Patchiness of grass mycorrhizal colonization in the Patagonian steppe

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ABSTRACT

In arid and semi-arid ecosystems vegetation is often arranged in high-density patches imbedded in an extensive matrix dominated by bare soil. This study explores the importance of vegetation pattern in the relationship between grasses and arbuscular mycorrhizal fungi (AMF). In shrub-dominated and bare-soil dominated patches of the Patagonian steppe, we quantified AMF colonization in the dominant grasses *Bromus pictus*, *Poa ligularis*, *Pappostipa speciosa* and *Pappostipa humilis*. Additionally, in the shrub-dominated patches, AMF colonization was measured in roots under the shrub canopy and off the shrub canopy. Soils in each patch type were also characterized. *B. pictus* showed the highest AMF colonization, followed by *P. speciosa, P. humilis* and *P. ligularis*. The shrub patch resulted to be a fertility hotspot for biological activity and soil attributes. Grass plants in the shrub patches showed in general the highest rates of AMF colonization. Conversely, we did not find consistent differences between the two types of microsites within the shrub patch. Shrub patches may be functioning as refuges for biological activity that preserve the occurrence of mycorrhizal symbiosis of grass species and alter nutrient dynamics.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with 75% of the plant species in most terrestrial ecosystems (Treseder and Cross, 2006). The main biological basis of this relationship is a bidirectional nutrient transfer: fungi provide minerals to roots in return for carbon substrates from the host plant. The AMF generate an extensive network of extraradical mycelium specialized in the acquisition of mineral nutrients (http://www.sciencedirect.com/science/article/pii/S0929139311001107, Smith and Read, 2008), particularly those with poor mobility and/or low availability (especially phosphorus, but also ammonium, copper, zinc and other microelements). Additionally, AMF generate a smaller carbon cost per unit absorbing area than roots, allow the exploration of soil volumes not accessed by roots, contribute to the formation of stable soil aggregates, and in some cases, improve plant water relations, accelerate decomposition of organic matter and reduce leaching losses of both organic and inorganic nutrients (van der Heijden et al., 2015). Thus, AMF facilitate plant recruitment and growth by mitigating nutrient deficiency and other environmental stresses (Smith and Read, 2008), and play a significant role in nutrient and carbon cycles in terrestrial ecosystems (van der Heijden et al., 1998, 2015).

In arid and semi-arid ecosystems, soil nutrients and water are scarce and plants are often distributed in patches, leading to a pattern with marked spatial and functional heterogeneity (Soriano et al., 1994; Aguilera et al., 1999). The shrub-grass Patagonian steppe is an interesting system in which to explore variations of biological interactions as it has a well-defined spatial pattern composed by two different structural patches: shrubs surrounded by a ring of grasses and grasses interspaced in a bare-soil matrix (Soriano et al., 1994). Shrubs represent points of higher organic matter accumulation and soil microbial activity (*i.e.* fertility hotspots) (Golluscio et al., 2009; Gonzalez-Polo and Austin, 2009). Additionally, they trap wind-blown seeds and facilitate the recruitment of grass species in relation to the more stressful

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conditions of the bare soil patches (Soriano et al., 1994). Moreover, in the Patagonian steppe nitrogen is particularly scarce and mainly present in the -less mobile-form of ammonium, the most preferred nitrogen form by grasses (Gherardi et al., 2013). Previous studies showed that AMF, apart from the well documented role in phosphorous uptake, can acquire preferably ammonium from the soil but also nitrate and/or organic compounds, which are assimilated as amino acids in the mycelium structures, then converted and transferred as ammonium to the plant (Hodge and Storer, 2015). In this context, it is possible to speculate that the association with AMF could be a relevant beneficial factor for plant growth and survival in this ecosystem. Fontenla et al. (2001) surveyed a high number of species in the region and classified them as mycorrhizal and non mycorrhizal. However, we currently lack information on the extent of root colonization by AMF and its distribution in the patchy shrub-grass Patagonian steppe.

The main objective of this work was to determinate the degree of variation in mycorrhizal colonization associated with different types of vegetation patches in the dominant grass species of the Patagonian steppe: *Bromus pictus*, *Poa ligularis*, *Pappostipa speciosa* and *Pappostipa humilis*. This study contributes to our understanding of the spatial distribution and environmental controls of AMF colonization in relation to the patchiness and composition of vegetation; additionally, it has implications on the conservation and functioning of mycorrhizal symbiosis in arid ecosystems.

