland

M. A. Lugo · M. Ferrero · E. Menoyo · M. C. Estévez · F. Siñeriz · A. Anton

Received: 14 November 2006 / Accepted: 8 August 2007 / Published online: 4 October 2007
© Springer Science + Business Media, LLC 2007

Abstract Rhizospheric soil samples were taken from Puna native grasses along an altitudinal gradient. Biodiversity of arbuscular mycorrhizal fungi (AMF) and associated bacteria was analyzed considering altitude and grasses photosynthetic pathways (metabolic type C_3, C_4). Cultivation-dependent approaches were applied to obtain further information about the phylogeny of the dominating cultivable aerobic–heterotrophic bacteria communities present in rhizospheric soil samples. In average, the bacterial count ranged between 1.30×10^2 and 8.66×10^4 CFU g^{-1} of dry weight of soil. Individual bacterial colonies of aerobic heterotrophic bacteria grown on R2A medium were morphologically grouped and identified as typical soil bacteria belonging to the genera *Bacillus*, *Pseudomonas*, and *Arthrobacter*. Ten AMF taxa were found: *Acaulospora* sp., *A. laevis*, *A. spinosa*, *Gigaspora* sp., *Gi. ramisporophora*, *Glomus* sp., *Gl. aggregatum*, *Gl. ambisporum*, *Gl. sinuosum*, and *Scutellospora biornata*. AMF diversity decreased with altitude.

M. A. Lugo (✉) · E. Menoyo
Cátedra de Diversidad Vegetal I, Fac. de Qca.,
Bioqca. y Farmacia, Universidad Nacional de San Luis,
San Luis, Argentina
e-mail: lugo@unsl.edu.ar

M. Ferrero · M. C. Estévez · F. Siñeriz
PROIMI (Planta Piloto de Procesos Industriales
Microbiológicos)–CONICET,
Tucumán, Argentina

F. Siñeriz
Cátedra Microbiología Superior, Fac. de Bqca.,
Qca. y Farmacia, Universidad Nacional de Tucumán,
Tucumán, Argentina

A. Anton
IMBIV–CONICET, Fac. Cs. Ex., Fís. y Nat.,
Universidad Nacional de Córdoba,
Córdoba, Argentina

Introduction

Arbuscular mycorrhizal fungi (AMF) comprise approx. 150 species of Glomeromycota, highly effective in the capture and translocation of soil nutrients, such as phosphorus, from soil to plant. AMF are the main components of the microbial soil community, and their diversity is associated with plant community structure and functioning [23]; AMF diversity also seems to be very important to determine productivity, and plant diversity in natural environments [52]. Arbuscular mycorrhiza (AM) is common in grasslands worldwide [25]. AMF sporulation is related to grass photosynthetic pathways (metabolic type C_3 or C_4) [34] and to host species [7].

Microbial populations are key components of the soil–plant system where they are immersed in a network of interactions affecting plant development. The so-called plant growth-promoting rhizobacteria in the rhizosphere participate in many key ecosystem processes such as those involved in the biological control of plant pathogens, nutrient cycling, and seedling establishment [30].

Mycorrhizal fungi and bacterial communities can be influenced by chemical and physical modifications in the environment surrounding the roots. Furthermore, the soil type, growth stage, cropping practices, and other environmental factors (pH, temperature, humidity, etc.) seem to influence the composition of the microbial community in the rhizosphere [21].

AMF sporulation and spore density associated to a specific plant host vary with the seasons, edaphic factors

(pH, soil moisture, and phosphate level), shade, host dependence, age of the host plant, sporulation capacity of AMF, dormancy, patterns of distribution of AMF spores in the soils, spatial distribution, or clamped distribution to stability effects. Soil bacteria can also affect AM formation and function. Particularly, the so-called mycorrhiza helper bacteria are known to stimulate mycelial growth of mycorrhizal fungi or to enhance mycorrhizal formation [18]. AMF interactions with bacteria have synergistic beneficial effects on plant growth, either by nutrient solubilization, N_2 fixation by nitrogen-fixing bacteria symbionts, or the production of plant hormones [2]. However, these interactions seem to be unclear. Phosphate-solubilizing bacteria can affect AM formation and function [3]. The microbiologically solubilized phosphate could be taken up by a mycorrhizal mycelium, thereby developing a synergistic microbial interaction. Mostly, interaction studies between AMF and bacteria have been done in pot and crop systems, but little is known about these interactions in field. In alpine environments, the fungi diversity of soil, including AMF, decreased with altitude. However, bacteria abundance and diversity is high in this environment [31].

Puna is a South American harsh biogeographical region with unique features, although it is considered an alpine environment by some authors [48]. In this study, we analyzed *in situ* the relationships between the biodiversity of bacteria and AMF in rhizospheric soil of Puna-native grasses along an altitudinal gradient considering altitude and grass metabolic type (C_3 and C_4 photosynthetic pathway of plants) as possible inter-related factors.

