

Communities of arbuscular mycorrhizal fungi associated with perennial grasses of different forage quality exposed to defoliation



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ABSTRACT

Vegetation management practices, such as defoliation may alter the composition of plant communities and/or the fungi-forming arbuscular mycorrhiza (AMF). We determined the species identity, density, frequency and diversity of AMF spores from soil under the canopies of three native perennial grass species in rangelands of Argentina: 1) *Poa ligularis* and *Nassella tenuis* (preferred by livestock) and 2) *Amelichloa ambigua* (not preferred). For each species, plants either remained undefoliated or were defoliated twice to a 5 cm stubble height during the growing season. Most active meristems remained on the plants after defoliations. AMF communities were sampled prior to (i.e., 48 soil samples) and following (i.e., 72 soil samples) each defoliation event. Spores were grouped in 15 morphospecies. Density, richness and diversity of AMF spores were not influenced by defoliation, and species richness and diversity of AMF were similar among species. Total spore density was greatest for *P. ligularis* at the sampling prior to defoliation, but this difference did not persist following the defoliation events. The most abundant AMF families were: Acaulosporaceae, Diversisporaceae and Glomeraceae. These results demonstrate that responses of the studied variables were insensitive to the defoliation treatments, and were largely unaffected by the studied grass species.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) affect the structure and dynamics of plant communities, especially in nutrient-poor soils (Van der Heijden et al., 1998). In turn, plant communities are important determinants in the distribution and composition of AMF in the soil (Johnson et al., 1992). Beneficial associations between individual plants or species and AMF are largely dependent on the species of the associated AMF, as AMF species vary in their demand for carbon from shoots and phosphorous translocation from roots to shoots (Pearson and Jakobsen, 1993).

Grazing management practices can alter plant species composition (Distel and Bóo, 1996; Augustine et al., 2017; Porensky et al., 2017), but their effects are largely unknown for fungal diversity in arid and semiarid rangelands. Herbivory affects mycorrhizal colonization by inducing changes in root morphology and soil physicochemical properties in addition to alterations in plant community structure (Su and Guo, 2007). For example, herbivory-induced changes in soil structure can reduce sporulation of AMF due to decrease in soil pore size (Allen and Allen, 1980). Lower infiltration rates from herbivory-induced changes to soil bulk density and plant basal cover (Thurrow et al., 1986) can reduce soil moisture, and consequently spore germination (Daniels

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and Trappe, 1980). Thus, reductions in VAM fungi sporulation and spore germination are often associated with drier and more compacted soil.

Rangelands in higher ecological states (i.e., greater abundance of higher seral species), can have a greater quantity and diversity of the AMF community (Mendoza et al., 2002). Thus, grazing mismanagement, which induces replacement of desirable (i.e. preferred) plant species by undesirable ones, and degrades the ecological state of vegetation community, results in reducing litter decomposition rate and nutrient mineralization of plant communities (Milton et al., 1994).

Effects of grazing or defoliation on the colonization by AMF have been inconsistent (Frank et al., 2003; Yang et al., 2013; Saravesi et al., 2014). For example, increased root growth of the C3 perennial grass *Poa pratensis* following defoliation suggests that greater belowground energy allocation also increased investment in mycorrhizal symbiosis which in turn increased spore abundance Frank et al. (2003). In contrast, defoliation of the legume *Medicago sativa* decreased plant biomass and AM colonization; the latter was likely limited by host carbon availability (Saravesi et al., 2014). Furthermore, grazing in an alpine-meadow on the Qinghai-Tibetan Plateau in China did not affect AM root colonization and spore density (Yang et al., 2013). These authors indicated that it is possible that the AM fungus *Rhizofagus intraradices* (= *Glomus intraradices*) shows a wide tolerance to environmental stress or there is functional diversity within this species. However, these three studies differ in the (1) defoliation characteristics (artificial, actual grazing, intensity, duration and frequency); (2) environmental set ups (greenhouse, growth chamber, field conditions); (3) study functional groups (grasses, legumes); (4) abiotic growth conditions (e.g., temperature: from a little more than 1 °C–22 °C), and (5) sampling time scales (from 3 to 9 weeks). Rodríguez Echeverría et al. (2008) reported that the number of AMF spores associated to *Ammophila arenaria*, an important sand dune-fixing species, varied significantly with sampling time at various locations of the European coast. Even more, Yang et al. (2013) pointed out that temperature differences of 1.2 °C during daytime and 1.7 °C at night determined that warming with grazing significantly increased AM fungal operational taxonomic units richness in roots of *A. arenaria*, compared to the grazing only treatment. These authors suggested that AM fungi might demonstrate complex responses under multiple global change factors in ecosystems.

In arid and semiarid rangelands, where nutrient availability is limiting, intensive grazing can negatively affect AMF (Cai et al., 2014). However, moderate grazing can maintain dominance of preferred species (Augustine et al., 2017; Porensky et al., 2017) with a resulting symbiosis with AMF stimulating organic matter decomposition and soil nutrient cycling (Nuccio et al., 2013). The key to the resilience of these higher seral plant communities is that AMF species in the genera *Glomus* and *Rhizophagus*, common in arid and semiarid environments, tolerate lower levels of carbohydrates coming from the host plants, which increase their growth and abundance (Eom et al., 2001; Saito et al., 2004; Su and Guo, 2007; Barto and Rillig, 2010; Yang et al., 2013). Since plants within a community can associate with different AMF species, it is expected that grazing and defoliation will result in a high variability in the various plant developmental morphology states and function of AMF species (Klironomos et al., 2004).