2. Materials and methods

Field work was carried out in a lightly grazed paddock at the Experimental Field Station of INTA (National Institute of Agricultural Technology) at Río Mayo, SW of Chubut province, Argentina (45° 41′ S, 70° 16′ W). The vegetation corresponds to the Occidental District of the Patagonian steppe where soils are Petrocalcic Calciorthids: sandy with presence of gravel and pebbles. Mean annual precipitation is 168 mm, mean monthly temperature ranges from 2 °C in July to 14 °C in January. The plant community is co-dominated by tussock grasses and shrubs (Soriano et al., 1994), which contribute more than 96% of total plant cover. The dominant grasses are *Bromus setifolius* var. *pictus* (Hook.f.) Skottsb. (hereafter *Bromus pictus*), *Poa ligularis* Nees ex Steud., *Pappostipa speciosa* (Trin. & Rupr.) Romasch. (ex *Stipa speciosa*), and *Pappostipa humilis* var. *ruiziana* (Parodi) Romasch. (ex *Stipa humilis*). They strongly vary in palatability and preference by domestic sheep, the dominant grazer in the area. *B. pictus* and *P. ligularis* are the most palatable and selected by sheep, followed by *P. speciosa* and finally by *P. humilis*, which is hardly consumed. The dominant shrubs are *Mulinum spinosum* (Cav.) Pers., *Senecio filaginoides* DC., and *Adesmia volckmannii* Phil. (ex *Adesmia campestris*).

We determined AMF colonization in the four grass species in three microsites of the structural pattern typical of this steppe (Fig. 1). One microsite was the vicinity of grasses in the bare-soil patches, where grasses grow scattered at low density. The other two microsites were in the shrub patch, where grasses form high-density rings around the shrub. One microsite was under the shrub canopy (microsite "inside shrub") and the other was in the margin with the bare-soil patch (microsite "outside shrub"). In all cases the shrub species was the most abundant *Mulinum spinosum*. The most palatable species (*B. pictus*) was sampled only in the inside shrub microsite because there were very few individuals in the other microsites.

In January (near growing season peak), we set a transect with random origin and direction and every 10 m we searched for the nearest *Mulinum spinosum* shrub patch. In that patch and in the surrounding bare soil patch we sampled the roots of one individual of the target grasses. We repeated the operation until reaching 8
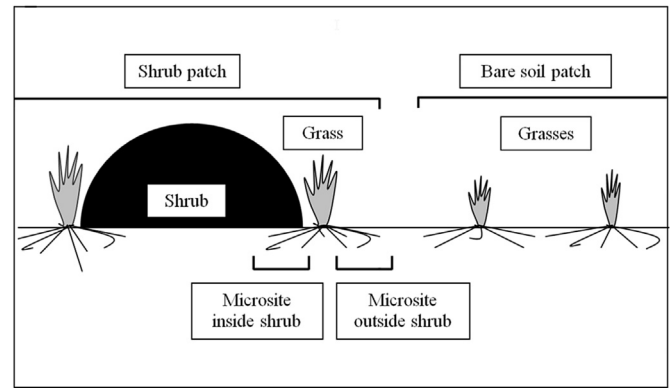

Fig. 1. Patch structure scheme of the Patagonian steppe (*sensu* Soriano et al., 1994). Two different vegetation patches were recognized: *i*) shrubs encircled by a ring of grasses (shrub patch) and *ii*) scattered grasses (bare soil patch). Samples of grass roots were taken from the bare soil patch and from microsites inside and outside of the shrub patch.

samples per species and microsite. Failure in sample processing in the laboratory resulted in a final number of replicates per species and microsite between 6 and 8. At the time of root sampling, for each target plant we made a narrow, horizontal pit (using spoons and brushes) around each of the selected roots (*i.e.* from the base to the apex). This procedure assured that the root samples belonged to the target plant. For each individual grass, roots were carefully unearthed to check identity, then extracted, washed and immediately fixed in formalin–acetic acid–alcohol (FAA) until processing for optical microscopy. In all cases, root samples were taken from the first 10 cm of the soil profile. Total root biomass and soil extractable phosphorus in the top 10 cm of the soil profile (excluding gravel and pebbles) were determined on ten samples per patch. Roots were separated from the soil by sieving through a 2 mm metal mesh, washed, and weighed after oven drying for 72 h at 80 °C. Extractable phosphorus was assessed by a non-reductive crystal violet colorimetric quantification (Bray and Kurtz method).

Mycorrhizal colonization was determined by histological detection of mycorrhizal structures (hyphae, arbuscules and/or vesicles) after root staining (Phillips and Hayman, 1970). Briefly, a sample of fresh root material was cleared in KOH (10% w/v, 1 h, 90 °C), acidified in HCl (1% v/v, 3 min) and then stained with Trypan Blue (0,05% w/v, 15 min, 90 °C) in acid glycerol. The percentage of root length with AMF colonization was assessed by evaluating 100 random intersections per sample at ×200 magnification (McGonigle et al., 1990). For the dominant grasses of the Patagonian steppe, the occurrence of symbioses with *Epichloë* endophytes was recently analyzed (Hernández-Agramonte and Semmartin, 2016; Gundel et al., 2016). At the same study site, none of the species included in our work harbored symbioses with *Epichloë* under different grazing treatments (Hernández-Agramonte and Semmartin, 2016).

