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Soil microbial structure and activity in a semiarid rangeland of Patagonia, Argentina: Plant species and defoliation effects

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ABSTRACT

Natural grasslands are an important renewable resource for livestock production. Grazing in these areas alters the plant community composition, litter quality, and soil microbial structure and activity. Three cool-season species were studied in a semiarid rangeland area of Argentina: *Poa ligularis* and *Nassella tenuis* (desirable/preferred for livestock) and *Amelichloa ambigua* (undesirable/non preferred). The objective was to analyze the effect of moderate defoliation and plant species on the structure and activity of soil microbial communities associated with their roots. In winter 2012, soil samples (0–10 cm) were taken underneath marked plant canopies of the three species (n = 8). Immediately thereafter, half of the plants (n = 4) were defoliated (5 cm stubble height) and the other half remained undefoliated (controls). The defoliation treatment was conducted again in the spring. Soil samples were taken 30 days after each defoliation event. The study was repeated in 2013, using a different plant set. Bacterial community structure and soil microbial activity were analyzed using PCR-DGGE analysis and basal soil respiration, respectively. Moderate and early defoliations allowed compensatory growth in the defoliated plants. Variations in the soil genetic profiles of *A. ambigua* suggest a higher dependence on its rhizospheric bacterial communities. Defoliation treatments did not substantially affect basal soil respiration but showed strong links between desirable species and soil microbial activity. Sustainable management practices that promote the persistence of these species are important for the development of microbial communities that respond quickly to stress conditions, favoring decomposition processes that maintain soil fertility in semiarid grasslands.

1. Introduction

Natural grasslands are an important renewable resource for livestock production, and most of them are located in arid and semiarid environments (Brown, 1995). Rangelands of Patagonia (Argentina) constitute one of the few cold semiarid regions in the world, and it has been degraded by overgrazing in the last 100 years (Busso and Fernández, 2018). Grazing in these areas alters the structure and composition of plant communities, litter quality and soil physicochemical properties (Distel and Bóo, 1996; Carrera et al., 2008). Desirable grasses (i.e., those preferred by animals/livestock) have tolerance mechanisms against herbivores, produce short-lived leaves, with high foliage appearance rates, and high litter quality (high N concentrations and low C:N ratios and lignin contents) (Campanella and Bertiller, 2008; Ambrosino et al., 2019). Selective grazing removes the desirable species and induces their replacement by undesirable ones (Distel and Bóo, 1996). These undesirable plants have avoidance mechanisms against herbivores, produce leaves with a longer life span, and low-quality litter (low N concentrations and high C:N ratios and lignin contents) leading to slower organic matter decomposition and mineralization (Saint Pierre et al., 2004;

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Ambrosino et al., 2019). Soils under intensive grazing lose organic matter and reduce carbon availability (Stark and Kytoviita, 2006). However, moderate grazing intensities can increase net primary productivity and soil fertility through increasing compensatory growth of grazed species and resource allocation (Bardgett and Wardle, 2003).

Defoliation can be defined as tissue removal from a standing plant by clipping, trampling, or browsing, causing an immediate change in the plant height and canopy density in the community (Heady and Child, 1994). Controlled defoliation improves grassland quality by stimulating rhizodeposition, increasing soil microbial biomass and activity, and consequently increasing the mineralization of soil nutrients (Wardle et al., 2004; Hamilton et al., 2008). In addition to defoliation effects, plant species identity affects the nutrient cycle and composition of soil microbial communities. Plant species with high leaf N concentration and fodder production are associated with microbial communities dominated by procaryotes that perform rapid rates of mineralization and nitrification (Grigulis et al., 2013). Conversely, species with opposite traits are associated with communities dominated by fungi, co-occurring with procaryotes with slow activities (Grigulis et al., 2013).

The studies mentioned above suggest that the characterization of soil microbial communities might help to explain the ability of plants to resist disturbances such as defoliation. The study of microbial communities requires the use of different techniques that provide complementary information. PCR-mediated denaturing gradient gel electrophoresis (PCR-DGGE) is a classical molecular ecological technique that has been widely used to characterize the structure of soil microbial communities (Das et al., 2007; Nakatsu, 2007). However, it provides little information about the functional aspects of microbial communities. Soil respiration is a widely used methodology to estimate the overall activity of soil microorganisms. It is also an indicator of the potential flow of CO_2 into the atmosphere that can be used to estimate the soil capacity for nutrient cycling (Gavrichkova et al., 2010). Both methodologies are still widely applied in studying the effect of disturbances on soil microbial communities (Guitian and Bardgett, 2000; Montecchia et al., 2011; Izumi, 2018).

Some grassland studies have shown that defoliation reduces soil respiration (Craine et al., 1999; Wan and Luo, 2003). High grazing pressures affect microbial activities related to the carbon cycle (Prieto et al., 2011), and the amount of N immobilized by the microbial biomass increases where plants with high C:N dominate (Grigulis et al., 2013). Moreover, the effect of defoliation on soil biota depends on the timing of defoliation; plants defoliated during active growth conserve resources, whereas defoliation after termination of growth results in the release of resources from the live roots (Dam and Christensen, 2015). The presence of desirable perennial grass promotes organic matter loss from aboveground litter and increases the potential N mineralization pool (Moretto and Distel, 2002; Ambrosino et al., 2019). The ability of plants to resist disturbances, such as defoliation, will depend on the microbial communities associated with their roots (Gavrichkova et al., 2010). Conversely, the type of vegetation will influence the activity of soil microorganisms and their resilience capacity after a disturbance (Fultz et al., 2016; Izumi, 2018). Therefore, studies that involve the combined action of defoliation and plant species characteristics (i.e., forage and litter quality) on the structure and functionality of microbial communities are important for the development of sustainable practices that maintain soil fertility in semiarid environments with low resilience levels.

