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Herbicide-mediated promotion of *Lotus tenuis* (Waldst. & Kit. ex Wild.) did not influence soil bacterial communities, in soils of the Flooding Pampa, Argentina

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ABSTRACT

Promoting the forage *Lotus tenuis* is an appealing alternative to meet the needs for cattle production in the Flooding Pampa region, Argentina. This agricultural practice requires herbicides application to remove plant species competing with *L. tenuis*. The use of chemical compounds, in addition to the removal of native vegetation, eventually may change the diversity of other ecosystem components such as bacterial communities. The objectives of this work were to examine the effect of *L. tenuis* promotion on the bacterial community composition and on specific water-related soil variables, and to detect specific bacterial taxa responding to the *L. tenuis* promotion. In order to achieve these objectives, here we studied three different rangeland sites of the Flooding Pampa region. At each site, two paddocks were compared, one managed to promote the forage legume *L. tenuis*, and the other lacking of management history and hence, covered by natural grasses. To asses bacterial diversity we used 454-FLX pyrosequencing technology of the V4 region of the 16S rRNA gene, on genomic DNA extracted from soil samples. We obtained 135.918 sequences, representing 3187 Operational Taxonomic Units (OTUs) distributed in 12 phyla and 45 classes. Overall, the main identified components of the bacterial community at the Phylum level were Acidobacteria, followed by Verrucomicrobia, Planctomycetes and Chloroflexi. Our results suggest that 5-6 years of land use with *L. tenuis* promotion does not affect the microbial community structure in this ecosystem. NMDS ordination in two dimensions based on Bray–Curtis distances and PERMANOVA test did not show differences in bacterial community composition between paddocks promoted or not with *L. tenuis,* although differences among sites were detected. In parallel, Pearson's correlation analysis suggested that *L. tenuis* promotion would indirectly affect members of classes Acidobacteria and Anaerolineae, through altering water-related soil properties.

1. Introduction

The Flooding Pampa is a vast region (90,000 km^2) of the Buenos Aires Province (Argentina), with about 70% of the area occupied by rangelands, 15% of which are used for mixed agricultural and livestock managements (rearing and wintering). Prominent features of this region are the recurrence of floodings and low soil fertility, what precludes the maintenance of cultivated pastures or crops (Sierra and Montecinos 1990; Soriano et al., 1992). As result, summer activities of cattle breeding such as rearing, wintering or dairy are often affected by the lack of forage quantity and quality (Rojas et al., 2011). This deficiency is compensated in some cases with sorghum or soybeans-derived forage, which is highly expensive and dependent on the matching between rains occurring during the growing season and the phenological period in that water is required by plants.

Promotion is defined as an agricultural practice consisting in removing competition from weeds and resident pastures using herbicides, so that the species of interest grows and fully develops.

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This practice has been experimentally used by Thom et al. (1993) to study the effect of herbicide on paspalum control and the growth and persistence of perennial ryegrass and white clover (*Trifolium repens* L.) plants over 5 years. The promotion of *Lotus tenuis*, a naturalized species with high forage value that reaches its maximum biomass in summer is currently being tested as alternative to overcome forage deficiency in this season. *L. tenuis* is a perennial legume that became naturalized in vast areas of the Flooding Pampa (Escaray et al., 2012). This species is characterized by a high capacity for natural reseeding, and the ability to withstand the water deficit that often occurs from late spring through summer, offering plenty of high quality forage.

L. tenuis is uncompetitive during the first stages of implantation, therefore, the application in winter of glyphosate (2-(phosphonomethyl) glycine, a post-emergence herbicide) removes broad leaf weeds, improving *L. tenuis* implantation, and hence, increasing its dominance.

Soil quality and fertility (major issues for sustaining livestock production in range management) greatly relay on soil bacterial communities (Gans et al., 2005), which may be impacted by herbicides (Lupwayi et al., 2009; Lancaster et al., 2010; Barriuso and Mellado, 2012; Cycón et al., 2013; Aguayo et al., 2014; Tang et al., 2014) and changes in botanical composition (Greacen and Sands 1980; Mulholland and Fullen, 1991; White et al., 2000). Also, livestock grazing may indirectly alter bacterial community composition, by increasing bulk soil density (Ford et al., 2013) and by reducing water infiltration (Blackburn et al., 1982; McCalla et al., 1984) and soil water retention, or water content (Carrero-González et al., 2012; Lei et al., 2012). On other hand, there may be an inverse relationship between the infiltration rate and bacteria, due to microbial growth and/or mechanical clogging (Rubol et al., 2014; Muirhead et al., 2006). Interestingly, *L. tenuis* plants present a highly developed tap root that allows a deeper soil exploration, which has been related to the improvement of soil water infiltration after several years of *L. tenuis* cultivation (Criado, 2014). On these bases, in this work we defend the hypothesis that the management with *L. tenuis* promotion alters soil bacterial communities, by affecting soil water-related properties.

We expect to gain knowledge on whether *L. tenuis* promotion affects diversity and community structure of soil bacteria, regardless of whether the effect is due to the herbicide-induced *L. tenuis* dominance or to herbicides themselves. This information could be useful to decide about the sustainability of this agricultural management before it becomes a common agronomical practice in the Flooding Pampa.

High-throughput pyrosequencing has emerged in the last years as a less labor intensive alternative method to examine highly diverse bacterial communities in different soils (Cristea-Fernström et al., 2007; Huse et al., 2007; Liu et al., 2007; Roesch et al., 2007; Sundquist et al., 2007; Dowd et al., 2008). In this work, three natural grass-based rangelands sites of the Flooding Pampa, continuously grazed by similar cattle charges were studied. Massive pyrosequencing of the V4 region of the 16S rRNA gene was used to examine the structure and diversity of the bacterial communities in paired paddocks with two different land uses: *L. tenuis* promotion with herbicides and natural grassland. Our objectives in this study were: (1) to examine the effect of *L. tenuis* promotion on the bacterial community composition and on the soil variables infiltration rate, water content and bulk density, and (2) to detect specific bacterial taxa responding to the *L. tenuis* promotion. In order to achieve these objectives, the bacterial taxonomic composition and diversity was analyzed and compared among different sites and land-management.