Our objectives were to (1) identify the species of AMF spores rhizospheric soil of three native perennial grasses with contrasting grazing preferences, and (2) to evaluate the effects of defoliation on AMF spore density, richness, frequency and diversity. We hypothesized that (1) soil closest to roots as possible contains a different density of AMF spores on the different studied grass species, and (2) AMF density, richness, frequency and diversity from soils directly beneath perennial grass plants will be non-responsive after three to four weeks from a moderate defoliation, when active (i.e., intercalary, apical) meristems remain on the plants after defoliation.

2. Methods

2.1. Study site

The study was conducted in 2012 within a 1.12 ha enclosure where domestic herbivores were excluded from grazing since 1996 in the Chacra Experimental Patagones, located at the south of the Province of Buenos Aires, Argentina (40° 39'S, 62° 54'W; 40 m.a.s.l.). This area is within the phytogeographical province of the Monte (Cabrera, 1976). The site was cleared of woody vegetation and undergrowth in 1951 and cropped until 1975. Desertification in this region is evident by a loss of plant cover, decreases in soil fertility, and increases in soil compaction and salinization (SAyDS, 2011).

Climate is temperate-semiarid, with 421 mm of mean annual precipitation (1981–2012) with a maximum of 877 mm in 1984 and a minimum of 196 mm in 2009 (Torres et al., 2013). Mean annual temperature is 14.1 °C (1981–2012; Torres et al., 2013).

Soil in the enclosure is a typical Haplocalcid (Giorgetti et al., 1997), with the 0–20 cm depth having pH of 8.26, organic matter content of 2.2%, total nitrogen of 0.12% and extractable phosphorus levels of 9.9 ppm.

2.2. Plant species

The plant community is dominated by perennial grasses with differing quality for grazing livestock and isolated shrub species (Giorgetti et al., 1997). *Poa ligularis* Ness. is a dominant, cool-season, preferred (i.e., desirable) perennial grass species. As grazing intensity increases (*Nassella tenuis* (Phil.) Barkworth replaces *P. ligularis* (Distel and Bóo, 1996), and with further increases in grazing intensity a non-preferred species (i.e. undesirable: *Amelichloa ambigua* (Speg.) Arriaga and Barkworth) becomes dominant (Giorgetti et al., 1997). These C₃ perennial grass species not only differ in grazing tolerance but also exhibit differences in forage and litter quality, and root morphology characteristics (Table 1).

2.3. Experimental design and treatments

Using a completely randomized experimental design, 12 individual plants per species and 12 sites approximately 1 m² in surface area without vegetation (controls) were randomly selected at the end of the growing season in December 2011. Unvegetated sites among vegetation patches were irregular as the result of topographic characteristics and previous grazing mismanagement. These sites were useful as a control for evaluating species-specific effects of the various perennial grasses.

Soil samples (2.5 cm diameter, 0–10 cm depth) were collected from beneath each individual plant and the control plots at the beginning of the experiment, prior to initiation of defoliation treatments (n = 48 samples). Soils were re-sampled beneath individual plants approximately 35–40 days following each defoliation event.

When plants were at the dormant stage of developmental morphology (January 2012: Giorgetti et al., 2000), they were all defoliated

Table 1

Major characteristics of the native perennial grass species under study (Distel and Bóo, 1996; Giorgetti et al., 1997; Saint Pierre et al., 2004a; Moretto and Distel, 2003).

	<i>Poa ligularis</i>	<i>Nassella tenuis</i>	<i>Amelichloa ambigua</i>
Successional stage	Late	Intermediate	Early
Forage quality	Highly Preferred	Preferred	Non preferred
Litter quality	High (high N, low C/N and lignin)	High (high N, low C/N and lignin)	Low (low N, high C/N and lignin)
Root morphology	Fine	Fine	Coarse

Table 2

Density (spore number/100 g soil, mean \pm 1 SE of $n = 12$) and frequency of occurrence (F: percentage of appearance from which spores of a particular species were recovered) of AMF species under plants of *Poa ligularis*, *Nassella tenuis*, and *Amelichloa ambigua*, and unvegetated sites (i.e., without vegetation) at the initial sampling, August 2012.

AMF species	<i>Poa ligularis</i>	<i>Nassella tenuis</i>	<i>Amelichloa ambigua</i>	Unvegetated sites	F (%)
Fam. Glomeraceae					
<i>Funnelformis mosseae</i>	7.5 \pm 2.5	2.2 \pm 1.1	1 \pm 0.4	1.7 \pm 0.7	100
<i>F. geosporum</i>	23.6 \pm 4.6	7.3 \pm 1.4	18.5 \pm 4.1	9.2 \pm 3.4	100
<i>Glomus</i> sp.	10.3 \pm 3.7	3.8 \pm 3.8	7.7 \pm 3	0	75
<i>G. microaggregatum</i>	0	0	0.2 \pm 0.2	0	25
<i>Rhizophagus clarus</i>	0	0	0.3 \pm 0.2	0	25
<i>R. irregularis</i>	1.2 \pm 0.9	2.7 \pm 1.4	2.3 \pm 1.7	8.7 \pm 2	100
Fam. Claroideoglomeraceae					
<i>Claroideoglossum etunicatum</i>	1.0 \pm 0.7	0	2.0 \pm 1.4	0	50
Fam. Diversisporaceae					
<i>Diversispora spurca</i>	12.3 \pm 2.7	11.2 \pm 3.9	6.5 \pm 2.5	9.7 \pm 2.7	100
Fam. Pacisporaceae					
<i>Pacispora</i> sp.	0	0.2 \pm 0.2	0	0	25
Fam. Acaulosporaceae					
<i>Acaulospora excavata</i>	0	0	0.2 \pm 0.2	0	25
<i>A. laevis</i>	0	0.2 \pm 0.2	0	0	25
<i>A. mellea</i>	5.0 \pm 2.4	1.8 \pm 0.6	0.7 \pm 0.4	2.5 \pm 0.6	100
Fam. Ambisporaceae					
<i>Ambispora leptoticha</i>	0.3 \pm 0.3	0.2 \pm 0.2	0	0	50
<i>A. gerdemannii</i>	0.2 \pm 0.2	0.2 \pm 0.2	0	0	50
Incertae sedis					
<i>Entrophospora báltica</i>	0	0	0.2 \pm 0.2	0	25