Materials and Methods

Sampling Area

The Puna is a highland that extends from the North West of Argentina to Southern Perú. It is limited by the Cordillera Real to the east and the Andes to the west, varying in altitude from 2,000 to 4,400 m above sea level (masl). The climate is cold and dry with a dry season in winter and an annual precipitation of 100 to 400 mm in the summer months (December, January, and February). At Iturbe (Jujuy province, Argentina), one of the sampling locations of the altitude gradient, the monthly mean precipitation during Summer were 88, 63, and 41 mm [48]. The annual mean temperature oscillated between 8.5 and 9.5°C [8]. The study area is located in the floristic district known as "Jujeño" or "Puna Seca." Solar radiation is intense, and relative air humidity is low (10–15%), existing large thermal differences throughout the day (16–20°C). The resulting climate is of a desert type [40]. The soils are superficial and immature, very poor in organic matter, sandy and rocky, and of the Aridisol (typical paleargid) and Entisol types (torriontente lithic) [53, 54].

Sampling Design and Methodology

Five sample sites were chosen along an altitudinal gradient (3,320, 3,370, 3,520, 3,700, 3,870 masl) in the Puna mountain grassland. The sample sites were located at 22° 51′35.2″S, 65°13′43.71″W; 23°00′06.8″S, 65°22′07.3″W; 22°52′14″S, 65°14′25.51″W; 22°53′34.1″S, 65°16′0.06″W; 22°53′23.3″S, 65°14′56.1″W in Jujuy and Salta provinces (Argentina). The physicochemical soil features (Lugo *et al.* 2005, Strasser *et al.* 2005) are detailed in Table 1.

Rhizospheric soil (soil adjacent to the whole root system) samples were collected from each grass species in the five sample sites (Table 3). In each site, five to six grass species were sampled as either of the C_3 or C_4 metabolic type. The most abundant grasses species (the same number of species for each grass metabolic type: three species with C_3 photosynthetic pathway and three with C_4) were sampled at each gradient site. The rhizospheric soils from five grasses individuals of each species in each sample site were mixed. Each mixed sample was fractioned, one for AMF spores extraction and another for bacterial diversity analysis. The samples were stored at 4°C before processing.

We chose to sample species belonging to one plant family (grasses) to reduce the effect of host species on AMF communities.

Table 1 Soil physicochemical characteristics

Altitude	Texture	HH (%)	$C_{ox.}$ (%)	OM (%)	pH	EC (mS/cm at 25°C)	P (ppm)	C_f (%)
3,320	Sand gravel silty	0.745	0.22	0.4	6.26	0.275	1.11	0.35
3,370	Sand silt gravel	0.835	0.5	1	6.8	0.334	2.77	0.32
3,520	Gravel sand silty	0.837	0.55	1	6.26	0.295	6.23	0.1
3,700	Sand gravel silty	1.564	1.15	2	7.1	1.081	6.85	0.32
3,850	Gravel sandy	1.479	0.64	1	5.9	0.334	1.94	0.31

HH Hygroscopic humidity; *Cox.* oxidable carbon, *OM* organic matter, *EC* electrical conductivity of extract at 1:2.5 water dilution, *P* Phosphorous, *Cf* free carbonates

AMF Methods

For the spore and sporocarp extraction, 100 mL of rhizospheric soil were taken and treated by wet sieving and the decanting method [20]. The material obtained was centrifuged with 80% sucrose [55]. The AMF spores and sporocarps accounted as a single spore were quantified at 500× magnification under dissecting microscope using the gridline method. For the taxonomic identification, fungal spores and sporocarps were mounted onto slides using polyvinyl alcohol with and without Melzer reagent [42] and followed International Culture Collection of Vesicular AMF descriptions (http://invam.caf.wvu.edu/index.html). AMF spore diversity was considered as richness composed by AMF spore richness and AMF spore density [38]. AMF spore richness was accounted as the number of AMF species forming spores for each grass species. Spore density of AMF was considered as the total spore number of AMF spores per 100 mL of rhizospheric soil for each grass species; at the AMF species level, this variable was named specific density.

