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Environmental Conditions and Grazing Exerted Effects on Arbuscular Mycorrhizal in Plants at Southern Patagonia Rangelands[☆]

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi play a major role in maintaining ecosystem functions. AM fungi are found in most ecosystems including rangelands currently under increasing pressures from human activities. Southern Patagonia (Argentina) is a semiarid region influenced by extensive livestock production in rangelands. There is a lack of information about the environmental conditions and functionality of AM fungi in plant species of Patagonia ecosystems associated with livestock grazing. We assessed how soil properties, climatic conditions, and grazing intensities influence the response of AM fungi colonization. We studied most palatable and representative plant species (*Poa dusenii, Rytidosperma virescens, Festuca gracillima, Nardophylum bryoides, Mulguraea tridens*, and *Carex argentina*) growing in main ecological areas of Southern Patagonia. Most of the studied plant species (except C. argentina) presented AM symbiosis. AM colonization showed a negative relationship with soil organic carbon and nitrogen and a positive relationship with soil bulk density and pH. Results suggest that plants promoted a higher root AM colonization when soil nutrients and water availability (rainfall) are limiting. Sheep stocking rates had a differential impact depending on the ecological areas and plant life forms. High grazing decreased the AM colonization in the ecological areas with palatable plant dominance, suggesting that the impacts of grazing could lead to further negative effects on the ecosystem. Moderate grazing allows to maintain higher AM colonization, which would probably benefit the aboveground production of palatable plant and, consequently, to herbivores, particularly in degraded rangelands like Patagonian steppes. This study improves the knowledge of AM association in Patagonian semiarid rangelands by increasing our understanding of the impacts of grazing on belowground ecology. This information becomes relevant for grazing sustainable management, which may contribute to food security.

Introduction

Mycorrhizal fungi are root symbionts that associate with ~90% of plant species in terrestrial ecosystems (Smith and Read 2008). Arbuscular mycorrhizal (AM) fungi are the most abundant types, forming symbiosis with 71—80% of vascular plants (Smith and Read 2008; Brundrett and Tedersoo 2018). Mycorrhizae contribute to plant nutrient and water uptake, soil formation processes (nutrient cycles and promoting C sequestration) and ecosystem productivity (Brundrett 2004; Smith and Read 2008; Mohan et al. 2014). Tedersoo et al. (2020) highlighted that mycorrhizal fungal hyphae networks connect the root systems of different plants (including different taxa and life forms) and regulate the flow of nutrients and the competitive interspecific and intraspecific interactions of plants in the ecosystem.

Southern Patagonia (Santa Cruz province, 46—52°S) is characterized as mostly an arid region, where the plant community structure varies according to rainfall gradient, soil type, and topographic variation (Jobbágy et al. 2002; Bisigato and Bertiller 2004; Peri et al. 2016). The arid environments of Southern Patagonia are constituted by 85% steppe grasslands, mainly characterized by the presence of native species such as tussock (*Festuca* spp., *Stipa* spp.), short grasses (*Poa* spp., *Rytidosperma* spp.), sedges (*Carex* spp., *Juncus* spp.), dwarf shrubs (*Nardophylum* spp., *Empetrum* spp.), and shrubs (*Berberis* spp., *Mulguraea* spp.) (Peri et al. 2013).

There are studies of AM host plant species throughout South America (Pagano and Lugo 2019), moreover in Northern Patagonian forests and steppe (Fontenla et al. 2001; Fernández et al. 2010;

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Velázquez et al. 2016; Dudinszky et al. 2019). However, there is a lack of information about the interaction and functionality of AM in the dominant plant species of Southern Patagonia ecosystems.

The different environmental conditions (rainfall and/or temperature) affect microorganisms such as mycorrhizal fungi that are associated with plants (Miller et al. 2012; Deveautour et al. 2018). Some studies have reported that soil moisture conditions affect AM fungi directly or indirectly by influencing plant root development or the soil nutrient cycles (Nouri et al. 2014; Deepika and Kothamasi 2015; Li et al. 2015). The physical-chemical soil properties that regulate the development of AM fungal communities have been reported (Tuomi et al. 2001; Mohammad et al. 2003; Hoeksema et al. 2010).