(clipped) to 5 cm height which removed senescent, dead shoots accumulated during the previous growing seasons. Half of the plants ($n = 6$) for each species were defoliated to 5 cm height on 6 August 2012 (mid-winter) during the vegetative developmental morphology stage. These same plants were again defoliated to 5 cm on 14 September 2012 (late winter-early spring) after the differentiation of the growth apex from vegetative to reproductive. This allowed apical and some intercalary, active meristems to remain on the plants after the defoliation treatments without affecting their biological growing capacity (Giorgetti et al., 2000; Briske and Richards, 1995). Control plants ($n = 6$ per species) were not defoliated at any time.

2.4. Spore isolation and identification

Spores were isolated from 100 g of dry soil using the wet sieving and decanting method (Gerdemann and Nicolson, 1963) and centrifugation in a sucrose gradient (Walker et al., 1982). Spores were isolated with Pasteur pipettes under a stereoscopic microscope. They were then separated in individual groups, according to common morphological features (e.g., size, color, hyphae connections, surface area spore characteristics), which were then transferred in water to glass watches. Each spore type was placed in polyvinyl-lactic acid-glycerin (PVLG; Koske and Tessier, 1983) and PVLG in a mixture 1:1 (v/v) with Melzer reagent (Brundrett et al., 1999). Spore identification was based on taxonomic criteria currently accepted for spore size, color, surface ornamentation and wall structure [Schenck and Perez, 1990; INVAM (International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi), <http://invam.caf.wvu.edu>].

2.5. Analysis of the AMF communities

After spore identification, the following determinations were made: 1) frequency of occurrence of the AMF species (percentage of samples from which spores of a particular species were recovered); 2) spore density of each AMF family (spore number of each AMF family per 100 g soil); 3) total spore density of AMF (total AMF spore number per 100 g soil); 4) richness of AMF (number of species per 100 g soil), and 5) Diversity index of Shannon and Weaver (1949), $H' = -\sum p_i \log_2 p_i$, where p_i is the relative species density in comparison with the total number of species identified per sample.

2.6. Statistical analysis

Data were analyzed using the software INFOSTAT (Di Rienzo et al., 2013). Principal component analysis (PCA) was used for correlating the major species of AMF with the perennial grass species or unvegetated sites, defoliation treatment and sampling date. Data corresponding to spore density of each family and total spore density were transformed to $\log_2(x + 1)$ to comply with the assumptions of normality and homocedasticity. At the initial sampling, spore density, richness and diversity were analyzed using one-way ANOVA with plant species/unvegetated site as the main factor. Remaining data were analyzed using two-way ANOVA with species and defoliation treatment as the main factors. When F tests were significant at the 5% significance level, mean comparisons were made using Fisher LSD test at a significance level of 5%.

3. Results

3.1. AMF species

The total number of isolated spores (5,297) during all sampling dates were grouped in 15 morphospecies in the Acaulosporaceae, Ambisporaceae, Claroideoglomeraceae, Glomeraceae, Diversisporaceae and Pacisporaceae families. Spores were identified to species level every time it was possible, and genus level when the species-level identification was unsuccessful. Unidentified spores of Glomeraceae and Pacisporaceae were named to a genus level (Tables 2 and 3).

Dominant species of AMF (100% frequency) across the three sampling dates were *Funnelformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & Schüßler, *F. geosporum* (T.H. Nicolson & Gerd.) C. Walker & Schüßler, *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & Schüßler, *Diversispora spurca* (C.M. Pfeiff., C. Walker & Bloss) C. Walker & A. Schüßler and *Acaulospora mellea* Spain & N.C. Schenck. Sub-dominant species were *Glomus* sp. (first sampling = 75% frequency; second and third sampling = 83%) and *Claroideoglossum etunicatum* Walker & Schüßler (first sampling = 50%; second and third sampling = 58%; Tables 2 and 3).

The first component of the PCA, major species, separated *D. spurca* and *R. irregularis* from *Glomus* sp., *C. etunicatum* and *F. geosporum* (Fig. 1). This is, *D. spurca* and *R. irregularis* appeared positively

Table 3
Density (spore number/100 g soil, mean ± 1SE, n = 6) and frequency of appearance (F: percentage of occurrence (F: percentage of appearance from which spores of a particular species were recovered) of AMF species under defoliated (D) and undefoliated (UD) plants of *Poa ligularis*, *Nassella tenuis* and *Amelichloa ambigua*, at the second (September) and third (October) sampling dates in 2012.