For some analyses, the two microsites from the shrub patch were averaged to compare with the bare-soil patches. For each species, comparisons of mycorrhizal colonization between patches were based on Student's *T*-tests, while comparisons of root sub-samples of the same individual plant were based on Student's *T*-test for paired samples. All data which did not comply with the assumptions of ANOVA were (arcsin $\sqrt{x}$) transformed. Statistical analyses were performed using Statistica package for Windows (StatSoft, Tulsa, OK, USA).

3. Results and discussion

All the dominant grass species of the Patagonian steppe assessed in this study formed symbiotic associations with AMF (Fig. 2). Although species of a same family tend to share the mycorrhizal status (Fontenla et al., 2001), AMF colonization strongly depends on the identity of plant and fungal partners and environmental conditions (van der Heijden et al., 1998). Accordingly, we found that mycorrhizal colonization was highly variable between species, decreasing in the order *Bromus pictus* (*c.a.* 65%), *Pappostipa speciosa* (*c.a.* 17%), *Poa ligularis* and *Pappostipa humilis* (both *c.a.* 5%) for the shrub patch (Fig. 2a). To our knowledge, this is the first quantification of this parameter for species of the shrub-grass Patagonian steppe.

The affinity of these species to form mycorrhizal associations is not surprising, because apart from the fact that AMF usually represent an advantage for phosphorus acquisition, other nutrients (specially nitrogen) and water strongly limit plant growth in this ecosystem (Table 1) (López et al., 2003; Golluscio et al., 2009). A previous study demonstrated that shrubs uptake nitrate from deep soil layers, while grasses, with shallower roots absorb preferentially ammonium from the upper layers (Gherardi et al., 2013). AMF uptake inorganic (preferably ammonium) and also organic forms of nitrogen through the extraradical hyphae and transfer it to the host plants (Hodge and Storer, 2015). Further work should experimentally quantify the eventual advantage of AMF to the dominant grasses of the Patagonian steppe.
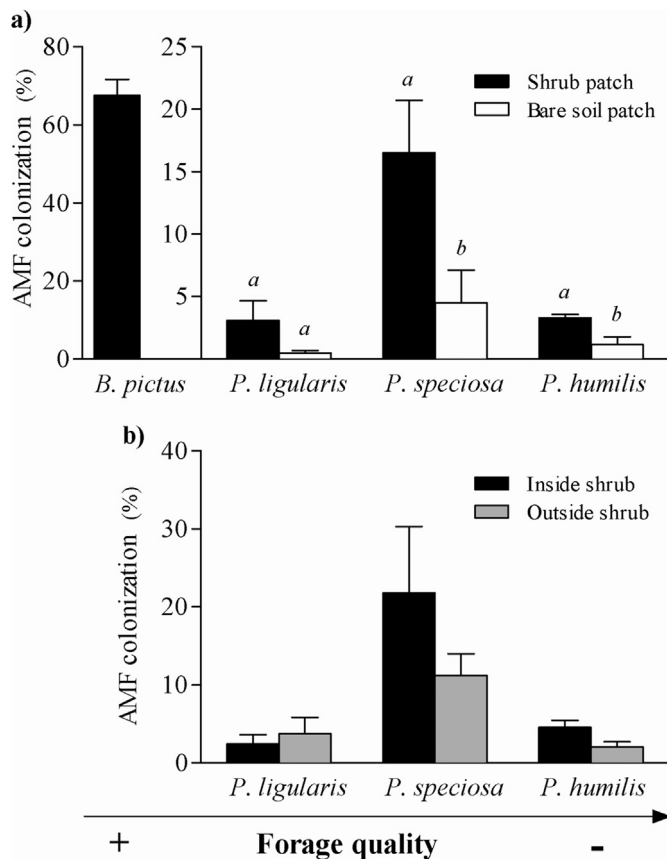
Paradoxically, in general, mycorrhizal colonization of dominant grasses was higher in the more fertile shrub patches than in the harsher bare-soil patches (Fig. 2a). Shrub patches showed more than ten times root biomass ($P < 0.01$), three times more extractable phosphorus ($P < 0.05$) and soil organic nitrogen (Golluscio et al., 2009), and higher water availability than the bare-soil patches (López et al., 2003; Gonzalez-Polo and Austin, 2009) (Table 1). In addition, shrub patches showed higher soil organic carbon (Golluscio et al., 2009) and microbial biomass (Gonzalez-Polo and Austin, 2009). In agreement with our finding, a greater root mycorrhizal colonization was registered in soil samples collected under *Adesmia bedwelli* (Fabaceae) shrub plants in the Chilean arid steppe, where organic matter, soil microorganisms and nitrogen increased as well (Aguilera et al., 1999). For the Patagonian steppe, our results are the first evidence that indicates that shrub plants acting as fertility hotspots (*i.e.* small soil volumes with much faster process rate and intensive interactions in comparison to average soil conditions) also harbor high levels of beneficial plant-microorganism associations. This pattern may stem from soil organic matter accumulation and protection against wind-related stressful conditions. At a finer scale, however, we did not find significant differences ($P > 0.3$ in all cases) in AMF colonization in roots growing towards different shrub-patch microsites (Fig. 2b), indicating that the environmental conditions under shrubs act at the whole-plant level instead of at the level of specific roots.