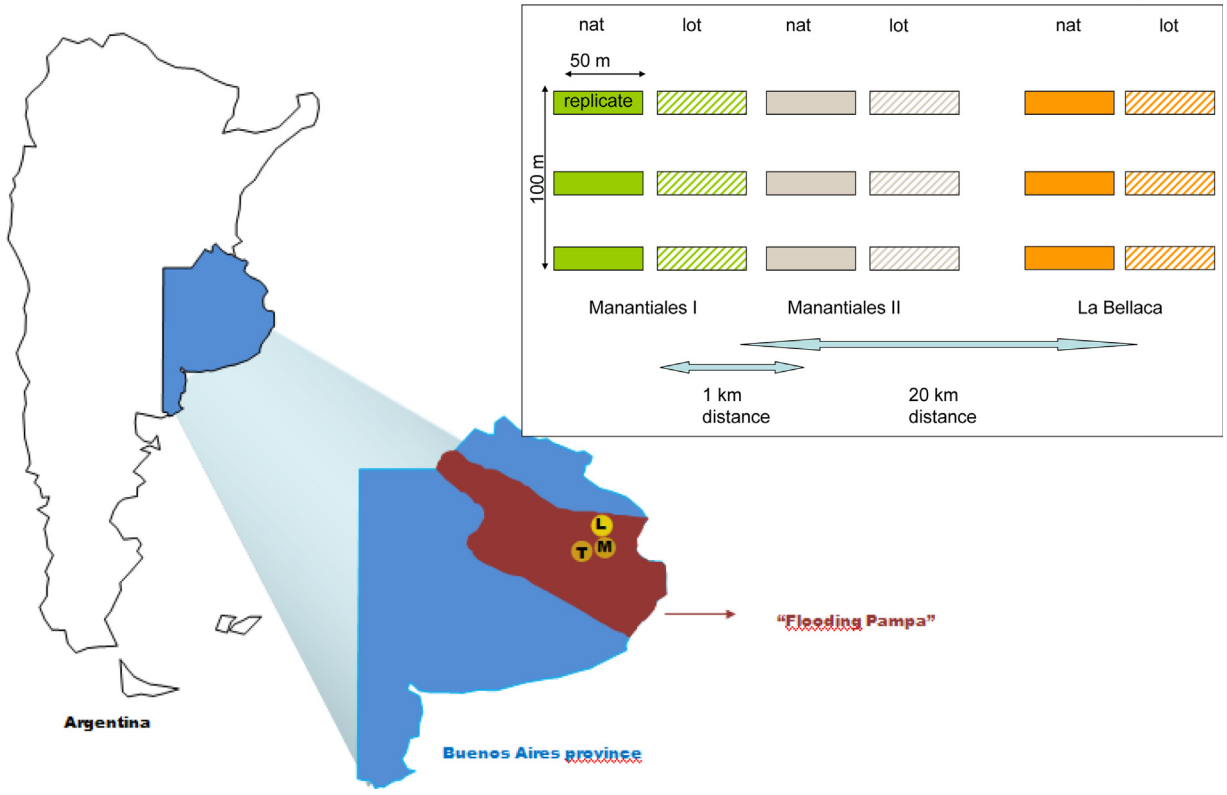

Fig. 1. Sampling sites for Flooding Pampa datasets. Soil samples were obtained in three different sites: Manantiales 1 (M), Manantiales 2 (T) and La Bellaca (L). In each site, two different land uses were studied: natural grass cover (nat) and promotion with *L. tenuis* and herbicides (lot). For sequencing purposes, three soil replicates per site were taken per site-land use combination, each consisting of 25 pooled soil cores.

2. Materials and methods

2.1. Study sites characteristics

Soil samples were obtained from three representative sites located at the Flooding Pampa area of Chascomús (Buenos Aires, Argentina: Manantiales 1 [(M) S 35°45′01′, W 58°02′22′]; La Bellaca [(L) S 35°35′55′′, W 57°56′44′′]; Manantiales 2 [(T) S 35°45′39′, W 58°03′38′.] Twenty kilometers separate (L) from (T) and (M), whereas the last two sites are at a 1 km of distance from each other (Fig. 1).

Soil samples were collected in November 2013 from neighboring paddocks (100 × 50 m, approximately) subjected to one of the two following types of land use: natural cover (nat) or *L. tenuis* promotion with herbicides (lot). Samples were named by the initial of each site followed by land use (e.g.: Mlot; Mnat, etc.).

All paddocks held 5 years of similar cattle grazing charge since November to March. Topography was similar among sites, with plain or concave relief. The soils studied in this research were Natraquoll, characterized by an A1 horizon with 3.5% organic carbon and 0.22 mg kg^{-1} of extractable Fe, and by a nitric B2t horizon at 17 cm of depth, with 53.3% clay content (Striker et al., 2005).

2.2. Herbicides treatment

L. tenuis promotion was achieved by the application of glyphosate (*N*-(phosphonomethyl) glycine; 3.5 l/ha), followed by two applications of 2,4 DB(4-(2,4-dichlorophenoxy) butyric acid, 1 l/ha) and a single dose of Quizalofop-*p*-ethyl(Ethyl(*R*)-2-[4-(6-chloro-2-quinoxalyloxy)phenoxy]propionate; 1.2 l/ha), in six or five annual cycles from June to August. Non-promoted (nat) paddocks received no management or agrochemicals since 1970. No fertilizer was applied in any case. After 4 or 5 years of herbicides application, plant species composition in promoted paddocks shifted, and *L. tenuis* became the dominated species, whereas in paddocks where no herbicide was ever applied, plant community was represented by native species and naturalized forages such as *Festuca arundinacea, Lolium multiflorum, Ambrosia tenuifolia, Esporobolus indicus, Paspalum vaginatum* and *L. tenuis.*

2.3. Samples collection

There were three soil replicates per site-land use combination. Each soil replicate was a composed sample consisting of 25 pooled soil cores. Soil cores were taken with a borer (20 cm length × 7 cm diam) every 10 m. Soil replicates were introduced into separated plastic bags and immediately transferred to the laboratory, where they were sieved through a 2 mm mesh and used for nucleic acid extraction. Genomic DNAs for PCR and pyrosequencing procedures were stored at −20C°.

2.4. Soil DNA extraction, PCR and pyrosequencing

Total DNA for amplicon sequences libraries was obtained from a 0.25 g aliquot of each soil replicate using PowerSoil DNA isolation kit (MO BIO Laboratories, Inc, CA, USA) following manufacturer's instructions. DNA quality and concentration in each sample was evaluated by spectrophotometer (Synergy TM H1, BIOTEK) and 1.5% agarose gel electrophoresis. Amplicon libraries were prepared on the base of the V4 hyper variable region of the 16s rDNA gene. This region has been proposed by the Ribosomal Database Project Data for diversity data collection, using 454-Roche technology (Olsen et al., 1992). Universal primers 515F: 5′ GTGCCAGCMGCCGCGGTAA 3′ and 806R: 5′ GGACTACVSGGGTATCTAAT 3′ were used and a re-amplification was performed to include the Roche 454 sequencing A and B adaptors and a nucleotide "multiple identifier" (MID) to sort samples. The PCR mixture (final volume 25 μl) contained 2.5 μl FastStart High Fidelity 10× Reaction Buffer (Roche Applied Science, Mannheim, Germany), 20 ng of template DNA, 0.4 mM of each primer, and 1.25 U FastStart High Fidelity Enzyme Blend (Roche Applied Science), and 0.2 mM dNTPs. The PCR conditions were 95 °C for 5 minutes for initial denaturalization, followed by 95 °C for 45 s, 57 °C for 45 s, 72 °C for 60 s in 30 cycles, and a final elongation step at 72 °C for 4 min. Two negative control reactions containing all components except for the template were performed. Libraries were purified using Agencourt AMPure XP. Purified PCR product was sequenced on a Genome Sequencer FLX (Roche Applied Science) using Titanium Chemistry according to the manufacturer's instructions. Analyses were performed at INDEAR (Argentina) genome sequencing facility.

