Role of Grazing Intensity on Shaping Arbuscular Mycorrhizal Fungi Communities in Patagonian Semiarid Steppes☆

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are vital for maintaining ecosystem structure and functioning and can be affected by complex interactions between plants and herbivores. Information found in the literature about how ungulate grazing affects AMF is in general contradictory but might be caused by differences in grazing intensities (GIs) among studies. Hence we studied how different GIs affect the composition, diversity, and abundance of AMF communities in a semiarid steppe of Patagonia. We predicted that 1) total AMF spore abundance (TSA) and diversity would decrease only under intense-grazing levels and 2) AMF species spore abundance would depend on their life-history strategies and on the GI. To test our predictions, we compared AMF communities among nongrazed (NG), moderately grazed (MG, 0.1–0.3 sheep ha[1]), and intensely grazed sites (IG, > 0.3 sheep ha[1]). GI was the most important factor driving changes in TSA and diversity, regardless of host plant identity. TSA, diversity, and evenness significantly decreased in IG sites but were not affected by MG. AMF species spore abundance varied depending on their life-history strategies and GI. Families with high growth rates like *Glomeraceae* and probably *Pacisporaceae* showed the highest spore abundance in all sites but decreased under IG. Species with higher carbon demands like *Gigasporaceae* showed low spore abundance and frequency in NG and MG sites and were absent in IG sites. In contrast, species with low growth rates, but efficient carbon usage, like *Acaulosporaceae,* showed low spore abundance in all sites but increased in IG sites compared with NG or MG sites. We conclude that intensification of grazing reduces AMF diversity and abundance, with the likely loss of AMF benefits for plants, such as improved nutrient and water uptake and soil aggregation. Therefore, sustainable grazing systems should be designed to improve or restore AMF communities, particularly in degraded rangelands, like the Patagonian steppes.

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Introduction

Complex interactions between plants and herbivores can affect soil microorganisms influencing ecosystem structure and functioning (van der Heijden et al., 1998a, 1998b; van der Heijden and Sanders, 2003) because changes or loss of belowground diversity can significantly reduce plant diversity, decomposition, nutrient retention, and nutrient cycling (Wagg et al., 2014). Particularly, soil diversity of arbuscular mycorrhizal fungi (AMF) is a major factor contributing to the maintenance of plant biodiversity and, therefore, to ecosystem variability and productivity (van der Heijden et al., 1998a, 1998b; van der Heijden and Sanders, 2003). AMF colonize about two-thirds of land plant species (Treseder and Cross, 2006; Smith and Read, 2008; Brundrett, 2009) developing what is currently considered the most ecologically important symbiosis on the planet (Fitter et al., 2005; van der Heyde et al., 2017).

External hyphae of AMF can grow up to 100 times longer than root hairs, representing a significant contribution to the plant belowground system (Miller et al., 1995). As a result, AMF can enhance host plant (HP) uptake of phosphate (Karandashov and Bucher, 2005; Javot et al., 2007) and nitrogen (both by accelerating decomposition and directly acquiring nitrogen from organic material) (Hodge et al., 2001), water, and micronutrients. In return, up to 20% of plant photosynthetic carbohydrates are transferred to the fungus (Parniske, 2008; Smith and Read,

2008). AMF can also play an important role in soil formation (Rillig and Mummey, 2006; Lehmann et al., 2017).

All beneficial effects of AMF are most apparent under conditions of limited nutrient and water availability (Parniske, 2008) and, therefore, are particularly important for plant survival or restoration in arid and semiarid ecosystems or degraded areas (Allen, 1989; Requena et al., 2001; Titus et al., 2002; Rillig and Mummey, 2006; Allen, 2007; Brundrett, 2009). Consequently, there is a growing interest in how AMF are affected, particularly in poorly grazed and degraded lands (Bethlenfalvay and Dakessian, 1984; Lugo et al., 2003; Su and Guo, 2007; Ba et al., 2012; Antoninka et al., 2015; Cavagnaro et al., 2017, 2018; Ambrosino et al., 2018).

Grazing by domestic herbivores is one of the most generalized disturbances in grasslands worldwide that can change ecosystems structure and functioning by affecting plant annual net primary production (ANPP), physiognomy, diversity (Milchunas et al., 1988; Milchunas and Lauenroth, 1993; Ren et al., 2018) and nutrient cycling (Golluscio et al., 2009; McSherry and Ritchie, 2013; Ren et al., 2018). Grazing can alter plant resources allocation to shoot or roots (Milchunas and Lauenroth, 1993), which can affect AMF colonization (Bethlenfalvay and Dakessian, 1984; Miller et al., 1995; Lugo et al., 2003; Barto and Rillig, 2010; van der Heyde et al., 2017; Cavagnaro et al., 2018), spore abundance, and diversity (Eom et al., 2001; Lugo and Cabello, 2002; van der Heyde et al., 2017). Intense grazing can also reduce AMF hyphal length density by reducing soil moisture, increasing bulk density, and decreasing soil organic C and plant-available N (Ren et al., 2018).