Bacteria Methods

Samples (0.5 g) were taken from each subsample, homogenized in 10 mL of 0.85% (w/v) saline, and serially diluted (tenfold) in saline. Aliquots (100 μL) were spread on R2A medium for heterotrophs [47] and incubated at 20°C. The number of colony-forming units (CFU) was determined as CFU g^{-1} of dry weight of soil performing three plates for each soil sample. CFU data considered were the mean values between plates, which the standard deviations were lower than 5%. All distinct colony morphotypes grown on these plates were separated and subcultured on R2A agar. Strains were stored at −70°C in 50% (v/v) glycerol for subsequent analysis. Strains identification were preliminary performed by morphological and biochemical test (catalase and oxidase production, casein, starch, gelatin, and urea hydrolysis) [6, 10, 11]. For phylogenetic characterization, single colonies of each strain was removed from the R2A plates and transferred to 2-mL microcentrifuge tubes for subsequent deoxyribonucleic acid (DNA) extraction. DNA was extracted by the cetyltrimethylammonium bromide (CTAB) method. Buffer A (1 mL of 100 mM Tris–HCl–100 mM ethylenediamine tetraacetic acid [pH 8.0]–100 mM phosphate buffer [pH 8.0]–1.5 M NaCl–1% CTAB) was added with 175 μg of proteinase K and 1 mg of lysozyme. Tubes were then agitated on a platform shaker at maximum speed for 20 min. Subsequently, 120 μL of 20% sodium dodecyl sulfate was added, and the samples were incubated at 65°C for 30 min. The samples were then centrifuged at 2,800×*g* for 2 min. The supernatant was transferred to a fresh tube, and the pellet was re-extracted with 300 μL of buffer A and centrifuged again. The combined supernatants were extracted with an equal volume of chloroform–isoamyl alcohol (24:1, v/v). The aqueous phase was precipitated with 0.6 vol of isopropanol at −20°C for 30 min. The pellet was washed in 300 μL of 70% (v/v) ethanol and air dried before resuspending in 100 μL of 10 mM Tris (pH 8.5). Almost full-length 16S ribosomal ribonucleic acid (rRNA) gene sequences were amplified from DNA extracted with oligonucleotide primers 27f (*Escherichia coli* 16S rDNA positions 8 to 27) and 1492r (*E. coli* 16S rDNA positions 1492 to 1512) [33]. The polymerase chain reaction (PCR) mix consisted of deoxynucleoside triphosphates at 200 μM each, 0.25 μM each primer, 3 mM $MgCl_2$, 1× PCR buffer, and 1 U of *Taq* DNA polymerase (Inbio Highway, Tandil, Argentina) in a total volume of 50 μL. Approximately 100 ng of target DNA was added to each reaction. The conditions for the amplification of full-length 16S rRNA genes were: 95°C for 15 min (for enzyme activation and target denaturation), followed by 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min 30 s, and a final extension at 72°C for 5 min. Thereafter, aliquots (10 μL) of the 16S DNA amplicons were visualized by electrophoresis on a 0.8% agarose gel after staining with ethidium bromide and then purified with Wizard PCR Preps (Promega, Madison, WI). Sequencing was performed directly on amplification products (Macrogen, Korea) and aligned with the reference 16S rRNA gene sequence using the basic local alignment search tool analysis according to the method of Altschul *et al.* [1].

Statistical Analysis

The variables (AMF spore richness and AMF spore density previous transformation with [log $(x+1)$]) were analyzed with analysis of variance and Tukey *a posteriori* test. Data out of the normal range (viable bacterial count, spore densities of *Glomus ambisporum* and *Glomus sinuosum*) were analyzed with the Kruskall–Wallis nonparametrical test and the *a posteriori* test of multiple comparisons [39] with $\alpha=0.05$. All analyses were performed considering two factors: altitude and grass metabolic type. Correlation analyzes (AMF spore density, AMF spore richness, rhizospheric bacteria CFU vs altitude and grass metabolic type) were carried out using the Pearson or Spearman test when data were normal or with a non-normal distribution. The Infostat version 2.0 statistic program was used.

Results

Arbuscular Mycorrhizal Fungi

Along the altitudinal gradient, ten AMF species were found (Table 2). The highest mean value of spore density was

Table 2 Specific density of AMF spores along altitudinal gradient

AMF taxa	Altitude (masl)				
	3,320	3,370	3,520	3,700	3,870
Acaulospora sp.	0	0	0.50±1.22	0	0
A. laevis	0	0	0.33±0.82	0	0
A. spinosa	0.40±0.89	0	0	0	0
Gigaspora sp.	0	1.00±2.45	1.17±2.86	0	0
Gi. ramisporophora	0.60±1.34	0.50±1.22	0	0	0
Glomus sp.	3.00±2.55	15.83±36.35	2.83±2.14	14.33±18.28	11.40±8.79
Gl. aggregatum	6.40±10.11	23.00±50.55	36.83±49.81	29.50±60.01	0.80±1.79
Gl. ambisporum	107.6±101.13 C[a]	73.67±62.04 BC	18.17±34.52 A	12.67±15.62 A	9.20±8.79 AB
Gl. sinuosum	4.80±2.49 B	2.00±2.45 AB	0.17±0.41 A	0 A	0.80±1.79 A
Scutellospora biornata	1.60±2.30	0.83±2.04	1.83±3.25	0	0

Data are mean values (*n*=5–6) and standard deviations of spore number for each AMF species (number of spores for each AMF species per 100 mL of rhizospheric soil).

[a] Different letters were used when the differences were significant ($p \leq 0.05$) for Kruskall–Wallis test and test *a posteriori* of multiple comparisons.

107.6 spore/100 mL of rhizospheric soil. AMF richness was negatively correlated with the altitude of the studied sites (p=0.0039, r=−0.5273). AMF spore richness was significantly different at each altitude (F=3.43, p=0.0254). Figure 1 shows the average of the richness among different grasses species at different altitudes. The highest absolute values for AMF richness (seven species) were found at the lowest altitude (3,320 masl). The lowest values were found at the highest altitudes (3,700 and 3,870 masl). Richness did not vary significantly with the metabolic type of grasses (F=0.43, p=0.5191).