2.5. Sequencing data analysis

Amplicon sequences were analyzed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline, version 1.8.0. Package (Caporaso et al., 2010). Sequences were demultiplexed and trimmed by quality using a split_libraries.py script from QIIME, and clustered into Operational Taxonomic Units (OTUs) using the pick_otus.py script and Uclust (Edgar, 2010) at 97% similarity. The most abundant sequence from each cluster was chosen as a representative. All representative sequences were aligned with the PyNAST method (Caporaso et al., 2010). The sequences from this study are available in GenBank under the Project accession code PRJNA2855990. Taxonomical identities were assigned to each representative sequence with the RDP classifier using the Greengenes Database update (McDonald et al., 2012), using a 50% bootstrap confidence. Singletons and unclassified sequences were removed from the obtained OTU table. Rarefaction curves of observed OTUs versus the number of sequences were generated with PAST 3.06 (Hammer et al., 2001). For further analyses, the final OTU table was normalized by sumbsampling down to the lowest number of reads, in order to correct for possible biases introduced by unequeal sequencing efforts (Vegan package (Oksanen et al., 2012), R environment (www.r-project.org)).

2.6. Diversity and statistical analyses

To compare OTUs diversity across the treatments, the Shannon diversity index (Shannon and Weaver, 1949) and Simpson index (Magurran, 1998) were calculated. The richness was estimated via extrapolation from observed patterns using the Chao-1 approach (Hughes et al., 2001). To test the hypothesis of "no differences in bacterial community composition (BCC) between treatments", we performed Hotelling paired comparisons on α-diversity indexes ($p < 0.05$, Hotelling, 1951).

For β-diversity analysis, the normalized OTU matrix was Hellinger transformed (Legendre and Gallagher, 2001) and then analyzed by computing the abundance weighted Bray–Curtis distances (Bray and Curtis, 1957) between samples at the 97% similarity OTU level, using Past 3.06 (Hammer et al., 2001). Patterns in the obtained similarity matrix were explored using nonmetric multidimensional scaling (NMDS) (Clarke and Green, 1988). Goodness of fit to the similarity matrix was assessed by using Kruskal stress formula 1 (Legendre and Legendre, 1998). Permutational MANOVA (PERMANOVA) (Clarke, 1993) was applied with 999 permutations to test the hypothesis of "no differences among sites". Vegan package (Oksanen et al., 2012) in the R environment (R Development Core Team, 2013).

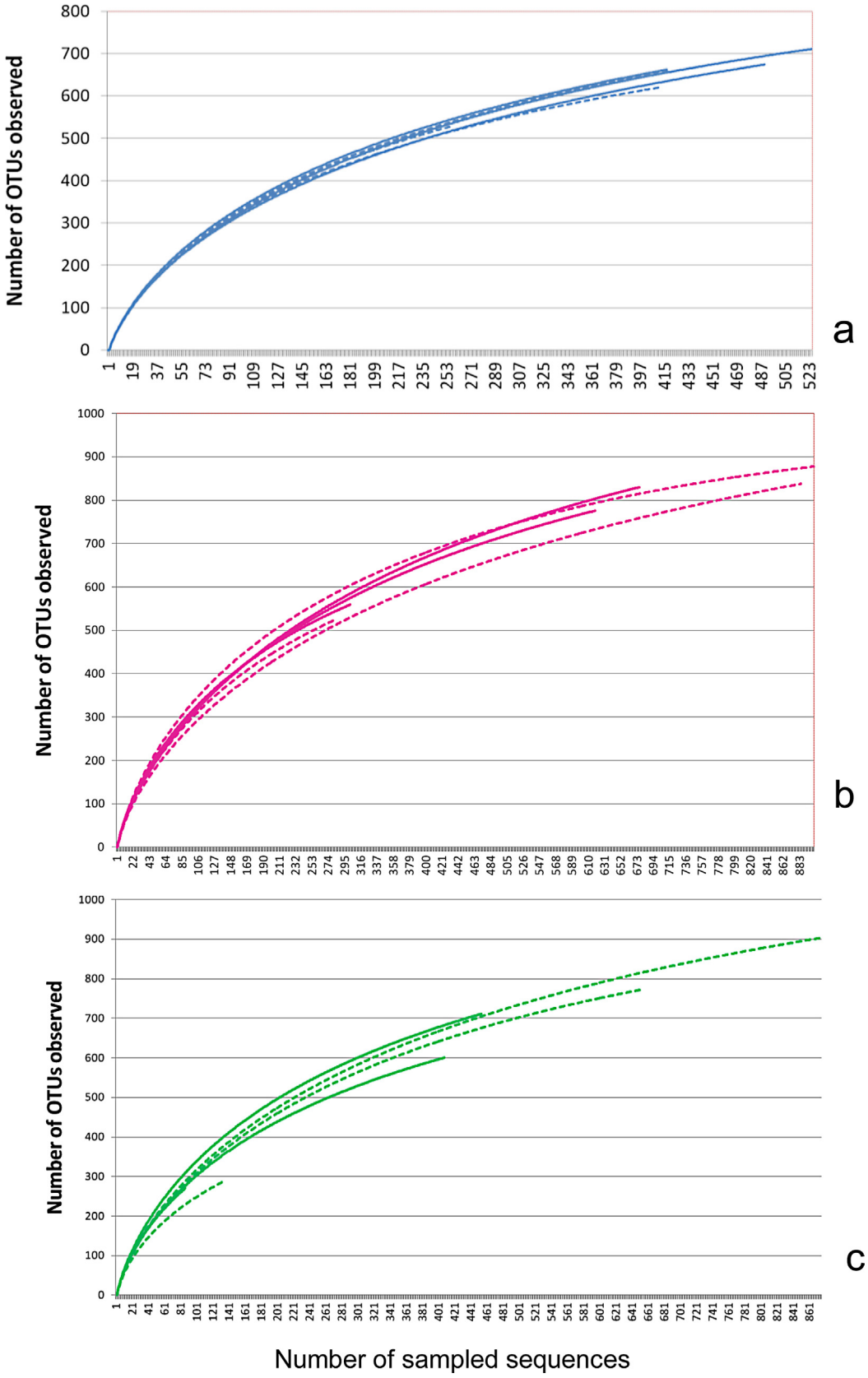

Fig. 2. Rarefaction curves of the number of observed OTUs vs the number of sequences per sample, for the three replicates of each site-land use combination: (a) Manantiales 1 (M), (b) La Bellaca (L) and (c) Manantiales 2 (T). ; dashed and solid lines correspond to natural grass cover (nat) and promotion with *L. tenuis* and herbicides (lot), respectively.