Information found in the literature about how grazing affects AMF is in general variable among studies (van der Heyde et al., 2017). In the case of AMF spore abundance, it ranges from a lack of effects, to increases or decreases, depending on the study considered (Eom et al., 2001; Klironomos et al., 2004; Su and Guo, 2007; Ba et al., 2012; Bai et al., 2013; Antoninka et al., 2015; van der Heyde et al., 2017; Ambrosino et al., 2018). Van der Heyde et al. (2017) have suggested that these inconsistencies could be caused by differences in experimental designs among studies and that sporulation might depend on grazing intensity (GI). Supporting this last statement, studies that report lack of effects—or increases—in AMF spore abundance and diversity have usually tested light or moderate GI or defoliation (Ba et al., 2012; van der Heyde et al., 2017; Ambrosino et al., 2018). A moderate removal of aboveground biomass can stimulate root exudations (von Alten et al., 1993; Buee et al., 2000), which could be beneficial for AMF and, thus, spore production (Ba et al., 2012; van der Heyde et al., 2017). On the other hand, studies reporting decreases of AMF spore abundance and diversity have usually tested higher GIs (Su and Guo, 2007; Ba et al., 2012). Decrease in spore abundance has been explained by the carbon limitation hypothesis, which states that intense defoliation can affect plant carbon availability, reducing the C transferred to the AM symbiont, which can compromise resources allocation of the fungus to spore production or root colonization (Wallace, 1987). Thus, there is an increasing indication of the importance of determining the role of GI on shaping mycorrhizal communities in rangeland ecosystems worldwide (Ba et al., 2012; van der Heyde et al., 2017; Ambrosino et al., 2018).

AMF response to disturbances can vary among species and families depending on their life-history strategies and type of disturbance (van der Heyde et al., 2017). The life-history classification system based on the grouping of functional traits, such as Grime's C-S-R (competitor, stress tolerant, ruderal) framework, can help to explain AMF successional dynamics, as well as biodiversity patterns in natural communities (Chagnon et al., 2013). Species from *Glomeraceae* have been suggested to have a ruderal strategy (sensu Grime, 1977) because of their high growth rates, early production of many asexual spores, high hyphal turnover rates, more efficient hyphal healing, and more efficient germination, both from spores and hyphae (Chagnon et al., 2013). According to these traits, *Glomeraceae* are in general tolerant to most disturbance types (van der Heyde et al., 2017) and usually are dominant in disturbed

ecosystems (Su and Guo, 2007; Ba et al., 2012; Stover et al., 2012; Brundrett and Ashwath, 2013). *Gigasporaceae* seem to be competitive species and have higher carbon demands, invest more biomass in extraradical hyphae, are favored by high N-to-P environments (Chagnon et al., 2013), germinate preferably or exclusively from spores after a dormancy period (Brundrett et al., 1999), and are intolerant to aboveground disturbances like forestry, agriculture, and herbivory (van der Heyde et al., 2017). *Acaulosporaceae* species are commonly stress tolerant, as they have lower carbon demands (Gehring and Whitham, 2002) because of their low growth rates and long-lived mycelium (Chagnon et al., 2013) and are particularly tolerant to soil mechanical disturbances (van der Heyde et al., 2017). In the case of *Pacisporaceae* and *Ambisporaceae,* ecological and phylogenetic information is still lacking (Oehl et al., 2011), although they seem to be disturbance tolerant (van der Heyde et al., 2017). To our knowledge, information about the specific response of AMF families to different intensities of grazing is still scarce.

If carbon is not a limiting resource (i.e., under moderate defoliation and grazing levels), *Glomeraceae* species might tolerate grazing better than other families because of their high growth and hyphal turnover rates (Chagnon et al., 2013). AMF are particularly stressed when the carbon supply from their host is consistently low (Chagnon et al., 2013), as can occur when the HPs are intensely defoliated (Wallace, 1987). Under carbon stress conditions, successful AMF may be those that use carbon most efficiently, through the slow production of high-cost, long-lived biomass. *Glomeraceae* species with high growth rates and, therefore, high carbon demands can be affected if severe defoliation produced by intense grazing affects the resources allocated by the HP to the AMF and, thus, can reduce spore production. Consistent with the expectation of stress tolerance, members of the *Acaulosporaceae* family are more efficient in C usage than members of the *Glomeraceae* and *Gigasporaceae* (Chagnon et al., 2013) and might cope better with the carbon limitation resulting from intense grazing. *Gigasporaceae* species that have large structures (spores and soil mycelium) and the highest carbon demands may be the least tolerant to carbon stress produced by intense grazing, particularly if soil N is low (Chagnon et al., 2013). *Pacisporaceae* species were formerly classified within *Glomeraceae* because they share many similarities in morphology and traits, such as spore structure and mechanisms of production (Oehl and Sieverding, 2004). Therefore, this family might respond to grazing similarly to *Glomeraceae*, although this is not very well known yet.

In our study we aimed to assess how GI affects AMF communities in Patagonian semiarid steppes. These ecosystems have been subjected to intense and continuous grazing regimes for more than a century (Aguiar, 2005), which have significantly reduced plant diversity (Perelman et al., 1997), cover, and N and C soil pools, (Austin and Vivanco, 2006; Golluscio et al., 2009). Intense grazing, together with the limited water availability (Sala et al., 1989; Jobbágy and Sala, 2000) and poor nutrient cycling and microbial activity (Austin et al., 2004), have ultimately favored desertification processes (León and Aguiar, 1985) that affect > 90% of the area (del Valle et al., 1998; Mazzoni and Vazquez, 2009). The composition of the AMF community and influence of grazing have never been studied in the Patagonian semiarid steppe, despite the potential importance of AMF diversity for restoration of plant communities in this degraded ecosystem.