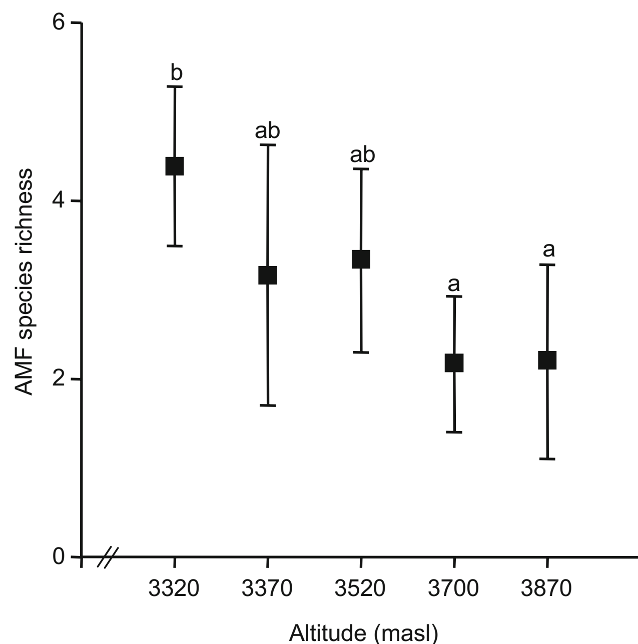

Fig. 1 AMF spore richness along the altitudinal gradient. Data are mean values and standard deviations of grasses species (n=5–6) at each site. Different letters were used when the differences were significant (p≤0.05) for one-way ANOVA and Tukey *a posteriori* test

Spore density of AMF showed a negative correlation with altitude (p=0.018, r=−0.4689). Representatives of Acaulosporaceae, Glomaceae, and Gigasporaceae were observed up to 3,520 masl. From 3,700 to 3,870 masl, only Glomaceae was found (Table 2). Spore density of AMF was not significantly different at each altitude (F=1.95, p=0.137). However, the spore densities of *Gl. ambisporum* and *Gl. sinuosum* were found significant differences with altitude (H=10.61, p=0.0299; H=9.63, p=0.0183, respectively, for Kruskall–Wallis test). In addition, *Gl. ambisporum* and *Gl. sinuosum* were the two species found at all altitudinal levels along the studied gradient. The abundance of *Gl. ambisporum* clearly decreased with altitude. However, the abundance of *Gl. sinuosum* also decreased with altitude without a clear inverse relationship between abundance and altitude (Table 2). Specific density of AMF did not change significantly with the plant metabolic type (F=1.30, p=0.2668).

Rizhospheric Soil Bacteria

Although few colony morphotypes predominated on R2A plates, different morphotypes isolated from 24 samples from the five sites were subcultured. All strains (32 aerobic–heterotroph bacteria) were grouped in three distinctive groups. Fourteen rod-shaped, motile, Gram-positive sporeformers were identified as belonging to the genus *Bacillus*, according to morphological and biochemical tests [10]. Phylogenetic analysis of the 16S rRNA gene confirmed that the bacteria belong to the low GC group within the genus *Bacillus*. Two coccoid, Gram-positive, nonspore-forming, nonmotile bacteria were morphological and biochemically identified as belonging to the Actinobacteria group according to morphological and biochemical

tests [11]. Phylogenetic analysis of the 16S rRNA gene indicated that the bacteria belong to the high GC group within the genus *Arthrobacter*. Finally, five Gram-negative, strictly aerobic, rod-shaped, motile cells were partially identified with morphological and biochemical test as Pseudomonadaceae [6]. Phylogenetic analysis of the 16S rRNA gene indicated that bacteria belong to the gamma-subclass of Proteobacteria within the genus *Pseudomonas*. Most closely related bacteria to the isolated strains are shown in Table 3.

Cultivation methods resulted in a low number of different taxa, all of which have 97 to 99% identity with the sequences described in databases, typical of soil bacteria recovered from soil environments.

The bacterial count values and bacterial diversity, defined in this article as the number of different genotypes, were low along the altitude gradient (Table 3). In average, bacterial count ranged between 1.31×10^2 and 8.66×10^4 CFU g^{-1} of dry weight of soil. The CFU did not show significant differences with altitude (H=4.52, p=0.3400) or with grass metabolic type (H=0.19, p=0.6642). In addition, this variable was not correlated to either the altitude (r=−0.22, p=0.30) or grass metabolic type (r=0.05, p=0.81).

Bacillus was the major bacterial group isolated in R2A solid medium constituting 44% of the total identified cultured bacteria. *Pseudomonas* (16%) and *Arthrobacter* (6%) were the other bacterial groups that would be identified. Whereas samples belonging to sites at 3,700 and 3,520 masl showed *Bacillus* as a single bacterial group obtained by this cultivation method, *Pseudomonas* was the dominant group found at 3,870 masl. At 3,320, only rhizospheric soil samples from *Aristida adscensionis* and *Lycurus setosus* yielded colonies that could be identified. No colonies were obtained on R2A agar medium from rhizospheric soil sampled from *L. setosus*, *Aristida asplundii*, *Bouteloua barbata*, and *Cynodon hirsutus* at 3,370 masl.

Nucleotide Sequence Accession Numbers

The GenBank accession numbers for the 16S rDNA sequences of the isolated bacterial strains examined in this study are as follows: AM237085 to AM237094.