2.7. Measurement of soil physical variables.

Water content (WC) and apparent bulk density were measured in four soil cores (9 cm length × 6 cm diam) per site-land use combination (Blake and Hartge, 1986). Each sample was immediately weighted and oven dried (105 °C). Water content and bulk density of soils were measured as:

$$\text{Water content}(\%) = \left(\frac{(\text{soil fresh weight} - \text{soil dry weight})}{\text{soil dry weight}}\right) \times 100$$

$$\text{Bulk density}(\text{g cm}^{-3}) = \frac{\text{soil dry weight}}{\text{soil volume}}$$

For infiltration rate measurements, 5 m^2 of sampling points were first cleaned by trimming the vegetation. A ring infiltrometer of 19 cm diam was introduced into the soil (without disturbing the soil surface), at a 10 cm penetration depth (Luters and Salazar, 1999). First, water was poured into the infiltrometer to moisten the soil. Then, additional water was added to a ponding depth of 10 cm. After 10 min and 1 h of infiltration, the ponding depth was measured again. With this data the infiltration rate was calculated as:

$$\text{Infiltration rate}(\text{cm/min or h}) = (\text{initial ponding depth} - \text{final ponding depth})/\text{min or h}$$

In order to investigate the relationship between soil physical variables and obtained patterns in BCC, NMDS axis scores were correlated with each soil physical variable using the Spearman Rank Order correlation coefficient. In addition, Pearson's correlations were performed between these measured soil variables and abundance of the different bacterial and archaeal classes.

3. Results

3.1. Analysis of alpha and beta diversity

We obtained 135.918 filtered sequences from the 18 studied samples, with a 336.43 (S.D. = 40) bp average length, totalizing 45.7 Mb. Filter parameters were set to reject reads that had mean quality score <20. The mean number of sequences per sample was 4967,17. Initially, OTUs number was 4656, but it decreased to 3187 after singletons removing and normalization. The rarefaction curve for OTUs (3% of dissimilarity) versus number of samples was not asymptotic, suggesting that the surveying effort did not cover the full extent of taxonomic diversity (Fig. 2).

The Shannon, Simpson and Chao1 indexes ranged from 4.29 to 5.13, 0.01 to 0.04 and 257 to 655, respectively (Figs. 3–5).

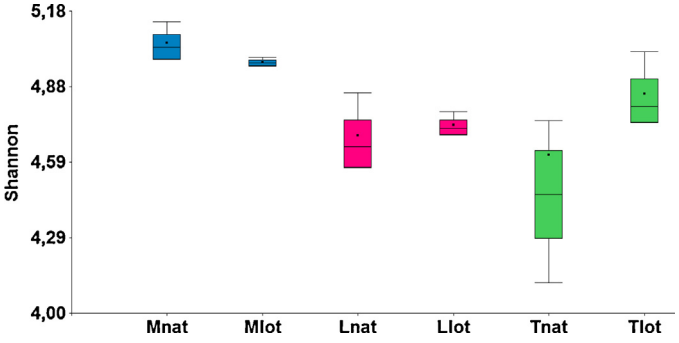

Fig. 3. Shannon index for the 18 site and land use combinations, based in 97% similarity of 16S rRNA gene sequences.

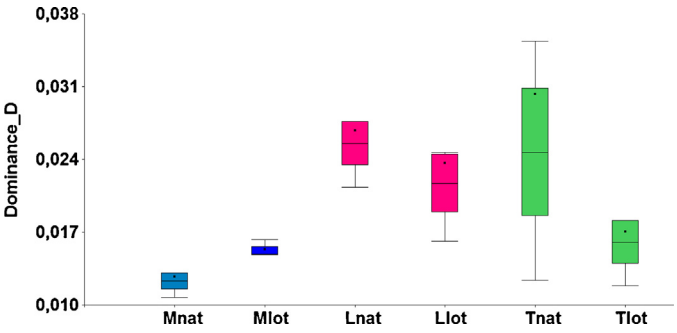

Fig. 4. Simpson index of dominance for the 18 site and land use combinations, based in 97% similarity of 16S rRNA gene sequences.

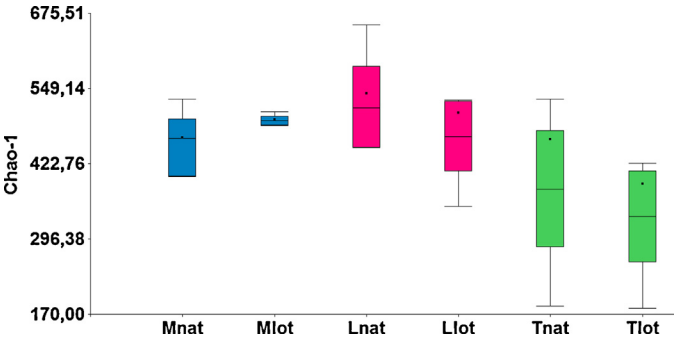

Fig. 5. Richness Chao 1 index for the 18 site and land use combinations, based in 97% similarity of 16S rRNA gene sequences.

According to the multivariate Hotelling test, there were not differences in α-diversity between promoted (lot) and non promoted plots (nat).

The NMDS ordination in two dimensions based on Bray–Curtis distances showed that (M) samples grouped separately from those of the other two sites, suggesting that they harbored a distinct bacterial community (Fig. 6). In contrast, samples from (L) and (T) partially overlapped, and there was not clear separation between "lot" and "nat" samples. The PERMANOVA test analysis with 999 permutations for significance of Bray–Curtis distances revealed differences among sites, but not between land uses ($F = 3.2658$; $p > 0001$).

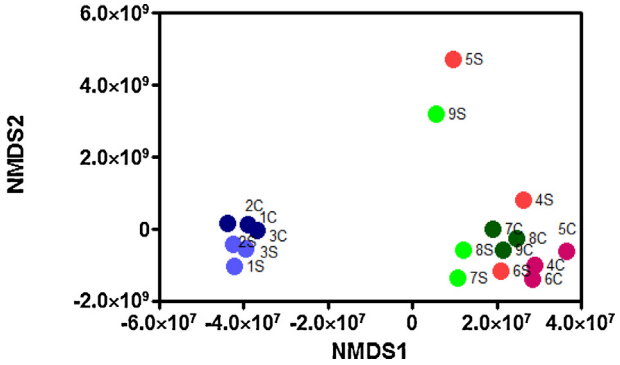

Fig. 6. Ordination by non parametric multidimensional scaling (NMDS) based in Bray–Curtis distances for the 18 samples. (1S, 2S and 3S = Mnat; 1C, 2C and 3C = Mlot; 4S, 5S and 6S = Lnat; 4C, 5C and 6C = Llot; 6S, 7S and 8S = Tnat, and 6C, 7C and 8C = Tlot) (Stress = 0.05703946).

Table 1
Relative abundance (%) of phyla at each evaluated site-land use combination, according to 16S rDNA sequences classified by Greengenes database.

Domain	Phylum	Manantiales 1		La Bellaca		Manantiales 2	
		Mnat	Mlot	Lnat	Llot	Tnat	Tlot
Archaea	Euryarchaeota	1.77	3.44	3.39	2.95	3.34	3.28
	Crenarchaeota	0.28	0.53	0.14	0.12	0.40	0.27
Bacteria	Acidobacteria	24.47	40.96	39.30	36.46	40.20	38.47
	Verrucomicrobia	3.53	5.56	17.98	24.16	20.31	19.17
	Planctomycetes	10.07	19.85	13.07	13.44	14.89	14.71
	Chloroflexi	5.26	9.55	9.19	7.10	7.29	6.85
	SPAM	4.92	4.98	3.12	3.14	3.21	4.31
	Other Bacteria	2.82	7.60	5.76	2.04	2.41	2.47
	Armatimonadetes	0.36	0.89	1.40	1.99	1.11	1.60
	Synergistetes	0.66	1.21	1.57	1.12	1.21	1.27
	Proteobacteria	1.19	1.67	0.70	0.80	0.81	1.74
	Actinobacterias	0.27	0.47	0.85	0.73	0.94	0.94
	Spirochaetes	0.44	0.28	0.22	0.43	1.36	0.77

Obtained 16S sequences were classified according Greengenes database (McDonald et al., 2012).