We conducted a field experiment to evaluate differences in AMF spore abundance and diversity of nongrazed (NG) versus MG and intensively grazed areas. According to Wallace's carbon limitation hypothesis (1987) and Connell's (1978) intermediate disturbance hypothesis, which states that the highest diversity is found under intermediate levels of disturbance, we predicted that:

1) Grazing effects on AMF communities will be different according to the GI: moderate grazing will not reduce AMF spore abundance but will increase diversity, while intense grazing will reduce them.

2) AMF families will show different responses to GI on the basis of their life history strategies: *Glomeraceae* and probably *Pacisporaceae* will tolerate moderate grazing better than the other families, *Acaulosporaceae* will be more successful under intense grazing conditions, and *Gigasporaceae* will be the least tolerant to intense grazing.

Methods

Study Area

The study was carried out at Río Mayo Experimental Ranch (9 895 ha) of INTA (Instituto Nacional de Tecnología Agropecuaria; in English: National Institute of Agricultural Technology) located in the Chubut province of Argentina (45°41′S, 70°16′W). Mean monthly temperatures range from 15°C in January to 1°C in July. Long-term mean annual precipitation is 150 mm (Golluscio et al., 1998), with 70% falling in autumn and winter, 18% in spring, and 12% in summer. Strong westerly winds dominate with mean annual speed values of $15 - 22$ km.h^{-1} and low humidity contents. Maximum wind speeds occur from September to January and can reach > 120 km.h^{-1}. Wind, precipitation, and temperature regimes result in high water deficits during the plant growing season (Paruelo et al., 1998). The sampling areas are representative of the semiarid steppe of the Patagonian Occidental District,

(ca. 12.7 M ha), which occupies 30% of the Patagonian phytogeographical province (León et al., 1998). The vegetation is characterized by relatively plain steppes dominated by perennial tussock grasses (32% basal cover) and shrubs (15% basal cover), with 55% of bare soil (Sala et al., 1989). Annual aboveground net primary production (ANPP) is 560 kg·ha^{-1}·yr^{-1} (Jobbágy and Sala, 2000). Soils are coarsely textured Aridisols with high gravel and pebble content throughout the soil profile (Soriano et al., 1983). The soil profile has an upper sandy texture layer (45 cm deep) with scarce soil organic matter content (0.4%) followed underneath (from 45 to 60 cm depth) by a sandy-loam texture layer containing 34.8% of CaCO$_3$ (Paruelo et al., 1988). Grass roots are concentrated in the upper soil layer until 30 cm of soil depth (Soriano et al., 1987).

Plant Species Selection

We evaluated AMF composition and spore density in the rhizosphere soil of the dominant native tussock grasses *Pappostipa speciosa* (Trin. & Rupr.) Romasch, *Pappostipa humilis* (Cav.) Romasch, *Poa ligularis* Nees ex Steud, and *Bromus pictus* Hook var. pictus (hereafter referred as *B. pictus*) (Golluscio et al.1998). According to the palatability for sheep, *Poa ligularis* and *B. pictus* are the most intensely selected and consumed grasses, while *P. humilis* is the least preferred one (Somlo et al., 1997). All the selected species show some degree of AMF root colonization in the same study area (Cavagnaro et al., 2017, 2018).

Experimental Design

In December 2006, five independent sampling areas (each one of ca. 1 ha) were carefully chosen. Within each area, three sites (each one of ca. 0.33 ha) subjected to different levels of GI were selected: one fenced to exclude large herbivores (NG), another subjected to moderate GI (MG; stocking rate: 0.1–0.3 sheep ha^1), and another subjected to intense grazing (IG; stocking rate: > 0.3 sheep ha^{-1}). The five sampling areas were considered as blocks within a complete randomized block design (as in Golluscio et al., 2009).

The selected sampling areas have similar slope, aspect, topography, and elevation. Hence, the replicates of the different NG sites were not completely comparable because they differed in the time since grazing cessation as follows: 52, 34, 23, 12, and 8 yr. Golluscio et al. (2009) found no significant differences in vegetation composition and cover or soil properties (soil organic matter, carbon and nitrogen soil pools) among these same NG sites and, therefore, it is expected that no major differences will occur in soil microbial properties. However, as van der Heyde et al. (2017) have found an effect of the time of recovery from grazing on AMF communities, this situation was considered in the data analysis to avoid confounding effects and to test for long-term effects of grazing on AMF parameters.

Because sheep selectivity and consumption of the dominant grasses vary according to their palatability (Elissalde et al., 2002), we aimed at determining if the effects on AMF communities were different depending on HP identity. In each of the five NG, MG, and IG replicates, we obtained samples for spore extraction from the rhizosphere soil of each of the four host grasses: *P. ligularis* (PL), *B. pictus (BP)*, *P. speciosa* (PS), and *P. humilis* (PH); and under bare soil patches (BS). Bare soil patches were considered because they represent an important percentage of soil cover (Golluscio et al., 2009) and are colonized underneath by roots from a mix of grass species (Leva et al., 2009). Samples were taken at the same soil depth in the upper soil layer, where the roots of the target grasses are located, and because grazing effects are usually more visible in this soil layer (Bai et al., 2013). Thus, the experimental design comprised five replicates of three GIs (NG, MG, IG) × five HP identities (PL, BP, PS, PH, BS).