Discussion

Different environmental factors affect both colonization and number of AMF [16, 28, 41]. Altitude is considered an important factor acting on diversity. Generally, an increase in altitude is accompanied by a decrease in temperature, affecting negatively both fungi in general and particularly AM [32]. This behavior was also found in the Puna grasslands. AMF richness and diversity diminished as the altitude increased in coincidence with the stressing effect of altitude. However, comparing altitude gradients of Puna grasslands with Pampa grasslands, altitude had no effect in the case of Pampa grasslands, probably because of the fact that the absolute gradient was smaller and the overall altitude did not surpass 1,025 masl [44].

Arbuscular mycorrhizal fungal richness and diversity values found in the Puna grasslands are low and are quite consistent with those found in arid environments [9, 12, 13, 28, 29, 35, 37]. The values found in this article are lower than those previously found in other high-altitude grasslands [34, 44].

We did not find correlation with grass metabolic type, although some authors showed differential response to host species [7, 49] and metabolic types [26, 27, 34]. Richness and abundance of AMF showed a negative correlation, as the altitudinal increase at the proposed gradient may be accompanied with by decrease in temperature. This factor, together with the decrease in light energy, could have an influence on these fungi, overriding host type [19].

Along the altitude gradient, an increase in the C_3/C_4 grasses species ratio was found, thus C_3 grasses were more frequent at high sites, whereas C_4 grasses species were more abundant at low altitudinal sites. Although we did not find significant differences between C_3 and C_4 grasses for AMF richness and abundance, it is clear that altitude influenced AMF richness because only Glomaceae spores were found at the highest altitude. In rain forests [56], representatives of *Acaulospora* and *Glomus* are usually found. In South American arid environments such as the "Jarillal" at 490 masl, *Gl. ambisporum* and *Gl. sinuosum* are the most abundant species [35]. Furthermore, *Glomus* was the most abundant genus in Namibia desert [28]. The predominance of *Glomus* in extreme environments [28, 35] agrees with the results obtained in Puna because *Glomus* was found at the highest altitude in the gradient.

Regarding bacteria, bacterial density in arid environment is usually low (0.22 10^5/32.67 10^5 CFU g^{-1}) [46]. In Puna grassland, however, density values of cultivable bacteria were much lower than these values. This may be due to the low content of organic matter and availability of water in soils of Puna grassland, both factors limiting microbial activity. pH, considered the major factor to influence bacteria diversity in soil [17], did not differ meaningfully in our sampling sites.

Several studies have indicated a plant-dependent composition of culturable bacteria. However, no differences in the bacterial density were found among studied grasses.

Bacteria diversity was surprisingly low as well. All of rhizospheric bacteria isolated at Puna grassland (*Bacillus*, *Pseudomonas*, and *Arthrobacter*) constitute bacteria groups

Table 3 *Poaceae* of different metabolic types collected at different altitudes and phylogenetic affiliation of rhizospheric soil bacteria

Altitude (masl)	Grass species	Metabolism Type	CFU g^{-1}	Isolates (acc. no.[a])	Most closely related bacteria[b] (acc. no.)	Identity (%)
3,320	*Aristida adscensionis* L.	C_4	8.21×10^2	21S1 (AM237085)	*Arthrobacter sp*. B2–6 (AJ785759)	99
	Bouteloua simplex Lag.	C_4	6.71×10^3	–	–	–
	Eragrostis nigricans var. *punensis* Nicora	C_4	1.09×10^4	–	–	–
	Jarava plumosula (Nees ex Steud.) F. Rojas	C_3	1.71×10^4	–	–	–
	Lycurus setosus (Nutt.) C. Reeder	C_3	2.98×10^4	22S1C (AM237086)	*Bacillus sp*. MSB2047 (AY275493)	98
3,370	*Aristida asplundii* Henrard	C_4	3.72×10^4	–	–	–
	Bouteloua barbata Lag.	C_4	1.82×10^4	–	–	–
	Cynodon hirsutus Stent	C_4	1.48×10^4	–	–	–
	Eragrostis nigricans var. *punensis*	C_4	1.25×10^4	25S5 (AM237092)	*Pseudomonas sp*. K94.08 (AY456703)	99
	Jarava plumosula	C_3	1.34×10^4	27S5A (AM237093)	*A. nicotinovorans* Ph49 (AY833102)	97
				27S5B (AM237094)	*B. anthracis* strain JH16 (DQ232744)	99
	Lycurus setosus	C_3	4.74×10^3	–	–	–
3,520	*Bouteloua barbata*	C_4	2.28×10^4	14S2	*Bacillus* sp.	–
	Cynodon hirsutus	C_4	7.95×10^2	15S2	*Bacillus* sp.	–
	Eragrostis nigricans var. *punensis*	C_4	1.55×10^4	16S2	*Bacillus* sp.	–
	Bromus catharticus Vahl	C_3	7.09×10^3	18S2	*Bacillus* sp.	–
	Jarava subaristata (Matthei) Matthei	C_3	1.77×10^4	13S2 (AM237087)	*B. thuringiensis* 4.3 MW-9 (AY826591)	98
	Nassella inconspicua (J. Presl) Barworth	C_3	1.26×10^4	17S2	*Bacillus* sp.	–
3,700	*Aristida asplundii*	C_4	7.98×10^3	11S4	*Bacillus sp.*	–
	Bouteloua barbata	C_4	4.34×10^2	–	–	–
	Cynodon hirsutus	C_4	2.44×10^2	9S4	*Bacillus sp.*	–
	Bromus catharticus	C_3	6.38×10^3	10S4	*Bacillus sp.*	–
	Deyeuxia rigescens (J. Presl) Türpe	C_3	9.06×10^3	12S4	*Bacillus sp.*	–
	Polypogon interruptus Kunth	C_3	4.64×10^2	8S4 (AM237091)	*Bacillus sp.* MT21(AY690689)	99
3,870	*Bouteloua barbata*	C_4	9.58×10^2	3S3A	*Pseudomonas sp.*	–
				3S3B	*Pseudomonas sp.* New10530 (AF456215)	97
				4S3	*B. thuringiensis* 4.3 MW-9 (AY826591)	98