This study aimed to analyze the effect of moderate defoliation and plant species on the structure and activity of soil microbial communities. We analyzed microbial communities associated with three native perennial grasses with contrasting grazing preferences using PCR-DGGE analysis of 16S rDNA genes and microbial soil respiration determinations. This study was performed using *Poa ligularis* Nees ex Steud and *Nassella tenuis* (Phil.) Barkworth, both desirable perennial grasses, and *Amelichloa ambigua* (Speg.) Arriaga & Barkworth, an undesirable perennial grass only consumed when more palatable forage is unavailable (Giorgetti et al., 1997). We hypothesized that (1) defoliation of the studied perennial grasses affects the structure and activity of microbial communities associated with their roots, (2) the structure of bacterial communities differs between desirable and undesirable grass species, and (3) the activity of microbial communities is higher under desirable than undesirable species.

2. Materials and methods

2.1. Study site

This two-year research study was carried out in 2012 and 2013 in a 16-year exclosure (1.12 ha) to domestic livestock, located at Chacra Experimental Patagones, in the south of Buenos Aires province (40° 39′S, 62° 54′W; 40 m a.s.l.). This area is within the Monte Phytogeographical Province (Cabrera, 1976). The site was under long-term continuous grazing by cattle and sheep until 1951. Thereafter, it was cleared of woody vegetation and undergrowth and cropped from 1951 until 1975. It was then excluded from domestic herbivores from 1975 to 1994 and afterward exposed to controlled grazing by cattle and sheep. It has been excluded from cattle grazing since 1996 to date. Combined environmental (i.e., droughts, strong winds, wildfires, irregular precipitation) and anthropogenic factors (i.e., clearing of woody vegetation and subsequent replacement by crops, overgrazing) have led the region to a degradation state (SAyDS, 2011).

Climate is temperate-semiarid, with precipitations concentrated in summer and autumn (Giorgetti et al., 1997). Climatic parameters were collected from an automatic weather station located 1 km from the exclosure (Fig. 1). The total annual precipitation values for 2012 and 2013 were 513 and 422 mm, respectively. Soil in the exclosure was classified as a typical Haplocalcid (Soil Survey Staff, 2014). Soil texture is loamy-clay-sandy in the first 20 cm from the soil surface, and in 2012, the chemical properties were pH 8.26, organic matter 2.19%, total N 0.12%, and extractable P 9.88 ppm (Ambrosino et al., 2019).

2.2. Plant species

Poa ligularis, a C_3 desirable perennial grass species (Distel and Bóo, 1996), is dominant in areas exposed to rotational grazing and low stocking rates (Giorgetti et al., 2006). When grazing intensity increases, this species is replaced by other C_3 desirable perennial grasses such as *N. tenuis* (Distel and Bóo, 1996). Under continuous grazing and high stocking rates, undesirable perennial grasses, such as *A. ambigua* replace the desirable ones (Distel and Bóo, 1996). This species is only consumed by livestock when more desirable forage is unavailable (Giorgetti et al., 1997). Both *P. ligularis* and *N. tenuis* produce high-quality aboveground litter (Moretto and Distel, 2003; Carrera et al., 2005). *Amelichloa ambigua* has a high fibrous content. These species start their growth in March–April, vegetate during winter, flower in mid-October (*P. ligularis*) and November (*A. ambigua* and *N. tenuis*), and fruit and disseminate seeds in late spring or early summer (Ithurrart, 2015).

2.3. Defoliation treatments and total aboveground biomass production

In January, 24 adult plants (8 plants per species) were randomly selected at different sites dominated by *P. ligularis*, *N. tenuis* or *A. ambigua*. Subsequently, the plants were defoliated (by cutting) at 5 cm stubble height when they were dormant (Fig. 2). This made it possible to eliminate all senescent and dead shoots, making conditions uniform at the beginning of the study, and only included aboveground biomass produced during the growing season. Each year, half of the plants (four replicates: n = 4) were defoliated to 5 cm height from the soil surface during the vegetative developmental morphology stage (winter) and immediately after differentiation of the growing apex from vegetative to reproductive (early spring) (Fig. 2). The plant material was collected in each defoliation event. The remaining plants were not defoliated