AMF species	Poa ligularis						Nassella tenuis						Amelichloa ambigua						F (%)						
	D		UD		Sept.		Oct.		D		UD		Sept.		Oct.		D			UD		Sept.		Oct.	
	Sept.	Oct.	Sept.	Oct.	Sept.	Oct.	Sept.	Oct.	Sept.	Oct.	Sept.	Oct.	Sept.	Oct.	Sept.	Oct.	Sept.	Oct.		Sept.	Oct.	Sept.	Oct.	Sept.	Oct.
Fam. Glomeraceae																									
<i>Funnelformis mosseae</i>	1.7 ± 1.1	3.7 ± 2.9	2.7 ± 1.8	2.0 ± 1	3.0 ± 1.5	1.0 ± 0.7	0.3 ± 0.3	3.7 ± 1.7	3.7 ± 1.7	3.0 ± 1.5	1.0 ± 0.7	0.3 ± 0.3	3.7 ± 1.7	3.7 ± 1.7	3.0 ± 1.5	1.0 ± 0.7	0.3 ± 0.3	3.7 ± 1.7	3.7 ± 1.7	3.0 ± 1.5	1.0 ± 0.7	0.3 ± 0.3	3.7 ± 1.7	3.7 ± 1.7	
<i>F. geosporum</i>	31.0 ± 6.1	18.0 ± 6.6	21.3 ± 8.8	16.0 ± 6.4	6.3 ± 2.1	3.7 ± 2.2	5.3 ± 2.7	10.0 ± 2.4	10.0 ± 2.4	6.3 ± 2.1	3.7 ± 2.2	5.3 ± 2.7	10.0 ± 2.4	10.0 ± 2.4	6.3 ± 2.1	3.7 ± 2.2	5.3 ± 2.7	10.0 ± 2.4	10.0 ± 2.4	6.3 ± 2.1	3.7 ± 2.2	5.3 ± 2.7	10.0 ± 2.4	10.0 ± 2.4	
<i>Glomus</i> sp.	21.7 ± 9.1	3.7 ± 1.2	8.0 ± 3	4.7 ± 1.8	8.3 ± 3.5	0	0	5.0 ± 2.4	5.0 ± 2.4	8.3 ± 3.5	0	0	5.0 ± 2.4	5.0 ± 2.4	8.3 ± 3.5	0	0	5.0 ± 2.4	5.0 ± 2.4	8.3 ± 3.5	0	0	5.0 ± 2.4	5.0 ± 2.4	
<i>G. microaggregatum</i>	0	0	0.3 ± 0.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Rhizophagus clarus</i>	0	0	0.3 ± 0.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>R. irregularis</i>	7.3 ± 3.8	7.7 ± 2	5.7 ± 2.8	3.7 ± 1.8	4.3 ± 1.7	2.3 ± 1	8.3 ± 2.9	5.0 ± 2.6	5.0 ± 2.6	4.3 ± 1.7	2.3 ± 1	8.3 ± 2.9	5.0 ± 2.6	5.0 ± 2.6	4.3 ± 1.7	2.3 ± 1	8.3 ± 2.9	5.0 ± 2.6	5.0 ± 2.6	4.3 ± 1.7	2.3 ± 1	8.3 ± 2.9	5.0 ± 2.6	5.0 ± 2.6	
Fam. Claroideoglomeraceae																									
<i>Claroideoglomus etunicatum</i>	2.7 ± 2.7	0.7 ± 0.7	2.7 ± 1.3	0	0.7 ± 0.7	0	0	1.3 ± 1	1.3 ± 1	0.7 ± 0.7	0	0	1.3 ± 1	1.3 ± 1	0.7 ± 0.7	0	0	1.3 ± 1	1.3 ± 1	0.7 ± 0.7	0	0	1.3 ± 1	1.3 ± 1	
Fam. Diversisporaceae																									
<i>Diversispora spurca</i>	10.0 ± 1.6	8.7 ± 2.2	6.3 ± 1.4	2.3 ± 1	4.3 ± 1.7	9.7 ± 1.5	8.3 ± 1.7	6.0 ± 1.4	6.0 ± 1.4	4.3 ± 1.7	9.7 ± 1.5	8.3 ± 1.7	6.0 ± 1.4	6.0 ± 1.4	4.3 ± 1.7	9.7 ± 1.5	8.3 ± 1.7	6.0 ± 1.4	6.0 ± 1.4	4.3 ± 1.7	9.7 ± 1.5	8.3 ± 1.7	6.0 ± 1.4	6.0 ± 1.4	
Fam. Acaulosporaceae																									
<i>Acaulospora mellea</i>	6.3 ± 2.4	2.7 ± 2.3	4.7 ± 2	2.7 ± 1	3.0 ± 1.2	2.3 ± 1.6	0.3 ± 0.3	1.7 ± 1.1	1.7 ± 1.1	3.0 ± 1.2	2.3 ± 1.6	0.3 ± 0.3	1.7 ± 1.1	1.7 ± 1.1	3.0 ± 1.2	2.3 ± 1.6	0.3 ± 0.3	1.7 ± 1.1	1.7 ± 1.1	3.0 ± 1.2	2.3 ± 1.6	0.3 ± 0.3	1.7 ± 1.1	1.7 ± 1.1	
Fam. Ambisporaceae																									
<i>Ambispora leptotricha</i>	0.3 ± 0.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