Mycorrhizal fungi receive plants' photosynthates as a source of energy and carbon (both from within the root or root exudates) (Bethlenfalvay et al. 1985; Smith and Read 2008; Barto and Rillig 2010). In addition, anthropic factors such as livestock grazing on natural rangelands affect plant productivity and AM fungi (Eldridge et al. 2016; Jiang et al. 2017; Fan et al. 2019a). Extensive livestock production is the main activity in Southern Patagonia rangelands (2.7 million sheep), where livestock varies from 0.13 to 0.75 heads ha^{-1} yr^{-1} depending on the ecological areas and use intensity (Peri et al. 2013). Thus, AM fungi are affected by livestock grazing, which has been informed across different types of rangelands (Barto and Rillig 2010; Van der Heyde et al. 2017; Yang et al. 2020). While some studies reported a decrease in AM colonization caused by grazing in semiarid grasslands (Bethlenfalvay and Dakessian 1984; Bethlenfalvay et al. 1985; Ren et al. 2018), other studies showed an increase (Hokka et al. 2004; Kula et al. 2005). These contrasting results in AM symbiosis are attributable to different effects of livestock grazing on the rangelands, where grazing impacts through plant defoliation (which reduces or increases the ability to supply carbohydrates to the roots), soil compaction, and animal feces and urine deposition (Bethlenfalvay and Dakessian 1984; Mikola et al. 2009; Barto and Rillig 2010). Gehring and Whitham (2002) found by studying more than 35 plant species that herbivory affects mycorrhizal colonization depending on plant life forms (annual, biannual or perennial, herbaceous or woody) by reallocating resources to the new tissue growth (Hawkes and Sullivan 2001; Davison et al. 2020; Tedersoo et al. 2020).

In this context, the objective of the present study was to determine the AM symbiosis in native plant species (grasses, dwarf-shrubs, and shrubs) in Southern Patagonia (Argentina) under different grazing intensities and environmental conditions present in the main ecological areas (Mata Negra Thicket, Dry Magellanic steppe, and Humid Magellanic steppe). The hypotheses were 1) AM fungal colonization will have a positive correlation with unfavorable environmental conditions (soil properties and climatic characteristics); 2) AM fungal symbiosis will have a negative relationship with high stocking rates; and 3) AM colonization responses to stocking rates will depend on the environmental characteristics and plant life forms (because grasses are more sensitive than dwarf-shrubs and shrubs).

Materials and Methods

Sampling study sites

This investigation was done in an East-West gradient that covered different vegetation communities and climatic conditions in the South of Santa Cruz province, Argentina, representing the natural grassland ecosystems of this region (Fig. 1). Six study sites were established in three ecological areas: Mata Negra Thicket (MMT), Dry Magellanic Steppe (DMS), and Humid Magellanic Steppe (HMS), where two long-term (> 50 yr) grazing intensities (moderate and high) were contrasted in each ecological area. The sites' soil types are related to Arenosols (HMS) and Durisols/Calcisols (MMT and DMS) (Soil Survey Staff 2010). Sheep stocking rates varied between 0.15 and 1.20 ($ewe{\bullet}ha^{-1}{\bullet}yr^{-1}$) depending on the ecological area (Table 1). In each site, three random replicate plots of 1 000 m^2 were established to capture variability of each environment.

The climatic variables (precipitation and temperature) that describe each study site were obtained from Worldclim (Hijmans et al. 2005). Mean annual temperature was similar between the ecological areas and ranged from 6.2°C (MMT) to 6.5°C (DMS and HMS). Mean annual precipitation varied from 150 to 258 mm yr^{-1} in MMT and HMS, respectively (Table 2).

Soil characteristics

In each site, a total of nine (3 per plot) composite soil samples (10 subsamples per plot) were randomly collected from the top soil (0–5 cm) using soil auger cores (5.6 cm in diameter), after removing the surface litter. Soil samples were placed in plastic bags and stored in refrigerated containers until transport to the laboratory.

The following methods were used for soil characteristics determination: The soil texture was determined through the Bouyoucos densimeter method and the sieving of the sand fractions (Gee and Bauder 1986). The pH of soils was determined in 1:2.5 water soil extract. Soil bulk density was estimated using a cylindrical core method ($n = 3$) by collecting a known volume of soil (intact core) and determining the weight after drying (Blake and Hartge 1986). Measurements of soil organic carbon (SOC) concentration were derived from the dry combustion (induction furnace) method, using the conversion factor of 0.58 (Peri et al. 2018); Kjeldahl digestion for soil total nitrogen (N) (Bremner and Mulvaney 1983); Olsen for soil available phosphorus (P) (Olsen 1954); and ammonium acetate extraction for potassium (K) with a plasma emission spectrometer (Shimadzu ICPS-1000 III, Kyoto, Japan).

Vegetation characteristics and plant species sampling

At each site, and within each 1 000 m^2 plot ($n = 3$), a 50 × 1 m wide transect was established for vegetation characterization and to determine the most dominant native plant species (> 10% landcover) representative in sheep diet (Borrelli and Oliva 2001; Manero et al. 2006; Andrade et al. 2015).