Spore Isolation and Identification

AMF spores were isolated from samples of 100 g (dry weight) of soil by using the wet-sieving and decanting method of Gerdemann and Nicolson (1963). Each sample was wet-sieved and then centrifuged in sucrose gradient (Walker et al., 1982). Quantification was carried out on Petri dishes (9-cm diameter) with a gridline of 1 cm per side, under a stereoscopic microscope at 50×. Ten divisions were counted and related to the total number of spores with the method of McKenney and Lindsey (1987). For taxonomic identification, fungal spores were mounted onto slides with polyvinyl alcohol with or without Melzer reagent (Morton, 1988). Specimens were compared with original species descriptions and reference isolates described by the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi, http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html. The observation of spores was corroborated with observations of freshly formed AMF spores in trap cultures. AMF species were identified from morphological rather than molecular—rDNA sequences—features because 1) this is the first study on AMF communities in the Patagonian semiarid steppes, with the correspondence between rDNA sequences and spore morphology entirely unknown; and in our view 2) a molecular approach will result in an oversized technique for the first assessment of the general effects of grazing on AMF diversity because of the large amount of genetic variation within single species of AMF and even within a single AMF spore (Landis et al., 2004; Sanders, 2004).

For each combination of GI and plant species composition, AMF diversity was calculated by the Shannon-Wiener diversity index as follows:

$$H = -\sum_{i=1}^{k} \ln(pi)pi \tag{1}$$

where *pi* is the proportion of the spores of *i*AMF species on the spores of all *k*AMF species. This index integrates the contribution of species richness or number of species (S) and evenness (E), $E = H/ln\,[S]$, to species diversity.

Data Analysis

All data analyses were performed with the R 3.4.0 free distribution software (R Development Core Team, 2017). We calculated the spore abundance and frequency of AMF species for each GI (NG, MG, and IG) and for each HP identity (PL, BP, PS, PH and BS). Indicator species analyses were performed with the "indicspecies" package of R. We evaluated the strength of association of AMF species or families with a particular GI or HP using the function "multipatt." This analysis

calculates an extended indicator value by looking for species that are indicators of both individual site groups and combinations of site groups (De Cáceres, 2013). We also used the function "signassoc" to test the association between each AMF species or family with each site (NG, MG, or IG), regardless of whether the indicator value was the highest or not. We used mode = 0 (site-based), alternative = two.sided (to identify either positive or negative associations) and report P values after Sidak's correction for multiple testing (Dufréne and Legendre, 1997; De Cáceres, 2013).

We tested the general effects of GI, HP identity, and the two-way interaction GI × HP on AMF spore abundance, for all spores together (AMF total spore abundance [TSA]), as well as for each one of the different AM fungal families separately, because changes on spore abundance are more evident at this taxonomical level (Chagnon et al., 2013; Ambrosino et al., 2018). We also tested GI, HP, and GI × HP effects on AMF spore diversity (H), richness (S), and Evenness (E). To test GI, HP, and GI × HP effects, we built mixed-effects models for each variable analyzed. All the models included the fixed categorical variables: 1) GI with three levels (NG, MG, and IG), 2) HP with five levels (BP, PL, PS, PH and BS), and 3) the two-way interaction GI × HP.

To evaluate if the NG areas were completely comparable and to test for any effect of the time passed after grazing cessation, we built a mixed-effects model including the fixed categorical variable time of recovery from grazing (TRG) with five levels (52, 34, 23, 12, and 8 yr after grazing cessation) and HP identity to test their effect on TSA, H, richness (S), and E. We found no significant effect of TRG or HP among NG sites on any of the dependent variables analyzed (see Section 1 and Table S1; available online at https://doi.org/10.1016/j.rama.2019.02.007). Therefore, we included NG sites in the main models built for evaluating the effects of GI, as results will not be biased by variances among NG sites.

To test if GI and HP effects on spore abundance varied across families (SAM), we built another mixed-effects model adding family (FAM) as a factor with four levels (Acaulosporaceae, Glomeraceae, Pacisporaceae, and Gigasporaceae). We were unable to test GI or HP effects on Ambisporaceae and Claroideoglomeraceae because of their low spore abundance and frequency.

In every case, to avoid pseudoreplication, and even though sites were similar in topography and slope, block was added as a random variable as it might include effects coming from other noncontrolled environmental variabilities.

The general models formula was Model = Y ~ (HP + GI + HP*GI + 1/block). For generalized mixed-effects models we used the "glmer" function, and for general mixed-effects models the "lmer" function from the "lme4" package (Bates et al., 2015). In the case of TSA, SAM, and S, the errors had a Poisson distribution and, accordingly, a log link function was used (generalized models). In the case of H and E, normal distributions were used (general models) and, in case of lack of normality, data were log-transformed to fit normality. We tested overdispersion using the "RVAideMemoire" package (Hervé, 2017) and, when present, we corrected it with the addition of an observation-level random effect factor (Harrison, 2014). For mixed-effects models, Chi^2 values were calculated using the "ANOVA" (analysis of variance) function of the "car" package (Fox and Weisberg, 2011). If significant effects were found, Tukey post-hoc tests were performed using the package "multcomp" (Hothorn et al., 2008). In the case of the analysis by functional groups, post-hoc tests were applied across grazing intensities within families and across families.