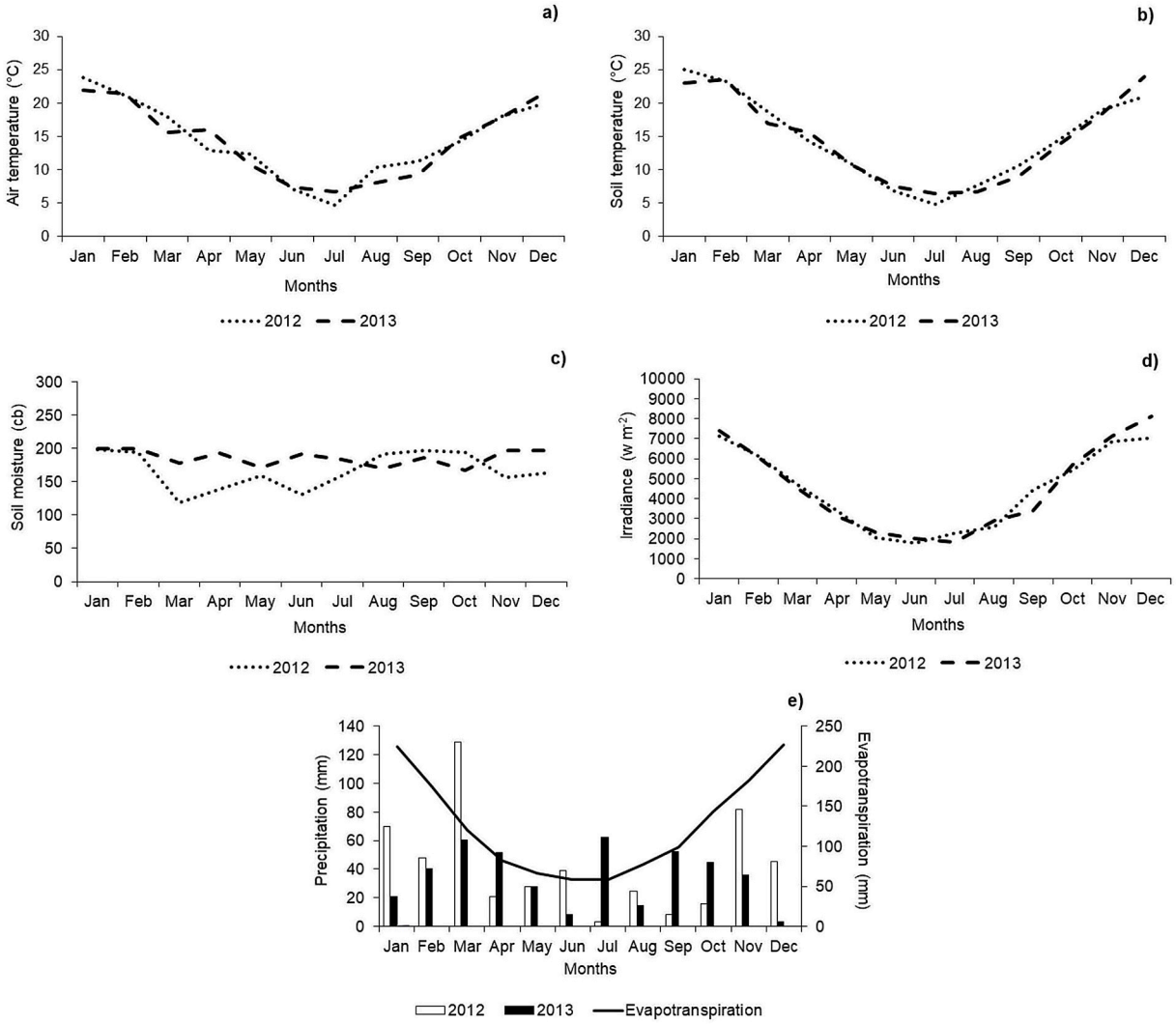

Fig. 1. Monthly climatic data at the Chacra Experimental Patagones during the study (2012–2013): (a) mean air temperature (°C), (b) mean soil temperature (°C; 0–20 cm), (c) mean soil moisture (cb; 0–10 cm), (d) mean irradiance (w m^{-2}), and (e) precipitation and evapotranspiration averaged over the study period (mm).

(control). Defoliation treatments simulated moderate grazing intensity (Quiroga et al., 2005) and rotational grazing management (Giorgetti et al., 2006). In this way, the active growth meristems (intercalary, apical) remained in the plants after the defoliation treatment, without affecting their biological growth cycle (Briske and Richards, 1995; Giorgetti et al., 2000). At the end of the growing season (December), plant circumference was measured and all the plants were cut to 5 cm stubble height (Fig. 2). The plant material was oven-dried at 70 °C for 72 h. Basal area of plants was calculated, and total aboveground biomass production (the sum of all the fodder produced during the growing season) was expressed as g cm^{-2} $year^{-1}$.

2.4. Soil sampling

The initial sampling was conducted during the vegetative developmental morphology stage (early August 2012). Soil samples (1 core per plant, 2.5 cm in diameter, 0–10 cm in depth) were taken underneath the canopy of the marked plants with a soil corer. To evaluate the defoliation treatment effect, new soil samples were taken approximately 30 days after each defoliation event (Fig. 2). In 2013, different plants were marked and the study was repeated using similar procedures to those conducted in 2012 (Fig. 2). In the laboratory, soil samples were sieved (2 mm) and stored until processing at −80 °C for the bacterial community structure analyses, and 4 °C for the microbial activity determinations.

2.5. DGGE analysis of 16S rDNA genes

Total microbial community DNA was extracted from 0.25 g of soil samples using a commercial kit (PowerSoil DNA isolation kit de MO BIO) according to the manufacturer's instructions and including the alternative lysis step (70 °C, 10 min). The extracted DNA was quantified by fluorometry (Qubit dsDNA BR Assay Kit, Invitrogen) and adjusted to 10 ng μl^{-1}. The V6–V8 regions of the 16S rRNA genes were PCR amplified using F984GC and R1378 universal bacterial primers (Heuer et al., 1997), following the conditions described by Montecchia et al. (2011). The size of the PCR products was checked by agarose gel electrophoresis and SYBR Safe staining. The amplified fragments were separated on 6% polyacrylamide gels with a 45–65% denaturing gradient of urea and formamide. The gels were run at 1700 V h^{-1} in Tris–Acetate–EDTA buffer at 60 °C, using the DGGEK-2001-220 kit (CBS Scientific). Gels were stained with SYBR Green I (Molecular probes) and photographed under UV light with InGenius LHR2 documentation system (Syngene).