For the AM evaluation, two plant samples were collected during spring (November–December) of 2016 and 2017, which is coincident with the peak plant growing season. In each transect, nine individuals per plant species and life forms (grasses, sedges, dwarf-shrubs, and shrubs) were selected. Plant communities differ in their strategies to cope with limited resources. While shrubs with a deep root system use resources (water and nutrients) mostly from lower soil layers, grasses, sedges, and dwarf-shrubs mainly use resources from upper soil layers (Soriano and Sala 1984; Reyes and Aguiar 2017).

This generated a total of ~400 sampled plants (3 ecological areas × 2 grazing intensities × 2 sampling years × 3–4 different life form species × 9 plants per species). While whole individuals of grasses (having similar sizes) were extracted, only fine roots (volume ~100 mL) of dwarf-shrubs and shrubs were collected. The samples were then placed in labeled plastic bags, stored at 4°C, and processed in the laboratory within 48 h of collection.

AM occurrence and quantification

Sampled roots, separated from the aerial part of the plant, were rinsed carefully with running water to clean and remove the soil and extra roots and then preserved in 70% alcohol. For the AM colonization, the fine roots (< 2 mm) were staining by the method

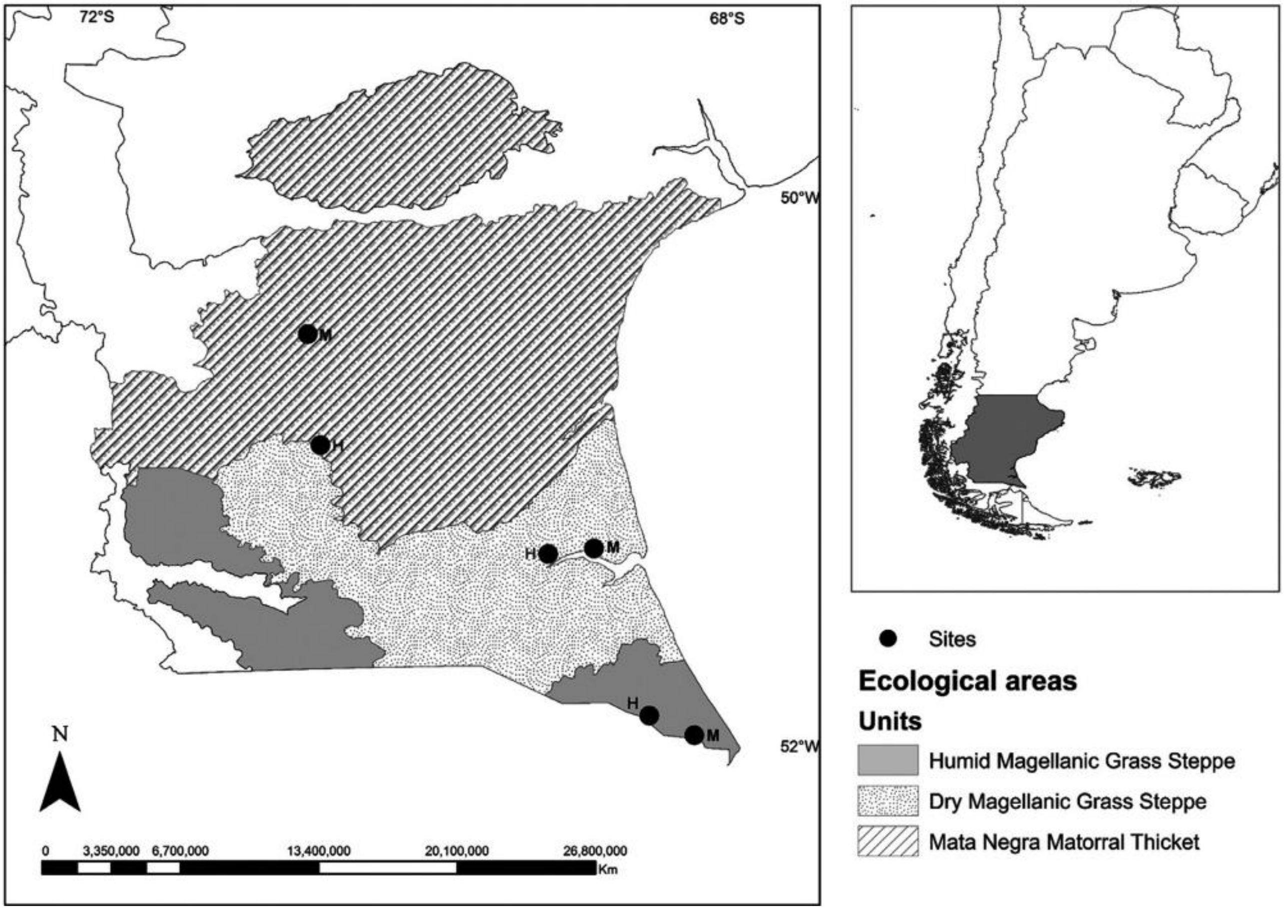

Fig. 1. Sampling schemes on the Southern Patagonia, Argentina, showing the three ecological areas studied and contrasting sheep stocking rates: moderate *(M)* and high *(H)* in each of the ecological areas.

Table 1
Average long-term grazing (> 50 yr) measured in sheep stocking rates (±standard deviation) for each of the sampling sites in the ecological areas in Southern Patagonia, Argentina (Peri et al. 2013).

Livestock land use	MMT		DMS		HMS	
	Moderate	High	Moderate	High	Moderate	High
Animal stocking[1] (ewe·ha^{-1}·yr^{-1})	0.32 (±0.06)	1.20 (±0.40)	0.13 (±0.02)	0.52 (±0.22)	0.21 (±0.05)	0.62 (±0.16)

[1] Average annual requirement of a 49 Kg live weight sheep in service, shorn in September, which gestate and wean a 20 Kg live weight lamb at 100 d of lactation and which has a consumption of 530 Kg DM·yr^{-1}. MMT incidates Mata Negra Thicket; DMS: Dry Magellanic Steppe; HMS: Humid Magellanic Steppe.

Table 2
Climatic variables for the sites of the ecological areas in Southern Patagonia, Argentina.