Results

Characterization of AMF Communities

AMF spores isolated from the soil samples were grouped into 14 different AMF morphospecies belonging to *Ambisporaceae, Acaulosporaceae, Gigasporaceae, Pacisporaceae, Claroideoglomeraceae,* and *Glomeraceae.* All AMF morphospecies were identified at genus level and 10 at species level (Table 1). Among the most frequent species, *Pacispora scintillans* was the most dominant (frequency = 100% in each of the NG, MG, and IG sites), followed in lower dominance by *Glomus* sp.1, *Glomus* sp.2, *Funneliformis mosseae, Acaulospora mellea, Ambispora fecundispora,* and *Claroideoglomus etunicatum* (see taxonomy in Table 1).

According to their indicator value (IndVal), *Glomus sp.*2 was strongly and significantly associated with NG and MG sites (IndVal = 0.73; P = 0.011), *A. mellea* was a good indicator of MG and IG sites (IndVal =

Table 1
Characterization of arbuscular mycorrhizal fungi communities.

Order	Family	Species	NG f (%)						MG f (%)						IG f (%)					
			BP	PL	PS	PH	BS	F (%)	BP	PL	PS	PH	BS	F (%)	BP	PL	PS	PH	BS	F (%)
Archaeosporales	*Ambisporaceae*	*Ambispora fecundispora* (Schenck et Smith) Walker						0	20			20		40	20			20	20	60
Diversisporales	*Acaulosporaceae*	*Acaulospora delicata* Walker, Pfeiff. & Bloss		20				20			20		20		0						0
		A. mellea Spain et Schenck						0		40		20	20	60	40		20	40	40	80
	Gigasporaceae	*Gigaspora* sp.				20		20	20					20						0
		Scutellospora sp1.	20	20				40					20	20						0
		S. calospora (Nicolson et Gerdemann) Walker et Sanders			20			20						0						0
		S. dipapilosa (Walker et koske) Walker et Sanders					20	20				20		20						0
		S. heterogama (Nicolson et Gerd.) Walker et Sanders					20	20						0						0
	Pacisporaceae	*Pacispora scintillans* (Rose et Trappe) Sieverd. et Oehl	40	60	40	20	80	100	20	60	40	80	60	100	20	20	20	40	40	100
	Claroideoglomeraceae	*Claroideoglomus etunicatum* (Becker et Gerdemann) Walker et Schüßler						0		20	20		20	60						0
Glomerales	*Glomeraceae*	*Funneliformis mosseae* (Nicolson et Gerdemann) Walker et Schüßler		20			20	40	20	20			40	60					20	20
		Glomus ambisporum Smith et Schenck	20			20	20	60		20			20							0
		Glomus sp.1	40	40	60	40	40	100	20	60	20	20	60	100		20	20	80	40	80
		Glomus sp.2	60	80	40	80	60	100	40	40	60	80		80		20	20	20		60

Frequency of occurrence (percentage of appearance from which AMF spores of a particular species were recovered) for each host species (*f*): *Bromus pictus* (BP), *Poa ligularis* (PL), *Pappostipa speciosa* (PS), *P. humilis* (PH), and below bare soil patches (BS); and for each grazing intensity (*F*): nongrazed (NG), moderately grazed (MG), and intensely grazed (IG).

0.52; $P = 0.012$), and *C. etunicatum* tended to be associated with MG sites (IndVal = 0.361; $P = 0.05$) (see Table 1). Other associations not mentioned (among GIs or HPs with AMF species or families) were weak and not significant ($P > 0.05$).

Indicator species analyses also showed (best P value, Sidak method) that *Acaulosporaceae* and *A. mellea* were least associated with NG sites, *G. ambisporum* was better associated with NG sites, and *Glomus sp.1* was better associated with NG and MG sites while *F. mosseae, Glomus sp.2, Glomeraceae, Pacisporaceae,* and *Gigasporaceae* were least associated with IG sites (see detailed P ranks in Table S2; available online at https://doi.org/10.1016/j.rama.2019.02.007).

Grazing Effects on AMF Spore Abundance and Diversity

GI was the most significant factor affecting AMF TSA, H, and evenness (E) (Table 2). TSA, H, and E were significantly different among NG and IG sites (see Figs. 1a, 2a, c). In IG sites TSA was four times lower (see Fig. 1a) while H and E were 50% lower than in NG sites (see Fig. 2a, c). We found no significant differences among NG and MG sites in terms of AMF TSA (see Fig. 1a), H (see Fig. 2a), or E (see Fig. 2c). AMF richness (S) was not significantly affected by GI (see Table 2), being on average low for all areas (1.65 ± 0.64 S.E.) (see Fig. 2b).

We found no significant differences in AMF TSA, H, richness (S), or E among HPs in general or according to each GI (GI × HP) (see Table 2).