2.6. Soil microbial activity

Basal soil respiration was determined according to the method of

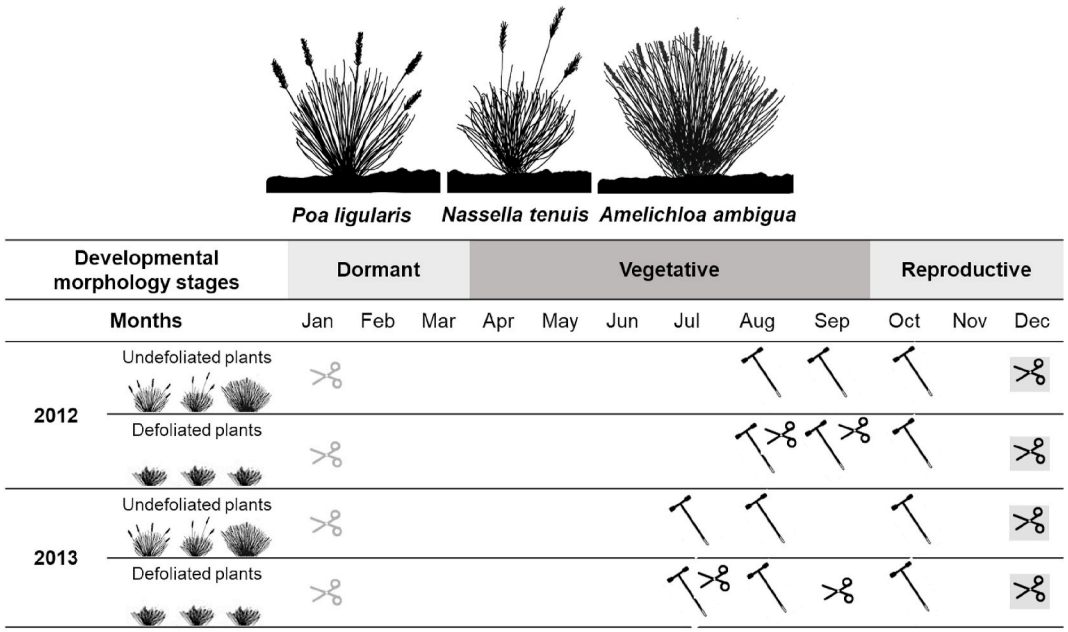

Fig. 2. Cutting to remove dead shoots (✂), defoliation treatments (✂), soil sampling dates (⟍; 0–10 cm in depth), and cutting to obtain the total aboveground biomass production (✂) in marked plants of the grass species (n = 4).

Isermeyer (Alef and Nannipieri, 1995). Each soil sample (25 g) was incubated in hermetic flasks for 7 days in darkness at 25 °C, together with a NaOH trap for CO_2 from soil respiratory activity. In addition, flasks without soil were used as blanks. After incubation, CO_2 recovered in each NaOH solution was measured by titration with HCl 0.2 M. The results were expressed in mg CO_2 g^{-1} dry soil $week^{-1}$ (Isermeyer, 1952).

2.7. Statistical analysis

For the study of the bacterial communities, comparative analysis of normalized DGGE profiles were performed using GelCompar II v. 6.5 (Applied Maths NV). Pearson's correlation coefficients were used to calculate pairwise similarity coefficients among densitometric profile patterns, and dendrograms were constructed using the unweighted pair group method with averages (UPGMA) algorithm (Rademaker et al., 1999).

Statistical analyses of total aboveground biomass production and soil microbial activity were conducted with INFOSTAT software (Di Rienzo et al., 2016). For total aboveground biomass production, data were analyzed using a three-way analysis of variance (ANOVA), taking (1) species, (2) defoliation treatment and (3) years as factors. Data corresponding to the initial analysis of basal soil respiration were analyzed using two-way ANOVA, taking (1) species and (2) year as factors. Subsequently, data were analyzed with multifactorial ANOVA, taking (1) species, (2) defoliation treatments, (3) sampling dates, and (4) years as factors. Because the data corresponded to repeated measures, linear mixed models with an order 1 autoregressive residual correlation and heteroscedastic residual variances over time were used for analysis. Where a significant interaction was detected among the study factors, each year was studied separately. Comparison of means was conducted using the protected test of Fisher (LSD), with a significance level of 0.05.

3. Results

3.1. Total aboveground biomass production

Significant interactions were detected between species, defoliation treatments, and years (Table 1).

In 2012, there were no significant differences ($P > 0.05$) between species, and defoliated plants had higher biomass production than control plants (Fig. 3, a). In 2013, the defoliation treatments did not affect the biomass production of *A. ambigua*. Defoliated plants of *P. ligularis* had higher production than undefoliated plants; the opposite was registered for *N. tenuis* (Fig. 3, b). Among the control plants, the highest values were found for *N. tenuis*, followed by *A. ambigua* and then *P. ligularis* (Fig. 3, b).

3.2. Bacterial community structure

Four analytical replicates were initially analyzed for each sampling site on each study year, but given the similarity between profiles among replicates, three replicates are shown to facilitate visualization of data (Fig. 4). Cluster analysis of DGGE profiles at the initial sampling in 2012 indicated that the structure of dominant bacterial communities was different among the studied plant species (Fig. 4, a). At a similarity level of 65%, 2 clusters were defined where desirable species (*P. ligularis* and *N. tenuis*) are separated from *A. ambigua*. Soil bacterial genetic profiles associated with the undesirable species roots were more homogeneous than those of the desirable species. At the second sampling date, 4 groups were defined at a similarity level of 75%. Defoliation treatments affected soil bacterial community structure under *A. ambigua*; defoliated plants of this species (except for one of them) clustered separately from

Table 1
Results of a three-way analysis of variance for total aboveground biomass production (factors: species, defoliation, and year).