correlated in the samples (i.e., when *D. spurca* was present in a sample, *R. irregularis* was also present in that sample most of the times). In the same way, *Glomus* sp., *C. etunicatum* and *F. geosporum* appeared together, while *D. spurca* and *R. irregularis* were absent, in the same sample most of the times (Fig. 1). The second PCA component grouped *A. mellea* and *F. mosseae* (Fig. 1). These two components explained 68.4% of the total variation. Proportions of *F. geosporum*, *Glomus* sp. and *C. etunicatum* associated with *P. ligularis* and *A. ambigua* were greater in August compared to October. At the same time, *N. tenuis* and the unvegetated sites had a greater proportion of *D. spurca* and *R. irregularis* (Fig. 1). At the second sampling (September), defoliated and undefoliated plants of *P. ligularis* and *A. ambigua* had a high proportion of *F. geosporum* and *Glomus* sp. spores compared to *N. tenuis* (Fig. 1). In October, defoliated and undefoliated plants of *N. tenuis* showed a high proportion of *D. spurca* and *R. irregularis*; the other two grass species did not appear to associate to any AMF species in particular (Fig. 1). In September, *Glomus* sp., *C. etunicatum* and *F. geosporum* tended to produce a greater proportion of spores in soil under the canopy of defoliated and undefoliated plants of *A. ambigua*, compared to *P. ligularis*. Within each study species, defoliation effects on the AMF community were obvious after two successive defoliations (i.e., in October) (Fig. 1).

3.2. Major AMF families

Defoliation treatments did not affect spore density in any of the three most abundant families associated with perennial grass species [Table 4 (b)]. For Glomeraceae, spore density was greater underneath the foliage of *P. ligularis* compared to *N. tenuis* and on unvegetated sites, in the August sampling [Fig. 2 (a)]. Spore density beneath *P. ligularis* and *A. ambigua* was similar for all sampling dates [Fig. 2 (a)].

Spore density of Acaulosporaceae among species and unvegetated sites did not differ neither in the initial sampling [Table 4 (a); Fig. 2 (b)] nor in October [Table 4; Fig. 2 (a)]. In September, however, spore density was lower under the foliage of *A. ambigua* than on that of *P. ligularis* [Fig. 2 (b)].

In August, AMF spore density was similar in soil under all three perennial grasses and unvegetated sites in Diversisporaceae [Table 4 (a); Fig. 2 (c)]. In September, *P. ligularis* and *N. tenuis* had similar spore density values, but greater values than *A. ambigua* [Fig. 2 (c)]. There were no differences ($p > 0.05$) in spore density among species in October [Table 4 (b); Fig. 2 (c)].

3.3. Total density, richness and diversity of AMF

Defoliation treatments did not affect total density, richness and diversity of AMF on any of the soil sampling dates [Table 5 (b)]. AMF density in August and September was greater under *P. ligularis* than *N. tenuis* (Fig. 3), and in the unvegetated sites in August (Fig. 3). In September, *P. ligularis* and *A. ambigua* did not differ in AMF spore density (Fig. 3). AMF spore density was similar for all plant species in October (Fig. 3). Plant species and unvegetated sites did not differ in AMF richness and diversity in August [Table 5 (a)], and no differences among plant species were observed in September and October [Table 5 (b)]. Mean values of AMF richness and diversity were 3.98 ± 0.12 species/100 g dry soil and 1.58 ± 0.04 , respectively.

4. Discussion

This study is the first report on the species identity and quantity of AMF spores in the infertile, semiarid soils of northeastern Patagonia, Argentina. We worked at the species and family of AMF in the analysis and presentation of the results in this study. AMF species were grouped to a family rather than as functional differences in these fungi occur at the family level than species level (Chagnon et al., 2013; Velázquez et al., 2013).