Ecological areas Grazing intensities	MMTDMS				HMS	
	Moderate	High	Moderate	High	Moderate	High
Climatic variables[1]						
MT annual	6.2	6.2	6.5	6.5	6.5	6.4
MT of warmest quarter	11.4	11.1	11.2	11.3	10.3	10.4
MT of coldest quarter	0.4	0.7	0.9	0.9	2.4	2.1
MP annual	150	164	231	217	255	258
MP of warmest quarter	35	42	76	71	79	81
MP of coldest quarter	40	42	49	45	59	58

[1] Data obtained from WorldClim (http://www.worldclim.com, Hijmans et al. 2005).MMT indicates Mata Negra Thicket; DMS, Dry Magellanic Steppe; HMS, Humid Magellanic Steppe; MT, mean temperature; MP, mean precipitation.

described by Phillips and Hayman (1970). AM occurrence and colonization were determined using the mycorrhizal structures: hyphae, arbuscules, and/or vesicles (Smith and Read 2008). Next, the method described by McGonigle et al. (1990) was followed, under optical microscope (Olympus BX40) at × 200 magnification. A total of three preparations ($n = 3$ replicates) were mounted, representing ~30 cm of roots, allowing 300 microscope observation points per sample. The criterion for confirmation of AM was the presence of at least one arbuscules per individual plant sample. Typical mycorrhizal structures were documented by taking images with a digital camera (Sony ExwaveHAD) and Image-Pro Plus 4.1.0.0 analysis software for Windows.

Table 3
Soil physical-chemical characteristics of the ecological areas of Southern Patagonia, Argentina with contrasting long-term (> 50 yr) grazing intensities. Mean values are shown. Different letters indicate significant differences between areas ecological and grazing intensity ($P < 0.05$)

Ecological areas	MMT		DMS		HMS	
Grazing intensities	Moderate	High	Moderate	High	Moderate	High
Soil parameter						
Textural class	Sandy Loam	Sandy Loam	Sandy	Sandy	Sandy Loam	Sandy
Bulk density ($g\cdot cm^{-3}$)	1.15 bc	1.21 c	0.96 ab	1.07 bc	0.80 a	0.82 a
pH	5.98 b	5.60 ab	5.60 ab	5.57 ab	5.20 a	5.16 a
SOC ($g\cdot kg^{-1}$)	12.70 a	16.70 ab	26.70 bc	17.10 ab	30.30 c	38.30 c
N ($g\cdot kg^{-1}$)	1.10 a	1.30 ab	1.80 abc	1.30 ab	2.60 c	2.40 bc
P available ($g\cdot kg^{-1}$)	0.025 b	0.025 b	0.012 a	0.012 a	0.016 ab	0.018 ab
K ($g\cdot kg^{-1}$)	0.160 c	0.086 ab	0.305 d	0.063 a	0.125 b	0.082 ab

MMT indicates Mata Negra Thicket; DMS, Dry Magellanic Steppe; HMS, Humid Magellanic Steppe.