Factor	numDF	F-value	p-value
Species	2	30.40	<0.0001
Defoliation	1	9.95	0.0032
Year	1	7.70	0.0087
Species x Defoliation	2	6.06	0.0054
Species x Year	2	8.48	0.001
Defoliation x Year	1	13.13	0.0009
Species x Defoliation x Year	2	14.71	<0.0001

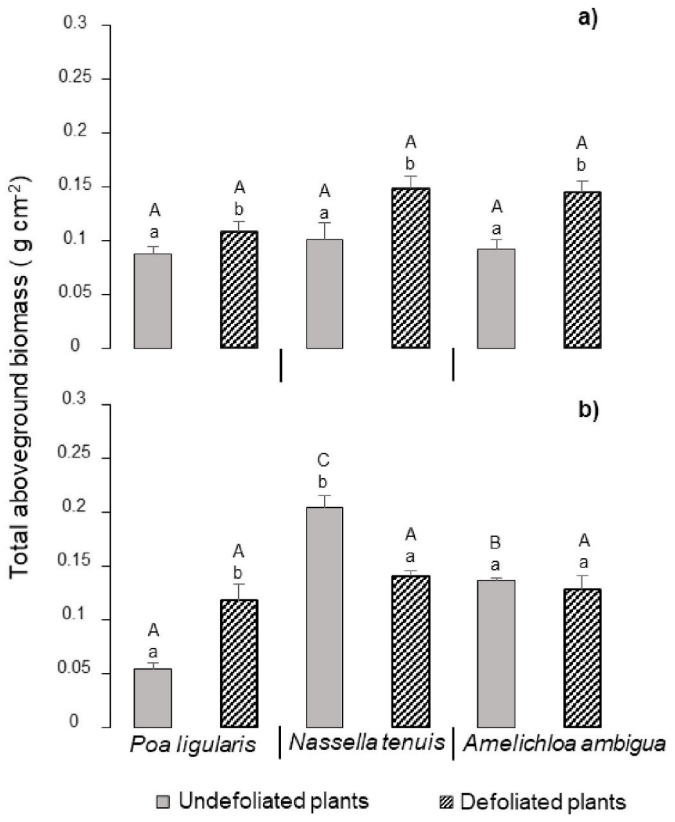

Fig. 3. Total aboveground biomass production per basal area unit (mean ± 1 S. E., n = 4; g cm^{-2} $year^{-1}$) of the species studied in (a) 2012 and (b) 2013. For each species, different upper and lower case letters above histograms indicate significant differences (P ≤ 0.05) between species and defoliation treatments, respectively.

control plants (Fig. 4, b). The opposite occurred with bacterial communities associated with the desirable species. However, *N. tenuis* formed a separate group from the rest. At the last sampling date, clusters were not defined either among species or among defoliated treatments; DGGE profiles of soil bacterial communities were homogeneous, with a similarity level greater than 87% (Fig. 4, c).

In 2013, at the initial sampling, 2 clusters were defined (similarity level of 70%). *Poa ligularis* and *A. ambigua* soils separated from those of *N. tenuis* (Fig. 4, d). Soil DGGE profiles of the undesirable species were more heterogeneous than those of the desirable ones (Fig. 4, d). At the second sampling date, two groups were defined at a similarity level of 85% (Fig. 4, e). Undefoliated plants of *A. ambigua* were separated from the rest, but there were no effects of defoliation on the other species (Fig. 4, e). At the last sampling date, cluster analysis defined 2 groups at a similarity level of 75%. Soil bacterial communities of either defoliated or undefoliated *N. tenuis* plants were separated from the other two species and defoliation treatments (Fig. 4, f).

3.3. Soil microbial activity

The initial sampling analysis did not detect a significant interaction between the studied factors (Table 2, a). For all species, basal soil respiration was higher in 2012 than in 2013 (Fig. 5).

In the analysis of the data obtained after conducting the defoliation treatments, significant two-way interactions were detected between species and years and between defoliation treatments and years (Table 2, b). In 2012, the soil microbial activity underneath the species showed higher values in October than in September, and there was no effect of defoliation at any sampling (Fig. 6, a). For all dates, *P. ligularis* and *A. ambigua* soils showed higher activity (mean ± 1 S.E., n = 16; 0.84 ± 0.06 and 0.71 ± 0.06 mg CO_2 g^{-1} dry soil $week^{-1}$ respectively; P ≤ 0.05) than *N. tenuis* soils (mean ± 1 S.E., n = 16; 0.52 ± 0.04 mg CO_2 g^{-1} dry soil $week^{-1}$).

In 2013, undefoliated plants of the desirable species showed higher basal respiration than defoliated plants, and *P. ligularis* soils exhibited the highest microbial activity in October (Fig. 6, b). In contrast, *A. ambigua* soils did not differ significantly between either sampling dates or defoliation treatments (Fig. 6, b). Within defoliated plants, *P. ligularis* and *A. ambigua* soils showed higher activity (mean ± 1 S.E., n = 8; 0.52 ± 0.05 and 0.55 ± 0.05 mg CO_2 g^{-1} dry soil $week^{-1}$, respectively; P ≤ 0.05) than *N. tenuis* soils (mean ± 1 S.E., n = 8; 0.39 ± 0.04 mg CO_2 g^{-1} dry soil $week^{-1}$). However, in undefoliated plants, *A. ambigua* soil registered lower values (mean ± 1 S.E., n = 8; 0.46 ± 0.04 mg CO_2 g^{-1} dry soil $week^{-1}$; P ≤ 0.05) than those underneath the desirable species (mean ± 1 S.E., n = 8; *P. ligularis* = 0.65 ± 0.05 mg CO_2 g^{-1} dry soil $week^{-1}$ and *N. tenuis* = 0.6 ± 0.05 mg CO_2 g^{-1} dry soil $week^{-1}$).