3.2. Taxonomical analysis

The RDP classifier assigned obtained sequences to 13 Phyla and 48 classes (Supplementary material. Table 1), whereas unclassified taxa represented 0,002% of the total OTUs.

Overall, the main identified components of the bacterial community at the Phylum level were Acidobacteria, Verrucomicrobia, Planctomycetes and Chloroflexi, in that order (Fig. 7; Table 1). Remaining Phyla were less abundant, although ubiquitous.

As a trend, our results showed that the land use did not significantly affect the abundance of OTUs affiliated to the most abundant Phyla, and that the relative abundance of some of the most abundant Phyla varied according with the site. This is the case of Verrucomicrobia presented at a higher relative abundance in (L) and (T) than in (M) (Table 1). OTUs belonging to identified classes with relative abundances >1% in any sample, that were shared by all sites-land use combinations accounted for 86–99% of the classified sequences (Table 2). The most abundant classes were Phycisphaerae, Chloracidobacteria, Acidobacteria and Planctomycea, in that order.

3.3. Soil physical variables

Infiltration rates were similar among non-promoted sites, whereas the (Tlot) site showed a lower infiltration rate than (Llot) and (Mlot) (Table 3). Lower WC and higher apparent soil density where also found in (T), comparing with (L) and (M). Promotion with *L. tenuis* consistently increased soil infiltration rates at 10 min in the three sites, and raised this rate at 1 h in (L) and (M). The water content was decreased by *L. tenuis* promotion in the three sites, in accordance with infiltration rates results, whereas the *L. tenuis* promotion induced different variations on bulk density, according with the site: increased in (M), diminished in (T) and did not change in (L).

The Spearman rank order coefficient showed no correlation between NMDS scores and soil physical variables ($p < 0.05$). Besides, no correlation between these variables and classes abundance was found, excepting for few, less frequent classes, as is the case of Ktedonobacteria, Soilbacteres, Opitutales, Acidobacteria, Anaerolineae, FFCH6980 and RB25 (Table 4).

4. Discussion

Increasing plant and livestock production are major targets for pasture-based systems, where bacteria are key components of numerous geochemical processes supporting production. Here we studied the bacterial diversity in six different site-land use combinations, in a relatively reduced area of the Flooding Pampa, the main region for cattle production in Argentina. Our results showed that *L. tenuis* promotion with herbicides did not show a significant influence on soil bacterial community structure, suggesting that it did not generate a selection pressure (e.g.: by reducing bacterial resources) capable of driving a change in bacterial community composition, towards a new community adapted to the new environmental condition. This result is congruent with studies where glyphosate was applied to cotton (Barriuso and Mellado, 2012) and maize (Barriuso et al., 2010, 2011), and with other works, in which reduction in plant diversity did not necessarily led to a reduction of the detected bacterial diversity (Kowalchuk et al., 2002; Grüter et al., 2006; Köberl et al., 2011; Zul et al., 2007). This information suggests that continuous *L. tenuis* promotion would be a sustainable practice that could be spread in the Flooding Pampa without significantly endangering the bacterial soil community. However, it is also possible that 5 or 6 years of this land use have not provided enough time for the bacterial community to reach a new equilibrium and therefore, further similar studies should be undertaken for longer periods.

Even though (M), (T) and (L) sites were relatively close to each other and shared the same climatic fluctuations, these sites presented different soil BCCs, as shown by the the NMDS with site (M) separating from the other two sites (Fig. 6). These sites also presented different soil structure in terms of water infiltration rate, water content and apparent bulk density (Table 3). Taken together, these results agree with the notion that the Flooding Pampa presents small topographic differences that determine important changes in soil characteristics (Ghersa et al., 2007).

To our knowledge, this work constitutes the first attempt to characterize and compare the structure of bacterial community in soils of the Flooding Pampa region. Our results revealed that the assemblages of bacterial species here found would be less rich and diverse than those reported for other rangeland systems, using similar techniques (Will et al., 2010; Castro-Silva et al., 2013). However, it is possible that our surveying effort did not cover the full extent of taxonomic diversity, as suggested by the OTUs rarefaction curve (Fig. 2).

Phyla found as the most dominant in this work, Acidobacteria, Verrucomicrobia, Planctomycetes and Chloroflexi were previously also reported as dominant in a survey of the global soil bacterial community based on libraries of 16S rRNA genes (Janssen, 2006). Our results differed from that survey with respect to the contributions to soil bacterial communities by different phyla. For example, Verrucomicrobia are reported to make up an average of 7% in soil bacterial communities (Janssen, 2006), whereas in this work, Verrucomicrobia reached averaged abundances as high as 24%, reinforcing the view that this group is more abundant in grasslands (Vandekerckhove et al., 2000, 2002; Wagner and Horn, 2006). On the other hand, members of the Phylum Planctomycetes are quite widely distributed in marine and hypersaline habitats (Fuerst, 1995). The ecosystem under study in this work is characterized by recurrent flooding (Sierra and Montecinos, 1990) and salinization of soil surface horizons, in connection with flood-drought cycles (Lavado and Taboada 1988). Therefore, recurrent flooding might also explain the relatively higher abundance of members affiliated to Planctomycetes found in this work (10–19.85%), compared with their contribution to libraries from soil bacterial communities (0–7.8%; Janssen, 2006) and other pasture-base systems (Acosta-Martinez et al., 2010; Will et al., 2010). Likewise, the high abundance registered for members of Acidobacteria (24.5–41%) reinforces the idea that recurrent flooding would be a major determinant of bacterial diversity in the

Table 2
Relative abundance (%) of identified taxa at the class level (percent of all detected OTUs belonging to each taxon).