We are not sure where the soil spores came from into our control,

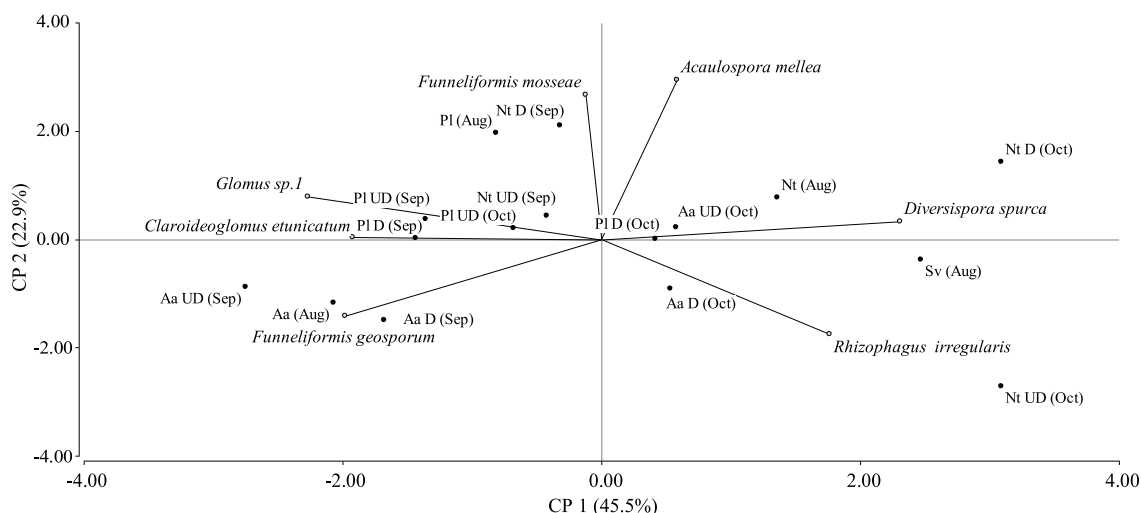


Fig. 1. Principal component analysis: Bi-plot graphic of the proportion of spores of the major AMF (number of spores of each species/total spore number) and its relationship with either defoliated (D) or undefoliated (UD) plants of *P. ligularis* (PI), *N. tenuis* (Nt), and *A. ambigua* (Aa) or unvegetated sites (Us) in August (Aug), September (Sep) and October (Oct) 2012. AMF species are graphed as vectors from the origin. Observations (grass species/unvegetated sites; treatments and sampling times) which are graphed in the same direction than AMF species represent high values for that variable, and low values for variables which are graphed in the opposite direction. Cophenetic correlation: 0.941.

unvegetated sites. Spores can persist in the soil during long time periods, thus not reflecting the current state of the symbiosis between the AMF and the plant species (Hijri et al., 2006). They could come from roots of neighboring plants; this might be because of the extensive, adventitious root system which is characteristic in perennial grasses (Caldwell and Richards, 1986). They could also be remnant spores bank of the vegetation that might have been previously there. This situation might also occur under the study plants. It was important to compare unvegetated sites with those where native, perennial grasses were established to evaluate the species-specific effects of the studied plants.

4.1. AMF species

Plant species *P. ligularis* and *A. ambigua*, which have contrasting seral stages, livestock preferences and litter quality, were associated with AMF *C. etunicatum* and *F. geosporum* (Fig. 1). Such fungi have been reported on other perennial grass species with similar plant characteristics to those of the studied species (Murray et al., 2010; Velázquez et al., 2013). *Claroideoglossum etunicatum* has been found (1) in degraded environments (Irrazabal et al., 2005), (2) to be very efficient when plants are exposed to high stress conditions (Lozano-Ruiz et al., 1995), and (3) unaffected by defoliation (Klironomos et al., 2004). *Funneliformis geosporum* is sensitive to high grazing pressures (Uhlmann et al., 2006). However, this AMF species has been found in restored, ungrazed or moderately grazed areas (Su and Guo, 2007). This might explain why the mentioned AMF species were positively correlated between themselves and showed tolerance to the first defoliation treatment (Fig. 1).

Nassella tenuis, the intermediate seral stage plant species with high litter quality and preferred by livestock, was more associated with the AMF species *D. spurca* and *R. irregularis* during August and October, and *A. mellea* and *F. mosseae* in September (Fig. 1). *Funneliformis mosseae* has been shown to be sensitive only to high grazing pressures (Su and Guo, 2007), and similarly to *D. spurca*, they tolerate drought conditions (Lozano-Ruiz et al., 1995). Spore density of *A. mellea* increases during spring (Lugo and Cabello, 2002), and this might explain its greater association with *N. tenuis* in September.

4.2. Defoliation

Despite the PCA allows to see some effect after the second defoliation, this effect did not show up when ANOVA were conducted with the

major AMF Families. Therefore, such effect was considered irrelevant. This emphasizes the importance of making analysis not only to the AMF species but also to a family scale. This is critical to the time of analyzing the ecological functionality of AMF. Defoliation treatments did not affect density, richness, or diversity of AMF spore density (as it was posted in our second hypothesis), despite the differential plant characteristics of the three grass species. Similarly, no consistent patterns in AMF spore counts were observed for two perennial, C₃ tussock grasses exposed to defoliation under various soil moisture regimes (Allen et al., 1989). Greater shoot relative growth rates on defoliated than undefoliated plants of *N. tenuis* and *A. ambigua* (Saint Pierre et al., 2004a) suggest that carbon is not a limiting factor on defoliated plants as to maintain mycorrhizal root colonization and subsequent reproduction. Defoliated plants may benefit from AMF functioning to stimulate compensatory growth (Kula et al., 2005), but if carbon is a limiting resource, the interaction between plants and AMF can become parasitic rather than mutualistic for plants (Fitter and Hay, 1983). Long-term defoliations, during at least 20 years, have reduced spore density of AMF (Frank et al., 2003; Su and Guo, 2007; Murray et al., 2010). Also, Van der Hayde et al. (2017) demonstrated that time since grazing cessation was an important factor to explain the dissimilarities between grazed and ungrazed AM fungal communities.

The lack of defoliation effects on AMF spore density in this study are in agreement with the second hypothesis, in accordance with previous studies in rangelands exposed to light or moderate grazing (Lugo and Cabello, 2002; Yang et al., 2013) or defoliation treatments (Allen et al., 1989; Bentivenga and Hetrick, 1992; Klironomos et al., 2004). They disagree, however, with results of Busso et al. (2001) who reported that defoliated plants of perennial, tussock grass species had a higher mean spore number than undefoliated plants. In our study, defoliation intensity might not have been sufficient to remove enough photosynthetic tissue as to reduce substrate availability to the fungi for sufficient periods to inhibit AMF colonization rates or induce mortality (Trent et al., 1988). Additionally, Van der Hayde et al. (2017) found that differences in the AMF communities between defoliated versus undefoliated plants increased with time from defoliation.