4. Discussion

The chemical composition of litter has a great influence on the biological interaction between soil bacterial and fungal communities. Changes resulting from these interactions can alter the organic matter decomposition process (Hossain et al., 2010). Moreover, plant roots play a fundamental role in modulating and conditioning the surrounding environment for the development of specific microbial communities on each plant species (Mawdsley and Bardgett, 1997). Regarding the first hypothesis proposed in our study, responses to the defoliation treatments differed according to the species considered; and contrary to the second hypothesis, only in August 2012 were genetic profiles of the desirable species grouped in clusters different from those of *A. ambigua*. Guitian and Bardgett (2000) reported that species with less developed grazing tolerance mechanisms are more dependent on extrinsic mechanisms to regrow, such as changes in radical exudation patterns for nutrient recycling that affect soil bacterial community structure (Fig. 7). This might explain why in both years, after the first defoliation, plants of *A. ambigua* were clustered separately from control plants. In response to stress conditions, plants release specific compounds in the rhizosphere, which enhance soil organic matter mineralization, increasing interconnections of bacterial species and promoting the development of a "cooperative effect" for coping with the stressful conditions imposed by grazing (Studer et al., 2016; Marcos et al., 2019). In contrast, for desirable species, no defoliation treatment effects were detected on DGGE genetic profiles. In these grasses, high leaf N concentration, and litter quality (Aerts and Chapin, 2000; Ambrosino et al., 2019) would produce a soil biota nutritional improvement and contribute to a high number of ecological niches (Wardle, 2002; Grigulis et al., 2013). This improvement confers great plasticity for responding to disturbances imposed by the environment to the soil bacterial communities without affecting their structure (Fig. 7).

We found no clear effects of defoliation treatments on soil microbial activity. These results are in contrast to the first proposed hypothesis and other studies where defoliation reduces soil respiration (Wan and Luo, 2003; Gavrichkova et al., 2010) or those that show effects of grazing on soil microbial activity (Bardgett and Wardle, 2003; Prieto et al., 2011; Peri et al., 2015). These differences may be due to the grazing characteristics (intensity, frequency, and timing) and the fact that the plants were clipped rather than grazed in our research (Heady and Child 1994). Grazing by cattle involves additional aspects, such as selection of plant species, trampling, and incorporation of feces and urine, which affect soil physicochemical and biological parameters (Sirotnak and Huntly 2000; Prieto et al., 2011). Trampling modifies physical soil properties, and urine input increases soil pH, inorganic N, and soil microbial activity (Prieto et al., 2011). Undesirable grasses could potentially have larger canopies than desirable grasses, simply as a consequence of more limited consumption by livestock. These larger, ungrazed canopies