Domain	Phylum	Class	M		L		T	
			nat	lot	nat	lot	nat	lot
Archaea	Crenarchaeota	C2	0,06	0,01	0,01	0,00	0,00	0,01
		Thaumarchaeota	0,62	0,51	0,13	0,12	0,40	0,26
	Euryarchaeota	Non classified	0,00	0,00	0,03	0,00	0,05	0,00
		Halobacteria	1,16	0,79	1,29	0,74	1,02	0,42
		Methanobacteria	2,72	2,28	1,65	1,96	1,78	2,56
		Methanomicrobia	0,04	0,10	0,07	0,00	0,25	0,07
		Thermoplasmata	0,08	0,27	0,35	0,24	0,25	0,22
Bacteria	Acidobacteria	Non classified	1,67	0,95	0,69	0,87	0,95	0,85
		Acidobacteria	19,79	18,63	6,92	4,74	12,13	9,43
		Chloracidobacteria	13,55	19,74	29,87	26,90	25,32	25,15
		iii1-8	1,56	1,30	1,72	3,51	1,85	3,10
		MVS-40	0,29	0,27	0,16	0,41	0,12	0,04
		RB25	0,06	0,09	0,08	0,02	0,06	0,00
		Soilbacteres	0,06	0,04	0,00	0,13	0,00	0,02
		Sva0725	0,02	0,02	0,00	0,03	0,02	0,00
	Verrucomicrobia	Non classified	0,00	0,01	0,01	0,00	0,00	0,00
		Methylacidiphilales	0,00	0,00	0,00	0,01	0,00	0,08
		Opitutales	0,07	0,07	0,05	0,18	0,10	0,10
		Spartobacteria	1,72	1,43	15,96	21,57	16,42	11,07
		Verrucomicrobiae	4,82	3,93	1,82	2,12	3,66	2,47
	Planctomycetes	agg27	2,32	2,18	0,44	0,39	1,40	0,79
		C6	0,32	0,23	0,00	0,03	0,00	0,00
		FFCH393	0,04	0,04	0,02	0,03	0,00	0,00
		Kueneniae	0,02	0,01	0,00	0,00	0,00	0,00
		Phycisphaerae	19,17	19,78	13,05	13,41	14,31	14,67
		Planctomycea	10,25	9,36	7,22	7,07	7,23	7,07
		PW285	0,10	0,23	0,08	0,05	0,00	0,10
		vadinHA49	0,20	0,26	0,00	0,00	0,00	0,01
	Chloroflexi	Non classified Chloroflexi	0,00	0,00	0,01	0,04	0,07	0,03
		Anaerolineae	6,22	6,20	3,32	1,76	2,22	1,64
		Bljii12	0,11	0,03	0,10	0,26	0,13	0,21
		Chloroflexi	0,03	0,18	0,15	0,22	0,16	0,43
		Chloroflexi-4	0,01	0,05	0,01	0,00	0,00	0,12
		Ktedonobacteria	0,22	0,18	0,03	0,44	0,00	0,47
		RA13C7	0,00	0,00	0,00	0,00	0,01	0,00
		SOGA31	2,66	1,17	3,54	3,13	3,13	2,33
		Thermomicrobia	0,68	0,70	0,69	0,51	0,70	0,65
		TK17	0,79	0,89	0,83	0,39	0,70	0,29
	Armatimonadetes	SJA-176	0,10	0,06	0,00	0,13	0,00	0,06
	Actinobacteria	Actinobacteria	0,50	0,47	0,85	0,73	0,94	0,94
	Proteobacteria	Alphaproteobacteria	0,48	0,43	0,14	0,40	0,34	0,79
		Betaproteobacteria	0,35	0,32	0,22	0,22	0,23	0,34
		Deltaproteobacteria	0,76	0,50	0,19	0,15	0,15	0,00
		Gammaproteobacteria	0,31	0,28	0,03	0,28	0,15	0,54
		Non classified Proteobacteria	0,00	0,04	0,00	0,00	0,00	0,00
	SPAM	0319-6G9	5,69	4,79	3,00	3,06	3,11	4,26
		FFCH6980	1,14	0,19	0,12	0,08	0,10	0,05
	Spirochaetes	Brachyspirae	0,45	0,27	0,22	0,43	1,36	0,58
		Leptospirales	0,04	0,01	0,00	0,00	0,00	0,19
	Synergistetes	Synergistia	1,17	1,21	1,57	1,12	1,21	1,27

M: Manantiales 1; L: La Bellaca; T: Manantiales 2; lot and nat mean *L. tenuis* promotion and natural grass, respectively.

Table 3
Water infiltration rate at 10 min and 1 h, water content and aparent bulk density.

Site	Land use	Infiltration rate after 10 min (cm/10 min)			Infiltration rate after 1 h (cm/1 h)			Water content			Aparent bulk density		
(L)	lot	2.57 a	±	0.98	9.5 a	±	3.81	328 b	±	7.7	0.92 d	±	0.02
	nat	0.2 b	±	0.12	1.01 b	±	0.33	385 a	±	3.9	0.98 d	±	0.01
(M)	lot	2.85 a	±	0.41	10.9 a	±	1.66	314 b	±	8	1.08 c	±	0.02
	nat	0.08 b	±	0.002	0.7 b	±	0.35	396 a	±	4.3	0.91 d	±	0.03
(T)	lot	2.93 a	±	0.59	2.7 b	±	0.51	189 d	±	18.4	1.18 b	±	0.02
	nat	0.13 b	±	0.02	0.2 b	±	0.04	268 c	±	7.1	1.37 a	±	0.06

Means (±EE; $N = 4$) with the same letter within each column are not significantly different (Duncan, $P < 0.05$). M: Manantiales 1; L: La Bellaca; T: Manantiales 2; lot and nat mean *L. tenuis* promotion or natural grass, respectively.

study area, as these bacteria are well adapted to tolerate fluctuations in soil hydration (Ward et al., 2009).

Despite the lack of significant changes in BCC due to *L. tenuis* promotion, the Pearson's correlation analysis between edaphic variables that were affected by *L. tenuis* promotion (Table 4) and

Table 4
Significant Pearson's correlations between measured soil physical variables, and relative abundance of bacterial classes.

Soil physical variables	Bacterial classes	*n*	Pearson	*p*-value
Aparent bulk density	Soilbacteres	18	−0.48	0.04
Infiltration rate (10 min)	Ktedonobacteria	18	0.47	0.05
Infiltration rate (1 h)	Ktedonobacteria	18	0.69	0
	Soilbacteres	18	0.63	0.01
	Opitutales	18	0.48	0.04
	Acidobacteria	18	−0.47	0.05
Water content	Anaerolineae	18	0.50	0.03
	FFCH6980	18	0.51	0.03
	RB25	18	0.47	0.05

bacterial classes, suggests an indirect influence of this land on specific bacterial taxa. Among such correlations, it is worthy to note the negative relationship between the class Acidobacteria and the infiltration rate (1 h). This, along with our result showing that *L. tenuis* promotion increased the infiltration rate (1 h, Table 3) would indicate that Acidobacteria were negatively impacted by this land use. It is known that pH is the most prominent environmental factor correlating with acidobacterial abundance in soils (Naether et al., 2012). In particular, low pH has been related with higher proportions of *Acidobacteria* (Mannisto et al., 2007; Griffiths et al., 2011; Jones et al., 2009). Taken together, this information along with results obtained in the present work, invite us to hypothesize that pH could be higher in the rhizosphere of *L. tenuis* plants, comparing with native grasses. On other hand, the positive correlation between the Anaerolineae class and water content, is in agreement with results presented by the relative abundance in Hollow soils, compared with hummock soils (Deng et al., 2014).

5. Conclusions

Further research is still needed in order to establish a link between *L. tenuis* effects on soil physico-chemical properties and observed changes in relative abundance of the above mentioned bacterial classes. Besides, future studies should be performed to determine whether other environmental soil variables, besides water content (like pH, organic matter, electric conductivity, etc.) play key roles in structuring bacterial populations in this economically important area. Also, shotgun sequencing analysis would be welcome in order to assess the ecological role of *L. tenuis* promotion on the functionality of bacterial communities.