4.3. Major AMF families

Identified families in the AMF communities agree with results previously reported in plant studies from semiarid rangelands (Yang et al.,

Table 4 Results of the ANOVA for spore density of the major AMF families (a) at the initial sampling date (i.e., August; Factor: species-unvegetated sites), and (b) after conducting the defoliation treatments (i.e., September and October; Factors: species and defoliation treatments).

	Glomeraceae		Acaulosporaceae		Diversisporaceae	
	F	p-value	F	p-value	F	p-value
August						
Species-Unvegetated sites	5.29	0.0033	1.95	0.1353	0.57	0.6365
Error						
df	3	44				
	Glomeraceae		Acaulosporaceae		Diversisporaceae	
	F	p-value	F	p-value	F	p-value
September						
Species	7.17	0.0029	3.38	0.0476	6.41	0.0048
Defoliation	0.47	0.4969	0.0004	0.9841	0.03	0.8581
Species x Defoliation	1.34	0.2768	1.02	0.3722	1.16	0.3264
Error						
df	2	30				
October						
Species	5.18	0.0117	0.71	0.5012	2.96	0.0673
Defoliation	0.46	0.5012	0.09	0.7619	2.54	0.1216
Species x Defoliation	2.01	0.1511	1.03	0.3682	1.56	0.2270
Error						
df	2	30				

Significant p-values (≤ 0.05) are in bold.

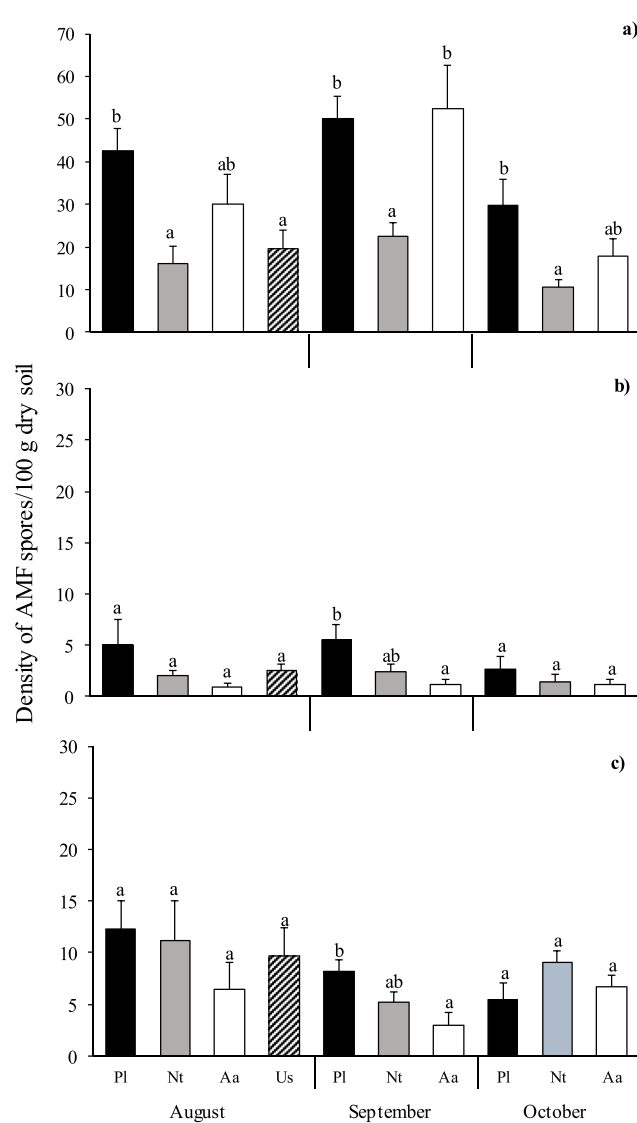


Fig. 2. Density (total spore number/100 g dry soil, mean \pm 1 S.E., n = 12) of AMF spores in soil under the canopy of *Nassella tenuis* (Nt), *Poa ligularis* (PI), and *Amelichloa ambigua* (Aa), and on unvegetated sites (Us) of (a) Glomeraceae, (b) Acaulosporaceae and (c) Diversisporaceae. Different letters indicate significant differences among plant species and unvegetated sites in August, and among species in September and October. Fisher LSD test was used to detect differences among means to a significance level of 5%. Note the differences in scales in the Y axes.

2013). Members of Glomeraceae are able to produce a large spore quantity during a short time period and regulate their growth as a function of host activity, which are typical characteristics of r-strategy species (Chagnon et al., 2013). Because of this, it is possible that seasonal or sampling effects were not detected on them. The genus *Rhizophagus* has a great tolerance to environmental stress and grazing (Yang et al., 2013), while *Glomus* is well adapted to a variable host quality, likely enabling sufficient capacity to establish symbiotic relationships with numerous plant species (Mendoza et al., 2002; Cai et al., 2014). Mendoza et al. (2002) demonstrated that *Claroideoglomus etunicatum* was in a greater proportion in the rhizospheres of *Deschampsia flexuosa* and *Poa rigidifolia* than in other grass species, and that the quantity and diversity of AMF is associated with a greater rangeland forage quality. These results are in agreement with ours, and with the first hypothesis, where *P. ligularis* showed a higher AMF spore density than *N. tenuis* and the uncovered sites in August, and that *N. tenuis* in September in the Glomeraceae [Fig. 2 (a)]. *Poa ligularis* has a

Table 5 Results of the ANOVA for total density, richness and diversity of AMF (a) at the initial sampling date (i.e., August; Factor: species-unvegetated sites), and (b) after conducting the defoliation treatments (i.e., September and October; Factors: species and defoliation treatments).