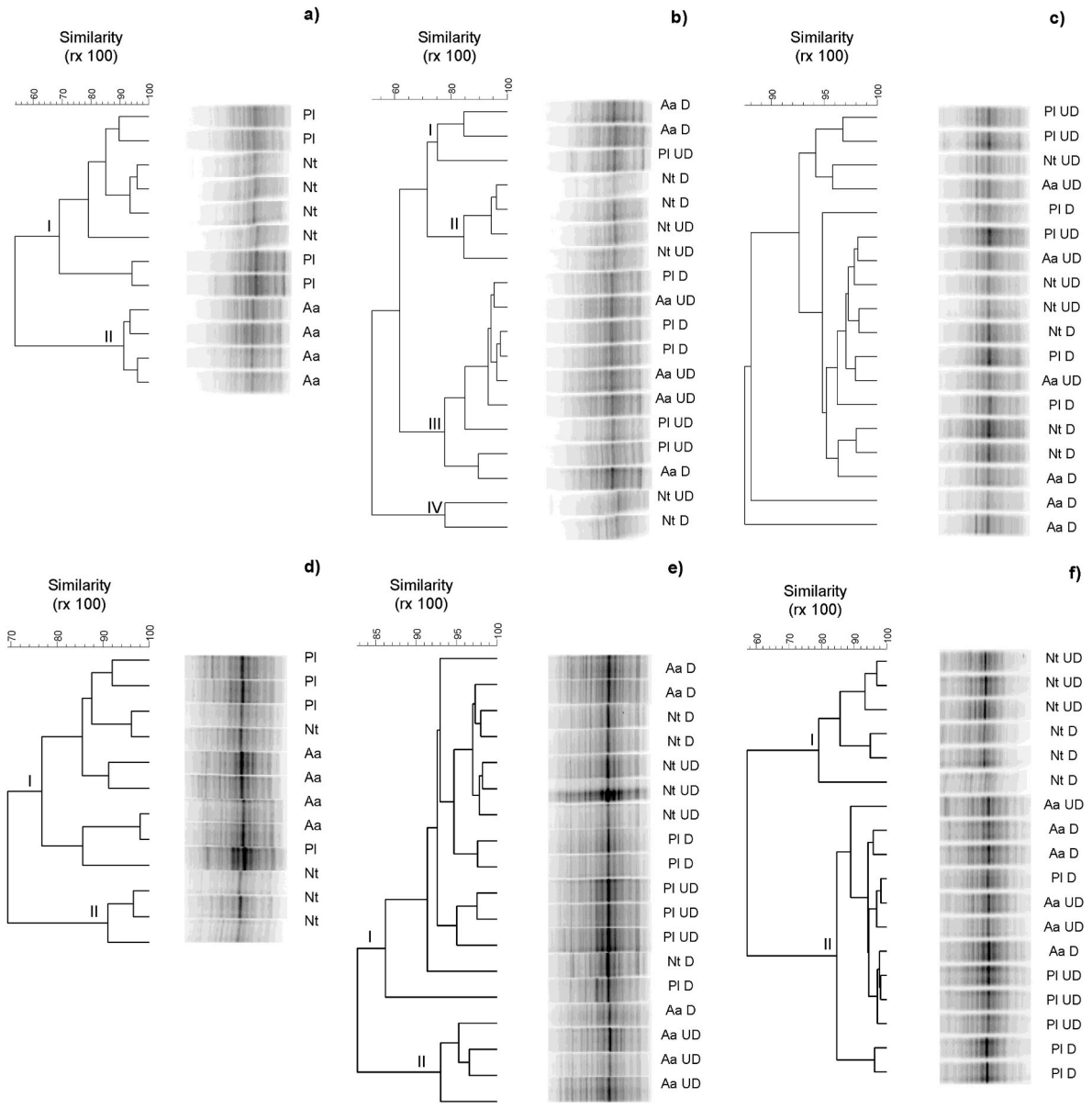

Fig. 4. Cluster analysis (Pearson/UPGMA) of 16S rDNA-DGGE fingerprints of soil bacterial communities in (a, b, c) 2012 and (d, e, f) 2013 under *Poa ligularis* (Pl), *Nassella tenuis* (Nt), and *Amelichloa ambigua* (Aa) at (a, d) the initial sampling and after (b, e) one or (c, f) two defoliations. D: defoliated plants, UD: undefoliated plants (control). I, II, III, and/or IV indicate the defined groups.

could produce greater amounts of litter and have a different microclimate below them than grazed canopies. However, Toledo et al. (2021) found no effect of grazing intensity on basal respiration in grasslands of southern Patagonia and demonstrated that microbial activity is modulated mainly by soil organic carbon and soil microbial biomass. Moreover, Dam and Christensen (2015) found that the effects of defoliation on soil biota depend on the timing of defoliation. In general, the defoliated plants in our study had similar or higher aboveground biomass production than control plants (Fig. 3), and only in 2013 did defoliation treatments affect the soil basal respiration associated with desirable species. Microorganisms are likely to degrade both new organic matter that enters the soil and native soil organic matter (Fontaine et al., 2003), increasing its mineralization. However, regular and slow exudation of organic compounds by plant roots also stimulates mineralization, while energy costs are minimized, and the microbial activity after a disturbance remains either unchanged or decreases (Fontaine et al., 2003). Therefore, moderate and early defoliations (i.e. those not removing intercalary and apical meristems) that allow compensatory growth in the defoliated plants would not substantially affect basal soil respiration. However, the results show strong links among desirable species and soil microbial activity (Fig. 7).

In general, the soil associated with the roots of *P. ligularis* showed higher basal respiration than *N. tenuis* soils. Its greater basal area determines a higher aboveground litter production (Ambrosino et al., 2019), and litter contribution with more labile compounds, stimulates organic carbon mineralization and soil microbial activity (Yan et al., 2018). However, the results did not support the third hypothesis proposed. Although we observed a slight tendency towards higher basal soil respiration values in *P. ligularis* than *A. ambigua* soils (mainly in 2012), these differences were not significant. Previous studies reported that belowground litter of *A. ambigua* had higher N content and decomposed faster than those of desirable species (Ambrosino et al., 2019). Differences in the decomposition dynamics of above- and belowground litter would contribute to explaining the similar soil basal respiration values among desirable and undesirable species.

In arid and semiarid environments, precipitation and temperature

Table 2
Results of the basal soil respiration. (a) Two-way analysis of variance at the initial sampling date (factors: species and year), and (b) linear model analysis with mixed effects for a multifactorial design after conducting the defoliation treatments (factors: species, defoliation, date, and year). Significant p-values ($P \leq 0.05$) are in bold.

a)			
Factor	numDF	F-value	p-value
Species	2	2.19	0.125
Year	1	45.83	**<0.0001**
Species x Year	2	0.91	0.4084
b)			
Factor	**numDF**	**F-value**	**p-value**
Species	2	10.21	**<0.0001**
Defoliation	1	0.0036	0.9522
Date	1	33.8	**<0.0001**
Year	1	19.18	**<0.0001**
Species x Defoliation	2	2.93	0.0596
Species x Date	2	1.64	0.201
Species x Year	2	3.6	**0.0322**
Defoliation x Date	1	0.05	0.8168
Defoliation x Year	1	5.04	**0.0279**
Date x Year	1	0.66	0.4208
Species x Defoliation x Date	2	1.32	0.2725
Species x Defoliation x Year	2	2.73	0.0716
Species x Date x Year	2	0.06	0.9384
Defoliation x Date x Year	1	0.21	0.6447
Species x Defoliation x Date x Year	2	0.18	0.8392

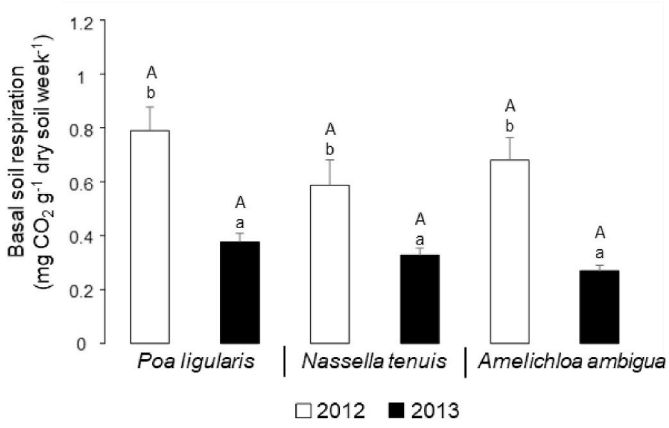

Fig. 5. Basal soil respiration (mean ± 1 S.E., n = 8; mg CO_2 g^{-1} dry soil $week^{-1}$) under the canopy of the studied species in the initial sampling (August 2012 and July 2013). Different upper and lower case letters above histograms indicate significant differences ($P \leq 0.05$) between species and years, respectively.