Table 3 (continued)

Altitude (masl)	Grass species	Metabolism Type	CFU g^{-1}	Isolates (acc. no.[a])	Most closely related bacteria[b] (acc. no.)	Identity (%)
	Bromus catharticus	C_3	7.23×10^3	1S3A	*Pseudomonas sp.*	–
				1S3B (AM237088)	*Pseudomonas sp.* New10530 (AF456215)	97
	Danthonia annableae P.M. Peterson & Rúgolo	C_3	2.27×10^5	5S3A	*Pseudomonas sp.*	–
				5S3B	*Pseudomonas sp.* New10530 (AF456215)	97
	Jarava pungens (Nees & Meyen) Matthei	C_3	8.53×10^2	2S3A	*Pseudomonas sp.*	–
				2S3B	*Pseudomonas sp.* New10530 (AF456215)	97
	Jarava subaristata	C_3	1.97×10^5	6S3A (AM237089)	γ-Proteobacterium LC-G2	99
				6S3C (AM237090)	*Pseudomonas sp.* 7–1	97

[a] Accession number
[b] Most closely related bacteria, include also morphologically assigned genera

associated to P solubilization and showed a synergic association with AMF in temperate grassland and also under laboratory conditions [3, 4, 50, 51].

Bacillus species were found as dominant populations in the photosphere of *Chrysanthemum* [14, 15], barley [43], and of grass [36]. *Arthrobacter* (nonsporulating actinomycetes) are considered to be ubiquitous and predominant members of culturable soil microbial communities of terrestrial subsurface environments and are sometimes the numerically predominant members. Because *Arthrobacter* utilize a wide variety of organic compounds, including aromatic hydrocarbons, they are widely believed to play a significant role in the transformation of organic matter in natural environments. *Arthrobacter* species were also found as dominant populations in rhizosphere DNA of maize grown in tropical soil [22] and from rhizosphere DNA of *Chrysanthemum* [14, 15]. In Puna grassland, bacterial diversity is not plant dependent as other authors found [5], and the low bacterial diversity would be related to soil poverty.

The stimulatory effect of AM extraradical hyphae was known in pot experiments in relation to bacterial genera *Arthrobacter*, *Pseudomonas*, and *Bacillus* [45], and this effect is already known for the relation of *Pseudomonas* over *Glomus* spp. in maize rhizosphere [24]. Although these interactions were not demonstrated between isolated bacteria and AMF communities in Puna grasslands, many publications listed in this article reported these kinds of interactions.

In this study, the description of bacteria diversity is based on the subset of culturable bacteria capable of growing on heterotrophic medium at 20°C under aerobic conditions. Therefore, this description constitutes only one part of the bacterial community in these samples. A culture-independent method using PCR combined with denaturing gradient gel electrophoresis is carrying out on whole DNA extracted directly from soil samples to provide a more complete description of bacteria diversity associated to rhizospheric soil in Puna grasslands.

Acknowledgments We are grateful to M. A. Negritto and E. M. Crespo for their field assistance. We are also indebted to G. R. de Fanjul for the linguistic corrections. This study was supported by grants from ANPCYT (PICT 11082-3 BID 1728/OC-AR) and UNT (CIUNT 26D/334), PROIPRO 2-0302 (SECyT, FQBYF-UNSL), and by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

References

1. Altschul SF, Madden TL, Schaffer AA, Zhang J, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402
2. Azcón R, Azcón-G de Aguilar C, Barea JM (1978) Effect of plant hormones present in bacterial cultures on the formation and responses to VA endomycorrhiza. New Phytol 80:359–364
3. Azcón-Aguilar C, Barea JM (1992) Interactions between mycorrhizal fungi and other rhizosphere microorganisms. In: Allen MF (ed) Mycorrhizal functioning. Chapman & Hall, London, pp 163–198
4. Bagyaraj DJ (1984) Biological interactions with VA mycorrhizal fungi. In: Powell, CL, Bagyaraj, DJ (eds) VA mycorrhiza. CRC, Boca Raton, pp 131–153
5. Berg G, Roskot N, Steidle A, Eberl L, Zock A, Smalla K (2002) Plant-dependent genotypic and phenotypic diversity of antagonistic