The interspecific differences in AMF colonization and the patterns associated with patch structure bear some implications on the conservation of the grass species (Fig. 2a). In particular, the most palatable grass, *B. pictus,* registered the higher AMF colonization levels by far and was mostly present deep inside shrubs, which act as a refuge from grazing, a key factor for conservation of palatable species in this ecosystem (Oesterheld and Oyarzabal, 2004; Graff and Aguiar, 2011). Our study suggests that the shrub patch represent a last refuge for the mycorrhizal function in this species. For *P. ligularis,* the other palatable species, we registered a clear trend ($P = 0.075$) to higher AMF colonization at the shrub patch. But remarkably, for *P. speciosa* (intermediate palatability) and *P. humilis* (not consumed at all) the results also showed significantly ($P = 0.030$ and $P = 0.014$, respectively) higher AMF colonization around the shrub in comparison to the bare soil patch (Fig. 2a). The effect of grazing on AMF colonization is often controversial (García and Mendoza, 2012; and references therein). The variety of responses may be related to the differential plant tolerance to the prevailing frequency (Ferraro and Oesterheld, 2002) and intensity (García and Mendoza, 2012) of defoliation, which could result in variable losses of carbon and nutrients. In our study, we could not discard a direct effect of defoliation on the degree of AMF colonization of the different species. But, as we found clear differences between patches for the non-palatable species (Fig. 2a), the defoliation regime of each patch type is not the only cause of the differences in AMF colonization.

4. Conclusions

Mycorrhizal colonization of Patagonian grasses varied among species (Fig. 2) and vegetation patches (Fig. 2a). The highest rate of AMF colonization was recorded on the most palatable species. The shrub patch, a fertility hotspot for soil nutrients and biological activity (Table 1 and Fig. 2a), harbored grasses with higher rates of AMF colonization in comparison to the bare soil patches. On the whole, for this system, we suggest that shrubs act as nurse plants forming resource rich patches (Table 1), and as a refuge for beneficial plant-microorganism associations (*i.e.* AMF) (Fig. 2a), which might facilitate recruitment and growth of other species. Often

Fig. 2. Arbuscular mycorrhizal fungi (AMF) colonization in grasses of the Patagonian steppe at a) different vegetation patches and b) microsites inside and outside the shrub patch. Species are sorted by decreasing forage quality: *Bromus pictus, Poa ligularis, Pappostipa speciosa* and *Pappostipa humilis*. Note that *B. pictus* was only present in the shrub patch. Values are means ± SE ($n = 6–8$). Different letters indicate significant differences ($P < 0.05$) between patches within each species based on Student's *T*-tests.

Table 1
Root biomass, microbial biomass and soil attributes for different vegetation patches (shrub and bare soil) of the Patagonian steppe. Values are means $\pm$ SE (n = 10 for our study).

	Type of patch		
	Shrub	Bare soil	Reference
Root biomass (g kg^{-1} soil)	3.47 $\pm$ 0.68	0.24 $\pm$ 0.09	This study
Microbial biomass (μg C g^{-1} dry soil)	691.1 $\pm$ 77.3	184.1 $\pm$ 25.9	Gonzalez-Polo and Austin 2009
Soil attributes			
Organic C (g kg^{-1} soil)	12.2 $\pm$ 1.5	4.5 $\pm$ 0.4	Golluscio et al., 2009
Organic N (g kg^{-1} soil)	1.0 $\pm$ 0.1	0.4 $\pm$ 0.1	Golluscio et al., 2009
Extractable P (mg kg^{-1} soil)	44.7 $\pm$ 1.3	16.4 $\pm$ 0.8	This study
Water content (% g H$_2$O g^{-1} dry soil)	7.23 $\pm$ 0.98	6.21 $\pm$ 0.85	Gonzalez-Polo and Austin 2009
	3.84 $\pm$ 0.22	2.43 $\pm$ 0.34	López et al., 2003

regarded as undesirable species in livestock systems, shrubs were depicted here as critical players of mycorrhizal symbiosis and nutrient dynamics. This is particularly relevant for conservation and revegetation programs in arid ecosystems.

Acknowledgements

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