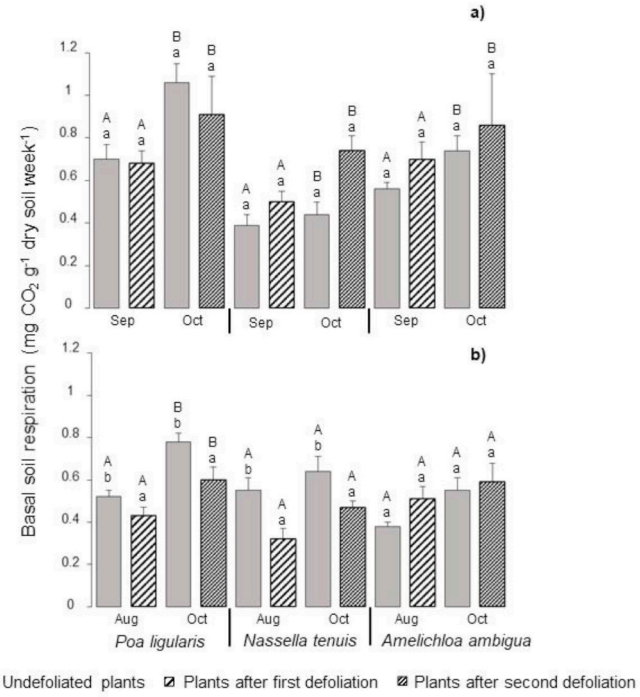

Fig. 6. Basal soil respiration (mean ± 1 S.E., n = 4; mg CO_2 g^{-1} dry soil $week^{-1}$) during (a) 2012 and (b) 2013 under the canopy of the studied species. Sampling dates: (a) September (Sep) and October (Oct); (b) August (Aug) and Oct. For each species, different upper and lower case letters above histograms indicate significant differences ($P \leq 0.05$) between sampling dates and defoliation treatments, respectively.

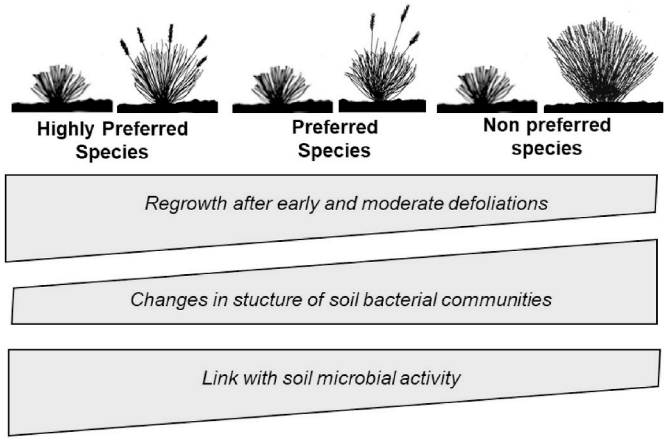

Fig. 7. Schematic of the effects of defoliation and perennial grasses with contrasting grazing preferences on soil microbial structure and activity associated whit their roots.

are important factors in controlling decomposition in soil microbial communities (Toledo et al., 2021). For all species, initial soil microbial activity was higher in 2012 than in 2013, and during the two growing seasons, the highest values of *P. ligularis* were observed at the third sampling date. These results could be related to differences in precipitation events and soil moisture (Fig. 1). In 2012, total annual precipitation values (523 mm) were above the long-term mean (1981–2012: 421 mm) and the highest monthly rains occurred in March (i.e., start of the species growing season; Fig. 1, e). Higher soil moisture can result in greater growth and allocation of belowground carbon, increasing the availability of labile substrates for microbial activity (Liu et al., 2007; Toledo et al., 2021), thus regulating basal soil respiration during the growing season. Moreover, early flowering in desirable species might explain the highest microbial activity in October. During flowering time, carbon allocation to reproductive organs in the plant is increased, foliage appearance rates are lower, and high litter quality production is maximized (Jones, 1992; Ambrosino et al., 2019). Hamilton and Frank (2001) demonstrated that *Poa pratensis* plants are capable of modulating their rhizospheric microbial activity and promoting organic matter decomposition. The highly desirable species could depend on this mechanism and stimulate soil microbial activity, increasing the nutrient availability required for reproductive structure formation. Therefore, the results show higher link with soil microbial activity underneath desirable than undesirable species (Fig. 7).

5. Conclusions

This study suggests a higher dependence of less desirable species on its rhizospheric bacterial communities for regrowth after defoliation.

The presence of highly desirable species favored the development of soil microbial communities that might respond quickly to stress conditions (such as defoliation), favoring decomposition processes and mineralization of organic matter without affecting their structure and composition. This highlights the importance the sustainable management practices that promote the persistence of desirable perennial grasses in the plant community to maintain soil fertility in semiarid grasslands. We suggest that further studies should (1) compare sites with different grazing histories and (2) examine the diversity and functional attributes of specific microorganisms in the rhizosphere of different plant species.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Carlos Alberto Busso reports financial support was provided by Universidad Nacional del Sur. Carlos Alberto Busso reports a relationship with National University of the South that includes: employment.

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