Differential Responses of Spore Abundance Among AMF Families and Grazing Intensities

Spore abundance varied significantly among FAMs and GIs (see Table 2, Fig. 1b − e). There was a significant interaction in GI x FAM (see Table 2), as spore abundance variance was different in magnitude and significance for each FAM and GI (see Table 2, Fig. 1b − e). *Glomeraceae* and *Pacisporaceae* were significantly affected by GI (see Table 2), with three to four times lower spore abundance in IG compared with NG sites (see Fig. 1b and c). For both families, spore abundance did not differ among NG and MG sites (Fig. 1b and c), but *Pacisporaceae* spore abundance tended to be slightly higher ($P = 0.05$) in MG sites than NG sites (see Fig. 1c). *Gigasporaceae* and *Acaulosporaceae* were not significantly affected by GI (see Table 2), but *Gigasporaceae* was completely absent in IG areas (see Fig. 1e). *Acaulosporaceae* spore abundance was the only one that did not decrease in IG sites (see Fig. 1d). *Glomeraceae* and *Pacisporaceae* showed the highest spore abundance under all GIs except in IG sites, where *Acaulosporaceae* and *Pacisporaceae* were similar (see Fig. 1b, e). In NG sites, *Glomeraceae* spore abundance was higher than *Pacisporaceae* (Fig. 1b, c). *Gigasporaceae* showed the lowest spore abundance values among all families in both NG or MG sites (Fig. 1b-e).

Spore abundance of the different families was not significantly affected by HP, and there was not a significant interaction in HP x FAM, indicating that associations among AMF families and HPs were not significant. We were unable to test the three-way interaction among GI × HP × FAM because of the characteristics of the data matrix. *Claroideoglomeraceae* was present only in MG sites with low spore abundance·100 g soil^{-1} (0.64 mean ± 0.36 S.E.), similar to *Ambisporaceae,* which was present only in MG (0.64 mean ± 0.41 S.E.) and IG sites (0.68 mean ± 0.39 S.E.).

Discussion

Grazing intensity exerted the strongest control over AMF spore abundance and diversity in the Patagonian steppes and, as predicted, intense grazing significantly reduced AMF spore abundance and diversity. Intense sheep grazing can significantly reduce aboveground biomass of the HPs (Golluscio et al., 2009). As a consequence, plants could reduce the C translocated to the root system, which can compromise the resources allocated to their AM fungal symbionts and, thus, to AMF spore production (Su and Guo, 2007; Ba et al., 2012). The low levels of AMF spore

Table 2

Statistics summary (mixed-effects models) of the effects of grazing intensity and host plant identity on arbuscular mycorrhizal fungi communities.

Variable	Factor	Chi²	P	DF	Level	Est	SE
TSA	GI	30.62	< 0.001***	2	Intercept-NG	1.86	0.42
					MG	0.32	0.56
					IG	−1.41	0.63
	HP	4.13	0.39	4			
	GI × HP	8.54	0.38	8			
H (Diversity)	GI	6.04	0.04	2	Intercept-NG	0.35	0.12
					MG	0.07	0.12
					IG	−0.28	0.11
	HP	7.61	0.11	4			
	GI × HP	12.12	0.14	8			
S (Richness)	GI	3.58	0.16	2			
	HP	7.89	0.09	4			
	GI × HP	6.11	0.63	8			
E (Evenness)	GI	8.36	0.02	2	Intercept-NG	0.25	0.19
					MG	0.14	0.26
					IG	−0.08	0.26
	HP	6.18	0.18	4			
	GI × HP	9.78	0.28	8			
SAF	GI	11.97	0.002**	2	Intercept-NG	3.61	1.52
	HP	10.54	0.06	4			
	FAM	75.12	<0.001***	3			
	GI × HP	10.58	0.22	8			
	FAM × HP	7.31	0.83	12			
	GI × FAM	13.31	0.038*	6	IG × GL	−3.81	1.27
					MG × GL	−1.86	1.84
					IG × PA	−3.75	1.32
					MG × PA	−1.24	1.26
					IG × GIG	−2.24	2.00
					MG × GIG	−2.46	1.40
Glomeraceae	GI	14.01	0.016*	2	Intercept-NG	1.72	0.24
					MG	−0.54	0.35
					IG	−1.58	0.38
	HP	9.21	0.33	4			
	GI × HP	9.89	0.18	8			
Pacisporaceae	GI	9.41	0.09	2	Intercept-NG	−0.35	0.23
					MG	−0.53	0.58
					IG	−1.27	0.53
	HP	5.74	0.24	4			
	GI × HP	4.50	0.81	8			
Acaulosporaceae	GI	0.11	1.00	2			
	HP	0.26	1.00	4			
	GI × HP	0.06	1.00	8			
Gigasporaceae	GI	0.51	0.92	2			
	HP	0.91	0.97	4			
	GI × HP	0.10	1.00	8			

Chi square (Chi²), P values, level of significance (***0.001, **0.01, *<0.05, and degrees of freedom (DF) of the effects of grazing intensity (GI), host plant identity (HP), and two-way interaction HP × GI on AMF total spore abundance (TSA), diversity (H), richness (S), evenness (E), spore abundance within each AMF family (*Glomeraceae, Pacisporaceae, Acaulosporaceae,* and *Gigasporaceae*), and across AMF families (SAF). SAF also includes the effects of the factor AMF family (FAM), GI × FAM, and HP × FAM. Model parameter estimates (Est) ± (SE) are presented for each factor level (if factor significant). If significant interactions were found, parameter estimates are presented only for the interaction levels. GI levels include intensive grazing (IG) and moderate grazing (MG). FAM levels include *Glomeraceae* (GL), *Pacisporaceae* (PAC), and *Gigasporaceae* (GIG).

abundance and diversity found under intense grazing support these assumptions. AMF diversity did not increase with moderate grazing but was maintained with this type of management. Stimulation of the amount of carbon translocated to the roots by moderate defoliation can benefit AMF (von Alten et al., 1993) and might help explain why AMF spore abundance and diversity did not decrease with moderate grazing (and why *Pacisporaceae* spore abundance tended to increase). Our results can provide an explanation for the contrasting results found in the literature about the effects of grazing on AMF, confirming that AMF spore community varies with GI, as suggested by other authors (Ba et al., 2012; van der Heyde et al., 2017; Ambrosino et al., 2018).