rhizobacteria isolated from different *Verticillium* host plant. App Environ Microbiol 68:3328–3338
6. Bergan T (1981) Human- and animal-pathogenic members of the Genus *Pseudomonas*. In: Starr MP, Stolp H, Trüper HG, Ballows A, Schlegel HG (eds) The prokaryotes, vol. 1. Springer, New York, pp 674–679
7. Bever JD, Morton JB, Antonovics J, Schultz PA (1996) Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. J Ecol 84:71–82
8. Cabrera AL, Willink A (1980) Biogeografía de América Latina. OEA, Washington, DC, pp 1–120
9. Camargo-Ricalde SL, Dhillion SS (2002) Endemic *Mimosa* species can serve as mycorrhizal "resource islands" within semiarid communities of the Tehuacán–Cuicatlán Valley, Mexico. Mycorrhiza 13(3):129–136
10. Claus D, Berkeley, RCW (1986) Genus *Bacillus* Cohn 1872. In: Sneath PHA, Mair NS, Sharpe, ME, Holt JG (eds) Bergey's manual of systematic bacteriology, vol. 2. Williams & Wilkins, Baltimore, pp 1105–1139
11. Crocker FH, Fredrickson, JK, White, DC, Ringelberg, DB, Balkwill, DL (2000) Phylogenetic and physiological diversity of *Arthrobacter* strains isolated from unconsolidated subsurface sediments. Microbiology 146:1295–1310
12. Cuenca G, Lovera M (1992) Vesicular–arbuscular mycorrhizae in disturbed and revegeted sites from La Gran Sabana, Venezuela. Can J Bot 70:73–79
13. Dhillion SS, Vidiella PE, Aquilera LE, Friese CF, De Leon E, Armesto JJ, Zak JC (1995) Mycorrhizal plants and fungi in the fog-free Pacific coastal desert of Chile. Mycorrhiza 5:381–386
14. Duineveld BM, Kowalchuk GA, Keijzer A, van Elsas JD, van Veen JA (2001) Analysis of bacterial communities in the rhizosphere of Chrysanthemum via denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA. Appl Environ Microbiol 67:172–178
15. Ellis RJ, Thompson IP, Bailey MJ (1999) Temporal fluctuations in the pseudomonad population associated with sugar beet leaves. FEMS Microb Ecol 28:345–356
16. Eom A, Wilson GWT, Hartnett DC (2001) Effects of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie. Mycologia 93:233–242
17. Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci USA 103:626–631
18. Garbaye J (1994) Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol 128:197–210
19. Gardes M, Dahlberg A (1996) Mycorrhizal diversity in artic and alpine tundra: an open question. New Phytol 133:147–157
20. Gerdemann JW, Nicolson TH (1963) Spores of a mycorrhizal *Endogone* species extracted from the soil by wet sieving and decanting. Trans Br Mycol Soc 46:235–244
21. Giri B, Giang PH, Kumari R, Prasad R, Varma A (2005) Microbial diversity in soil. In: Buscot F, Varma A (eds) Soil biology, vol 3. Microorganisms in soils: roles in genesis and functions. Springer, Berlin, pp 19–55
22. Gomes NCM, Heuer, H, Schönfeld, J, Costa, R, Hagler-Mendonca, L, Smalla, K (2001) Bacterial diversity of the rhizosphere of maize (*Zea mays*) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant Soil 232:167–180
23. Grime JP, Mackey JML, Hillier SH, Read DJ (1987) Floristic diversity in a model system using experimental microcoms. Nature 328:420–422
24. Gryndler M, Vosátka M (1996) The response of *Glomus fistulosum*–maize mycorrhiza to treatments with culture fractions of *Pseudomonas putida*. Mycorrhiza 6:207–211
25. Hartnett DC, Wilson GWT (2002) The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. Plant Soil 244:319–331
26. Hetrick BAD, Kitt DG, Wilson GWT (1988) Mycorrhizal dependence and growth habit of warm-season and cool-season tallgrass prairie plants. Can J Bot 66:1376–1380
27. Hetrick BAD, Wilson GWT, Todd TC (1990) Differential responses of C_3 and C_4 grasses to mycorrhizal symbiosis, phosphorus fertilization, and soil microorganisms. Can J Bot 68:461–467
28. Jacobson KM (1997) Moisture and substrate stability determine VA-mycorrhizal fungal community distribution and structure in an arid grassland. J Arid Environ 35:59–75
29. Khan AG (1974) The occurrence of mycorrhizas in halophytes, hydrophytes and xerophytes, and of *Endogone* spores in adjacent soil. J Gen Microb 81:7–14
30. Kloepper JW, Zablotowicz RM, Tipping EM, Lifshitz R (1991) Plant growth promotion mediated by bacterial rizhosphere colonizers. In: Keister DL, Cregan PB (eds) The photosphere and plant growth. Kluwer, Dordrecht, The Netherlands, pp 315–326
31. Körner C (1999) Alpine soils. In: Körner C (ed) Alpine plant life. Springer, Berlin, pp 63–76
32. Koske RE (1987) Distribution of VA mycorrhizal fungi along a latitudinal temperature gradient. Mycologia 79:55–68
33. Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, New York, pp 115–175
34. Lugo MA, Cabello MN (2002) Native arbuscular mycorrhizal fungi (AMF) from mountain grassland (Córdoba, Argentina) I. Seasonal variation of fungal spore diversity. Mycologia 94 (4):579–586
35. Lugo MA, Anton AM, Cabello MN (2005) Arbuscular mycorrhizas in the *Larrea divaricata* scrubland of the arid "Chaco", Central Argentina. J Agric Tech 1(1):163–178
36. Mc Caig AE, Glover, LA, Prosser, JI (1999) Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. Appl Environ Microbiol 65:1721–1730
37. Mc Gee P (1986) Mycorrhizal associations of plants species in a semiarid community. Aus J Bot 34:585–593
38. Magurran AE (1988) Ecological diversity and its measurement. Croom Helm, London, pp 1–160
39. Marascuilo LA, McSweeney M (1977) Post-hoc multiple comparisons in sample preparations for test of homogenesity. In: McSweeney M, Marascuilo LA (eds) Non-parametric and distribution free methods the social sciences. Books/Cole, California, pp 141–147
40. Martínez Carretero E (1995) La Puna Argentina: delimitación general y división en distritos florísticos. Bol Soc Arg Bot 31:27–40
41. Miller RM (1979) Some occurrences of vesicular–arbuscular mycorrhiza in natural and disturbed ecosystems of the Red Desert. Can J Bot 57:619–623
42. Morton JB (1988) Taxonomy of VA mycorrhizal fungi: classification, nomenclature, and identification. Mycotaxon 32:267–324
43. Normander B, Prosser, JI (2000) Bacterial origin and community composition in the barley phytosphere as a function of habitat and presowing conditions. Appl Environ Microbiol 66:4372–4377
44. Pérez CA, Frangi JL (2001) Grassland biomass dynamics along an altitudinal gradient in the Pampa. J Range Manage 53:518–528
45. Posta K, Marschner H, Römheld V (1994) Manganese reduction in the rhizosphere of mycorrhizal and nonmycorrhizal maize. Mycorrhiza 5:119–124
46. Rao AV, Venkateswarlu B (1983) Microbial ecology of the soils of Indian desert. Agric Ecosys Environ 10:361–369