		Density		Richness		Diversity	
df		F	p-value	F	p-value	F	p-value
August							
Species-Unvegetated sites	3	4.60	0.0069	1.08	0.3678	0.82	0.4909
Error	44						
b)							
df		Density		Richness		Diversity	
		F	p-value	F	p-value	F	p-value
September							
Species	2	7.90	0.0018	1.10	0.3473	0.92	0.4084
Defoliation	1	1.28	0.2661	0.00	> 0.9999	0.01	0.9423
Species x Defoliation	2	0.91	0.4152	0.82	0.4492	0.51	0.6084
Error	30						
October							
Species	2	2.87	0.0723	2.89	0.0709	1.42	0.2586
Defoliation	1	0.24	0.6251	0.08	0.7809	0.19	0.6690
Species x Defoliation	2	0.83	0.4455	0.14	0.8718	0.05	0.9504
Error	30						

Significant p-values (≤ 0.05) are in bold.

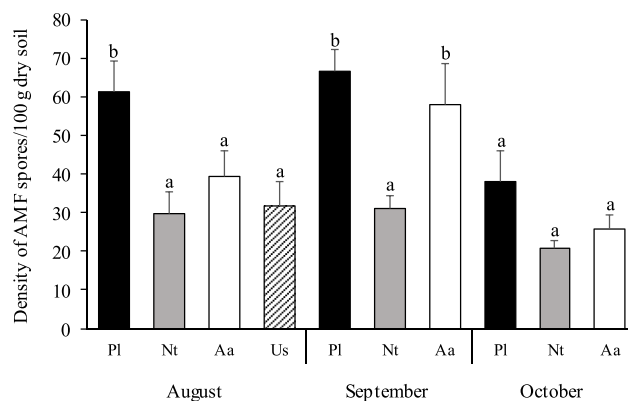


Fig. 3. Density (total spore number/100 g dry soil, mean \pm 1 S.E., n = 12) of AMF in soil under the canopies of *Poa ligularis* (PI), *Nassella tenuis* (Nt) and *Amelichloa ambigua* (Aa) and on unvegetated sites (Us). Each histogram is the mean \pm 1 S.E. of n = 12. Different letters indicate significant differences among species and unvegetated sites in August, and among species in September and October. Fisher LSD test was used to detect differences among means to a significance level of 5%.

greater forage value than *N. tenuis* (Cano, 1988).

Developmental morphology (i.e., phenological) stage differences of the perennial grasses were hypothesized to affect spore density in the Acaulosporaceae and Diversisporaceae. At the second sampling date in early spring [Fig. 2 (b, c)], rapid differentiation of *P. ligularis* apical meristems from vegetative to reproductive (20% at sampling time) elongated internodes (Giorgetti et al., 2000), which increases stem growth but reduces production of new leaves (Busso and Richards, 1995). This results in a reduced allocation of carbon resources to roots and an associated decrease in root biomass (Dawson et al., 2000). This in turn translates into lipid translocation from fungus vesicles to resistance structures (i.e., AMF spores: Bentivenga and Hetrick, 1992). The greater density of the Acaulosporaceae and Diversisporaceae spores in *P. ligularis* compared to *A. ambigua* in the September sampling highlights the influence of the differential plant characteristics on AMF (Bentivenga and Hetrick, 1992; Lugo and Cabello, 2002).

4.4. Total density, richness and diversity of AMF

The late seral, highly preferred and high litter quality plant species *P. ligularis*, exhibited a consistent trend for a greater AMF density than the other two grass species across the sampling dates (Fig. 3). Values were only significantly different at the first sampling date (August), and at least 12.2% or 32.6% greater in *P. ligularis* than in the other two species in the September and October samplings, respectively. Because preferred perennial grasses facilitate AMF spore production, and then their propagation through a higher litter quality and a more rapid aboveground litter decomposition (Moretto and Distel, 2003; Cai et al., 2014), this could have important consequences on the communities of soil microorganisms.

Plant size can be an important factor in structuring AMF communities (Piippo et al., 2011), and results herein provide support that mean basal area for *P. ligularis* was more than 3-fold greater than that in *N. tenuis*. This might also contribute to the greater AMF density in *P. ligularis* than in *N. tenuis* (Fig. 3). The greater basal area and tiller density in *P. ligularis* than in *N. tenuis* (Saint Pierre et al., 2004b) gave a greater photosynthetic leaf length in the first than in the second species (Ithurrart, 2016). This might contribute to an increased photosynthetic carbon input in *P. ligularis*, which might facilitate partitioning of a greater amount of carbon resources to roots, thus increasing its capacity for associating with AMF (Piippo et al., 2011). This might be because AMF spore density was either greater or similar, but not lower, in *P. ligularis* than in the other two species.

5. Implications

Defoliation did not significantly affect AMF spore diversity, richness nor density in soil sampled under perennial grass canopies with contrasting plant characteristics. Rather, the particular characteristics of the grass species such as plant size, litter quality and plant developmental morphology stage were influential in altering AMF communities. Rangeland management strategies in northeast Patagonia should focus on increasing the abundance of the highly preferred *P. ligularis* relative to the less preferred species *N. tenuis* (Distel and Bóo, 1996). We suggest that further studies should address the (1) comparison of sites with different grazing histories, and (2) effects of various defoliation characteristics (e.g., frequency, intensity, timing) on AMF spore viability under perennial grasses.

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