For each GI, the responses of the different AMF functional groups (families) varied according to their life-history strategies. *Glomeraceae* and *Pacisporaceae* were the most dominant families in the entire grazing gradient. Most *Glomeraceae* species seem to have ruderal strategies
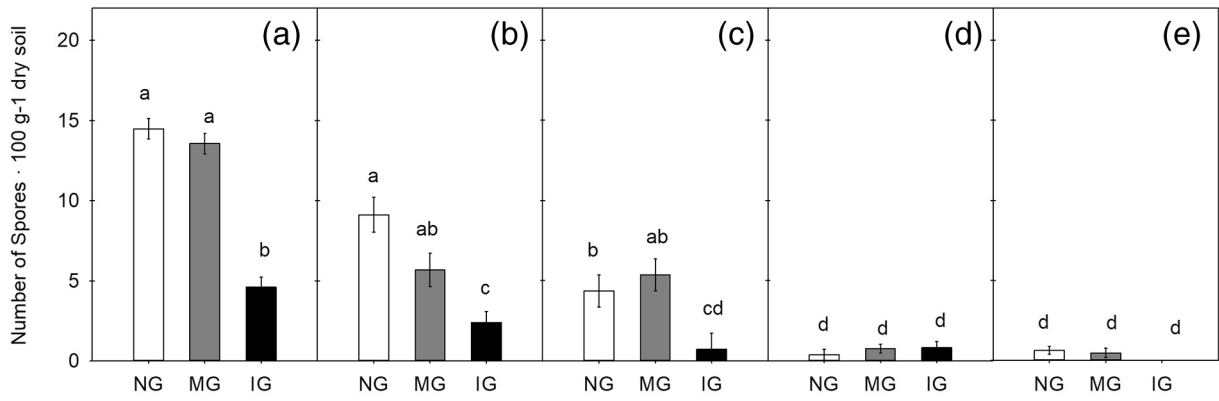
Figure 1. Main effect of grazing intensity (GI) on arbuscular mycorrhizal fungi (AMF) spore density. Number of spores·100 g^{-1} of rhizosphere soil (mean ± S.E.) of (**a**) AMF total spore abundance (TSA), (**b**) *Glomeraceae*, (**c**) *Pacisporaceae*, (**d**) *Acaulosporaceae*, and (**e**) *Gigasporaceae* in nongrazed (NG), moderately grazed (MG), and intensely grazed (IG) sites (*n* = 75). In (**a**) different letters indicate significant differences in TSA among GI and from (**b**) to (**e**) indicate significant differences within and across AMF families and among GI (after Tukey post-hoc analysis, *P* < 0.05).

(Chagnon et al., 2013), which explain their dominance along disturbed areas (Longo et al., 2016), ranging from steppes (Su and Guo, 2007; Ba et al., 2012), prairies (Stover et al., 2012), and woodlands to wetlands and rainforests (Brundrett and Ashwath, 2013). In the case of

Figure 2. Main effect of grazing intensity (GI) on diversity, richness, and evenness. Arbuscular mycorrhizal fungi (**a**) Shannon-Wiener diversity index, (**b**) species richness, and (**c**) species evenness (mean ± S.E.) in nongrazed (NG), moderately grazed (MG), and intensely grazed (IG) sites (*n* = 75). Different letters indicate significant differences among GI (after Tukey post-hoc analysis, *P* < 0.05).

Pacisporaceae, their ecology is not well known yet (Oehl et al., 2011), but the dominance of *Pacisporaceae* in the entire grazing gradient suggests that this family has adapted to the poor nutrient- and water-deficient soils of Patagonian steppes. The lack of effects of moderate grazing on *Pacisporaceae* spore abundance supports the prediction that this family would respond to grazing, similarly to *Glomeraceae*. The fact that these two families share strong similarities in spore morphology and type of production (Oehl and Sieverding, 2004) might indicate that *Pacisporaceae* also presents ruderal strategies, which remains to be studied. The reduction of *Glomeraceae* and *Pacisporaceae* spore abundance in IG sites supports our prediction that AMF families characterized by fast growth rates and high carbon demands (Chagnon et al., 2013) might be more sensitive to the carbon limitation produced by intense grazing. According to our expectations, A*caulosporaceae,* which is in general characterized by low growth rates and, thus, is more efficient to cope with carbon limitation (Chagnon et al., 2013), was more successful under intense grazing than the other families. *Gigasporaceae* species that have a high carbon demand because of their bigger structures compared with other families and are sensitive to N limitation (Chagnon et al., 2013) showed a low spore abundance in all sites, probably because of the low N:P ratio of Patagonian steppe soils. This N limitation, together with the carbon limitation produced by severe defoliations, might explain why *Gigasporaceae* species were completely absent from IG sites. Given the low spore frequency, abundance, and richness of *Claroideoglomeraceae* and *Ambisporaceae,* we were unable to determine their response to grazing, but *Claroideoglomus etunicatum* was a relatively good indicator species of MG sites.

