

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 assess 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.

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1. Introduction

The Flooding Pampa is a vast region (90,000 km²) 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 rely 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; Cychón 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.

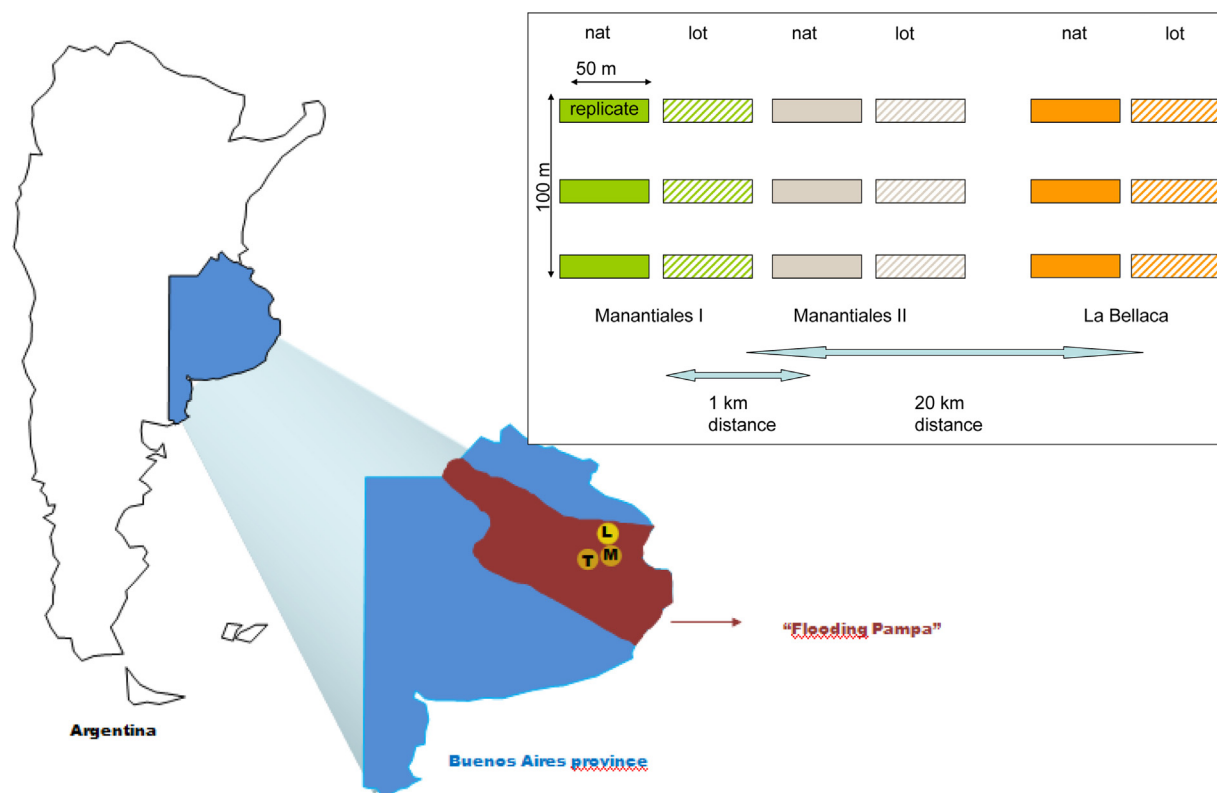


Fig. 1. Sampling sites for Flooding Pampa datasets. Soil samples were obtained in three different sites: Manantiales 1 (M), Manantiales 2 (T) and La Bellaca (L). In each site, two different land uses were studied: natural grass cover (nat) and promotion with *L. tenuis* and herbicides (lot). For sequencing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

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