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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.apsoil.2015.09.011.

References

Acosta-Martinez, V., Dowd, S.E., Sun, Y., Wester, D., Allen, V., 2010. Pyrosequencing analysis for characterization of soil bacterial population as affected by an integrated livestock-cotton production system. App. Soil Ecol. 45, 13–25.
Aguayo, P., González, C., Barra, R., Becerra, J., Martinez, M., 2014. Herbicides induce change in metabolic and genetic diviersity of bacterial community from a cold oligotrophic lake. World J. Microbiol. Biotechnol. 30, 1101–1110.
Barriuso, J., Marín, S., Mellado, R., 2010. Effect of the herbicide glyphosate on glyphosate-tolerant maize rhizobacterial communities: a comparison with pre-emergency applied herbicide consisting of a combination of acetochlor and terbuthylazine. Environ. Microbiol. 12, 1021–1030.
Barriuso, J., Valverde, J., Mellado, R., 2011. Estimation of bacterial diversity using next generation sequencing of 16S rDNA: a comparison of different workflows. BMC Bioinf. 12, 473.
Barriuso, J., Mellado, R.P., 2012. Glyphosate affects the rhizobacterial communities in glyphosate-tolerant cotton. Appl. Soil Ecol. 55, 20–26.
Blackburn, W.H., Knight, R.W., Wood, M.K., 1982. Impact of grazing on watersheds: a state of knowledge. Texas Agr. Exp. Sta. Pub. MP-1496.
Blake, G.R., Hartge, K.H., 1986. Bulk density, In: Klute, A. (Ed.), Methods of Soil Analysis. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI, pp. 363–375.
Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of Southern Wisconsin. Ecol. Monogr. 27, 249–325.
Caporaso, G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Gonzalez Pena, A., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.
Carrero-González, B., De La Cruz, M.T., Casermeiro, M.A., 2012. Application of magnetic resonance techniques to evaluate soil compactation after grazing. J. Soil Sci. Plant Nutr. 12, 165–182.
Castro-Silva, C., Ruíz-Valdiviezo, V.M., Valenzuela-Encinas, C., Alcántara-Hernández, R.J., Navarro-Noya, Y.E., Vázquez-Nuñez, E., Luna-Guido, M., Marsch, R., Dendooven, L., 2013. The bacterial community structure in an alkaline saline soil spiked with anthracene. Electron. J. Biotechnol. 16, 10.
Clarke, K.R., Green, R.H., 1988. Statistical design and analysis for a biological effects study. Mar. Ecol. Prog. Ser. 46, 213–226.
Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Aust. Ecol. 18, 117–143.
Criado, C.A., 2014. Lotus PAMPA INTA. Una herramienta de trabajo para los suelos bajos—inundables. Publicación Técnica Instituto Nacional de Teconología Agropecuaria, Argentina.
Cristea-Fernström, M., Olofsson, M., Chryssanthou, E., Jonasson, J., Petrini, B., 2007. Pyrosequencing of a short hypervariable 16S rDNA fragment for the identification of nontuberculous mycobacteria—a comparison with conventional 16S rDNA sequencing and phenotyping. APMIS 115, 1252–1259.
Cycón, M., Wójcik, M., Borymski, S., Piotrowska-Seget, Z., 2013. Short-term effects of the herbicide napropamide on the activity and structure of the soil microbial community assessed by the multi-approach analysis. Appl. Soil Ecol. 66, 8–18.
Deng, Y., Cui, X., Hernández, M., Dumont, M.G., 2014. Microbial diversity in hummock and hollow soils of three wetlands on the Qinghai-Tibetan plateau revealed by 16S rRNA pyrosequencing. PLoS One 9 e 103115.
Dowd, S.E., Callaway, T.R., Wolcott, R.D., Sun, Y., McKeehan, T., Hagevoort, R.G., Edrington, T.S., 2008. Evaluation of the bacteria diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). BMC Microbiol. 8, 125.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.
Escaray, F.J., Menéndez, A.B., Garriz, A., Pieckenstain, F.L., Estrella, M.J., Castagno, L. N., Carrasco, P., Sanjuan, J., Ruiz, O.A., 2012. Ecological and agronomic importance of the plant genus Lotus. Its application in grassland sustainability and the amelioration of constrained and contaminated soils. Plant Sci. 182, 121–133.
Ford, H., Rousk, J., Garbutt, A., Jones, L., Jones, D.L., 2013. Grazing effects on microbial community composition, growth and nutrient cycling in salt marsh and sand dune grasslands. Biol. Fertil. Soils 49, 89–98.
Fuerst, J.A., 1995. The Planctomycetes: emerging models for microbial ecology, evolution and cell biology. Microbiology 141, 1493–1506.
Gans, J., Wolinsky, M., Dunbar, J., 2005. Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science 309, 1387–1390.
Ghersa, C.M., Perelman, S.B., Burkart, S.E., León, R.J.C., 2007. Floristic and structural changes related to opportunistic soil tilling and pasture planting in grassland communities of the Flooding Pampa. Biodivers. Conserv. 16, 1575–1592.
Greacen, E.L., Sands, R., 1980. Compaction of forest soils: a review. Aust. Soil Res. 18, 163–189.
Griffiths, R.I., Thomson, B.C., James, P., Bell, T., Bailey, M., Whiteley, A.S., 2011. The bacterial biogeography of British soils. Environ. Microbiol. 13, 1642–1654.
Grüter, D., Schmid, B., Brandl, H., 2006. Influence of plant diversity and elevated atmospheric carbon dioxide levels on belowground bacterial diversity. BMC Microbiol. 6, 68.
Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST-Paleontological statistics. www.uv.es/~pardomv/pe/2001_1/past/pastprog/past.pdf (acessado em, 25.09.).
Hotelling, H., 1951. A generalized *T* test and measure of multivariate dispersión. Second Berkeley Symposium on Mathematical Statistics and Probability 1, 23–41.
Hughes, J.B., Hellmann, J.J., Ricketts, T.H., Bohannan, B.J., 2001. Counting the uncountable: statistical approaches to estimating microbial diversity. Appl. Environ. Microbiol. 67, 4399–4406.
Huse, S.M., Huber, J.A., Morrison, H.G., Sogin, M., Welch, D.M., 2007. Accuracy and quality of massively parallel DNA pyrosequencing. Genome Biol. 8, R143.
Janssen, 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA Genes. Appl. Environ. Microbiol. 73, 1719–1728.
Jones, R.T., Robeson, M.S., Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. ISME J. 3, 442–453.