47. Reasoner DJ, Geldreich EE (1985) A new medium for the enumeration and subculture of bacteria from potable water. Appl Environ Microbiol 49:1–7
48. Ruthsatz B (1977) Pflnzengesellschaften und ihre lebensbedingungrn in den andinen halbwiisten Nordwest Argentiniens. Dissertactiones Botanicae. Band 39. J. Cramer Fl 9490, Vaduz, pp 1–168
49. Sanders IR, Fitter AH (1992) Evidence for differential responses between host-fungus combinations of vesicular–arbuscular mycorrhizas from a grassland. Mycol Res 96(6):415–419
50. Souchie EL, Saggin-Júnior OJ, Silva EMR, Campillo EEC, Azcón R, Barea JM (2006) Communities of P-solubilizing bacteria, fungi and arbuscular mycorrhizal fungi in grass pasture and secondary forest of Paraty, RJ-Bazil. An Acad Bras Cienc 78:183–193
51. Toro M, Azcón R, Barea JM (1997) Improvement of arbuscular mycorrhiza development by inoculation of soil with phosphate-solubilizing rhizobacteria to improve rock phosphate bioavailability (^{32}P) and nutrient cycling. Appl Environ Microbiol 63:4408–4412
52. van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IA (1998) Mycorrhizal fungal diversity determine plant biodiversity, ecosystem variability and productivity. Nature 396:69–72
53. Vargas Gil JR, Culto JP, Quiroga I, Corvalán E, Nieva JJ, Nuñez P, Galán V, De La Paz C, Rodríguez T, Yanez A, Chachaua G, Álvarez M, Zelaya M, De Suárez S, De Rhetti MM (1990a) Atlas de los Suelos de la República Argentina. Tomo I, Jujuy. Secretaría de Agricultura, Ganadería y Pesca. Proyecto PNUD ARG. 85/019. INTA, Argentina, pp 685–731
54. Vargas Gil JR, Culto JP, Quiroga I, Corvalán E, Nieva JJ, Nuñez P, Galán V, De La Paz C, Rodríguez T, Yanez A, Chachaua G, Alvarez M, Zelaya M, De Suárez S, De Rhetti MM (1990b) Atlas de los Suelos de la República Argentina. Tomo II. Salta. Secretaría de Agricultura, Ganadería y Pesca. Proyecto PNUD ARG. 85/019. INTA, Argentina, pp 289–350
55. Walker C, Mize W, Mc Nabb HS (1982) Populations of endogonaceous fungi at two populations in central Iowa. Can J Bot 60:2518–2529
56. Zhao Z-W, Xia Y-M, Qin X-Z, Li X-W, Cheng L-Z, Sha T, Wang G-H (2001) Arbuscular mycorrhizal status of plants and the spore density of arbuscular mycorrhizal fungi in the tropical rain forest of Xishuangbanna, southwest China. Mycorrhiza 11:159–162