The responses of different AMF FAMs according to their life-history strategies (Chagnon et al., 2013) can help explain the change in spore abundance and diversity in each GI. If intense grazing persists, few species of *Glomeraceae, Pacisporaceae,* and *Acaulosporaceae* would dominate—though in low abundance—while *Gigasporaceae* would tend to disappear from the ecosystem. Considering that *Glomeraceae* and *Pacisporaceae* were the most diverse, frequent, and abundant families in NG and MG areas and that intense grazing significantly reduces them, this type of management would imply a significant reduction of AMF diversity in the ecosystem.

The lack of effects of HPs on AMF spore abundance suggests that there is not a strong preference among AMF species and the different host grasses. Also, the fact that in each GI AMF spore abundance was not significantly different among HPs indicates that grazing effects were independent of the sheep preference of the different host grasses studied. These results suggest that grazing effects on AMF symbioses can occur at plant community level. This assumption is supported by the significant reduction of AMF root colonization of both palatable and unpalatable host grasses, which occurs in response to intense
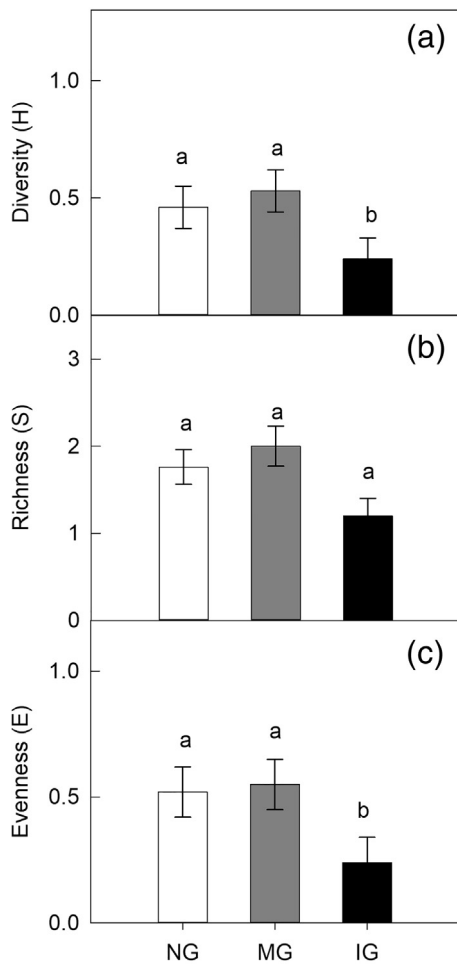
sheep grazing (Cavagnaro et al., 2018). There is considerable evidence that plants can be linked into common mycorrhizal networks and that N, P, and C transfer can occur from one plant to another through AMF hyphae (Johnson et al., 2001; Smith and Read, 2008; Ren et al., 2013). Therefore, intense grazing might have affected the C transfer in the whole mycorrhizal network, independently of any specific effect on the individual host plants.

Our results highlight the importance of the role of GI in shaping AMF communities and the negative consequences that grazing mismanagement can have on the ecosystem. If continuous and intense grazing persist and AMF abundance and diversity is reduced, benefits for plants, such as improved nutrient and water uptake (Hodge et al., 2001; Karandashov and Bucher, 2005; Javot et al., 2007; Parniske, 2008; Smith and Read, 2008) and soil aggregation (Rillig and Mummey, 2006; Lehmann et al., 2017) could be lost. Some functional groups provide particular benefits for their host plants. *Glomeraceae* can provide better protection of the hosts plants against herbivores, and *Gigasporaceae* is more efficient in nutrient and water uptake (Chagnon et al., 2013). As intense grazing significantly reduces *Glomeraceae* abundance and considering that *Gigasporaceae* are absent under this situation, their particular benefits for the host plants would also be reduced or lost. Exclusion of AMF from the soil could be also relevant because it reduces the rate of organic matter decomposition by reducing the substrate supply to free-living decomposers (Averill et al., 2014), contributing to constrain nutrient cycling. As AMF benefits are particularly important under conditions of limited nutrient and water availability (Parniske, 2008), the loss of AMF abundance and diversity can be critical for ecosystem functioning.

We conclude that maintaining high grazing pressures under continuous regimes will continue to reduce the abundance and diversity of AMF communities, contributing to the ongoing ecosystem degradation of Patagonian steppes.

Implications

Grazing regime is important to regulate the effect of grazing on plant communities (Zhang et al., 2018), and our results suggest that it would also be key to control the effects on AMF. Our findings indicate that MG could not only improve vegetation cover and soil nutrient content (Golluscio et al., 2009) but also maintain AMF diversity. Other types of adaptive management like seasonal grazing (with inclusion of periodic resting periods) could increase aboveground production of palatable plant species (Golluscio et al., 2015; Zhang et al., 2018), which would probably benefit both herbivores and AMF. Restoration and maintenance of AMF diversity can increase the positive effects on plant diversity and productivity and, consequently, on ecosystem structure and functioning, particularly in degraded rangelands like Patagonian steppes.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rama.2019.02.007.

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