Köberl, M., Müller, H., Ramadan, E.M., Berg, G., 2011. Deser farming nenefits from microbial potential in arid soils and promotes diversity and plant health. PLoS One 6, e24452.
Kowalchuk, G.A., Buma, D.S., de Boer, W., Klinkhamer, P.G.L., van Veen, J.A., 2002. Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. Anton. Leeuw. Int. J. Gen. Mol. Microbiol. 81, 509–520.
Lancaster, S.H., Hollister, E.B., Senseman, S.A., Gentry, T.J., 2010. Effects of repeated glyphosate applications on soil microbial community composition and the mineralization of glyphosate. Pest Manag. Sci. 66, 59–64.
Lavado, R.S., Taboada, M.A., 1988. Water, salt and sodium dynamics in a natraquoll in Argentina. Catena 15, 577–594.
Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination species data. Oecologia 129, 217–280.
Legendre, P., Legendre, L., 1998. Numerical Ecology, 2nd English edn. Elsevier, Amsterdam.
Lei, G.A.N., Xin-Xua, P.E.N.G., Peth, S., Horn, R., 2012. Effects of grazing intensity on soil water regime and flux in inner mongolia grassland, China. Pedosphere 22, 155–177.
Liu, Z., Lozupone, C., Hamady, M., Bushman, F., Knight, R., 2007. Short pyrosequencing reads suffice for accurate microbial community analysis. Nucleic Acid Res. 35, E120.
Lupwayi, N.Z., Harker, K.N., Clayton, G.W., O'Donovan, J.T., Blackshaw, R.E., 2009. Soil microbial response to herbicides applied to glyphosate-resistant canola. Agric. Ecosyst. Environ. 129, 171–176.
Luters, J.C., Salazar, J.P., 1999. Guía para la evaluación de la calidad y salud del suelo. United States Department of Agriculture, CRN-CNIA-INTA, Buenos Aires 88 p..
Magurran, A.E., 1998. Population differentiation without speciation. Phil. Trans. R. Soc. Lond. B 353, 275–286.
Mannisto, M.K., Tirola, M., Haggblom, M.M., 2007. Bacterial communities in Artic fields of finnish lapland are stable but highly pH/dependent. FEMS Microbiol. Ecol. 59, 452–465.
McCalla, G.R., Blackburn, I.I., Merril, W.H., LB, 1984. Effects of livestock grazing on infiltration rates, Edwards Plateau of Texas. J. Range Manag. 37, 265–268.
McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., Hugenholtz, P., 2012. An improved greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J. 6, 610–618.
Muirhead, R.W., Collins, R.P., Bremer, P.J., 2006. The association of E. coli and soil particles in overland flow. Water Sci. Technol. 54, 153–159.
Mulholland, B., Fullen, M.A., 1991. Cattle trampling and soil compactation on loamy sands. Soil Use Manag. 7, 189–193.
Naether, A., Foesel, B.U., Naegele, V., et al., 2012. Environmental factors affect acidobacterial communities below the subgroup level in grassland and forest soils. Appl. Environ. Microbiol. 78, 7398–7406.
Oksanen J., Blanchett F.G., Kindt R., Legendre P., Minchin P.R, O'Hara R.B., Simpson G. L., Solymos P., Stevens M.H.M., Wagner H., 2012. Vegan: community Ecology Package. R Package 2.0.3 Downloaded from http://CRAN.R-project.org/package=vegan on 9 May 2012.
Olsen, G.J., et al., 1992. The Ribosomal Database Project. Nucleic Acids Res. 20 (Suppl), 2199–2200.
R Development Core Team, 2013. R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, Vienna, Austria) Available at http://www.R-project.org.
Roesch, L.F.W., Fulthorpe, R.R., Riva, A., Casella, G., Hadwin, A.K.M., Kent, A.D., Daroub, S.H., Camargo, F.A.O., Farmerie, W.G., Triplett, E.W., 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J. 1, 283–290.
Rojas, M.C., Vázquez, P.M., Verdier, M., Noseda, R., 2011. Componentes del paisaje que favorecen la aparición de carbunco en la Pampa Deprimida (provincia de Buenos Aires, Argentina). Rev. Sci. Tech. Off. Int. Epiz. 30, 897–909.
Rubol, S., Freixa, A., Carles-Brangari, A., Fernàndez-Garcia, D., Romaní, A.M., Sanchez-Vila, X., 2014. Connecting bacterial colonization to physical and biochemical changes in sand box infiltration experiment. J. Hidrol. 517, 317–327.
Shannon, C.E., Weaver, W., 1949. The Mathematical Theory of Communication. The University of Illinois Press, Urbana, II.
Sierra, E.M., Montecinos, E.R., 1990. Cronología de inundaciones y sequías en la Depresión del Salado. Rev. Fac. Agric. 11, 35–45.
Soriano, A., León, R.J.C., Sala, O.E., Lavado, R.S., Deregibus, V.A., Cahuepe, M.A., Scaglia, A., Velasquez, C.A., Lemcoff, J.H., 1992. Río de la Plata grasslands: ecosystems of the world 8A. In: Coupland, R.T. (Ed.), Natural Grasslands. Introduction and Western Hemisphere. Elsevier, New York, pp. 367–407.
Striker, G.G., Insausti, P., Grimoldi, A., Ploschuk, E., Vasellati, V., 2005. Physiological and anatomical basis of differential tolerance to soil flooding of *Lotus corniculatus* L. and *Lotus glaber* Mill. Plant Soil 276, 301–311.
Sundquist, A., Ronaghi, M., Tang, H., Peyzner, P., Batzoglou, S., 2007. Whole-genome sequencing and assembly with high-throughput, short-read technologies. PLoS One 2, e484.
Tang, C.Y., Mao, S.F., Ma, X.Q., Qiu, M.M., Ma, K., Zhu, M.X., Wang, Z.J., 2014. The influence of three different types of herbicides on biodiversity. Adv. Mater. Res. 838–841, 2417–2426.
Thom, E.R., Wildermoth, D.D., Taylor, M.J., 1993. Growth and persistence of perennial ryegrass and white clover directdrilled into a paspalum-dominant dairy pasture treated with glyphosate. N. Z. J. Agric. Res. 36, 197–207.
Vandekerckhove, T.T., Willems, A., Gillis, M., Coomans, A., 2000. Ocurrence of novel verrucomicrobial species, endosymbiotic and associated with parthenogenesis in Xiphinema americanum-group species (Nematoda, Longirodirae). Int. J. Syst. Evol. Microbiol. 50, 2197–2205.
Wagner, M., Horn, M., 2006. The Planctomycetes, Verrucomicrobia, Chlamydiae and sister phyla comprise a superphylum with biotechnological and medical relevance. Curr. Opin. Biotechnol. 17, 241–249.
Ward, N.L., et al., 2009. Three genomes from the Phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. App. Environ. Microb. 75, 2046–2056.
White, R.P., Murray, S., Rohweder, M., 2000. Pilot Analysis of Global Ecosystems: Grassland Ecosystems. World Resources Institute, Washington, DC.
Will, C., Thürmer, A., Wollherr, A., Nacke, H., Herold, N., Schrumpf, M., Gutknecht, J., Wubet, T., Buscot, F., Daniel, R., 2010. Horizon-specific bacterial community composition of german grassland soils, as revealed by pyrosequencing-based analysis of 16S rRNA genes. App. Environ. Microb. 76, 6751–6759.
Zul, D., Dezel, S., Kotz, A., Overnann, J., 2007. Effects of plant biomass, plant diversity, and water content on bacterial communities in soil lysimeters: Implications for the determinants of bacterial diversity. App. Environ. Microb. 73, 6916–6929.