Table 4
Classification and distribution of dominant native plants with consumption preference by sheep used to study arbuscular mycorrhizal (AM) symbiosis in the ecological areas of Southern Patagonia Argentina.

Family	Genus and species[1]	Life form	Forage[2] preference (%)	Location	AM (%)[3]
Poaceae	*Poa dusenii* Hack.	Grass	~ 12	MMT DMS HMS	5.5 ± 2.8 a
Poaceae	*Rytidosperma virescens* (E. Desv.) Nicora var. virescens	Grass	~ 8	DMS HMS	30.3 ± 9.5 b
Cyperaceae	*Carex argentina* Barros	Sedge	~ 7	MMT DMS	NM[4]
Asteraceae	*Nardophyllum bryoides* (Lam.) Cabrera	Dwarf-shrub	~ 5	MMT DMS	49.7 ± 11.4 c
Verbenaceae	*Mulguraea tridens* (Lag.) N. O'Leary & P. Peralta	Shrub	~ 3	MMT	41.2 ± 6.2 c
Poaceae	*Festuca gracillima* var. *Glacialis*	Grass	~ 2	MMT DMS HMS	27.9 ± 14.3 b

[1] Classification by Anton et al. (2012).
[2] Importance in the sheep diet taken from Manero et al. (2006) and Andrade et al. (2015).
[3] AM colonization in the native plant species. Means values and standard deviations (±) are shown. Different letters indicate significant differences ($P < 0.05$) between the plant species.
[4] NM indicates nonhost plant.MMT indicates Mata Negra Thicket; DMS, Dry Magellanic Steppe; HMS, Humid Magellanic Steppe.

Statistical analysis

Statistical analyses were performed using INFOSTAT software (Di Rienzo et al. 2013). AM colonization data were analyzed by nonparametric Kruskal-Wallis test. Significant differences between means were separated in all cases by Tukey's test with a significance level of $P < 0.05$. Only host plant species were considered to determine the mycorrhizal colonization; the nonmycorrhizal (NM) species was not included in the comparative statistical analyses.

General analysis of dataset

To study the ecosystem of Southern Patagonia and its relationship to AM colonization, all host plant species sampled were used. Analysis of variance was performed with multifactorial analysis of variance, using plant species (*Poa dusenii, R. virescens, F. gracillima, Nardophylum bryoides,* and *Mulguraea tridens*); ecological areas (MMT, DMS, and HMS); grazing intensities (moderate and high); and yr (2016 and 2017) of measurement as factors. To analyze all factors together (soil physicochemical variables [pH, bulk density, SOC, N, P, and K], climatic variables [precipitation and temperature], and AM colonization of all the host plants in the three ecological areas studied [MMT, DMS, and HMS]), a principal component analysis (PCA) was used. Pearson's correlation coefficients between soil variables (pH, bulk density, SOC, N, P, and K) and AM colonization of the studied host species were determined. Relationships between AM colonization and soil nutrients were established by simple linear regressions; the dependent variable was the AM colonization of all analyzed host species, and the independent variables were SOC, N, P, and K.

Grazing intensities comparison of the AM colonization

In each ecological area, the response of the AM colonization to the livestock stocking rates factor with its two levels (moderate and high) was analyzed; all the plant species sampled within each ecological area were considered.

Within each ecological area, AM colonization for each native plant species was examined. We analyzed the effect of the livestock stocking rates factor with its two levels (moderate and high) in the plants.

Results

Soil characteristics

The MMT presented a sandy-loam soil texture, while the DMS and HMS steppes presented a sandy texture. The pH values were slightly acidic, decreasing from 5.98 in the MMT to 5.16 in the HMS. Soil bulk density values were lower in HMS compared with the other ecological areas. In contrast, SOC and soil N content were higher in the HMS, followed by DMS and MMT. Additionally, soil P content was higher in the MMT. Soil K varied between the different ecological areas and sheep stocking rates (Table 3).

Plant species and mycorrhizae

The dominant and most representative plant species consumed by sheep in Southern Patagonian ecosystems were *P. dusenii*, *R. virescens,* and *F. gracillima* (grasses); *Carex argentina* (sedge); *N. bryoides* (dwarf-shrub); and *M. tridens* (shrub). Shrub and dwarf-shrub species are less preferred in the sheep diet than grasses from the Poaceae family, except *F. gracillima* (Table 4). The presence of dominant plant species varied between ecological areas: the shrub *M.*

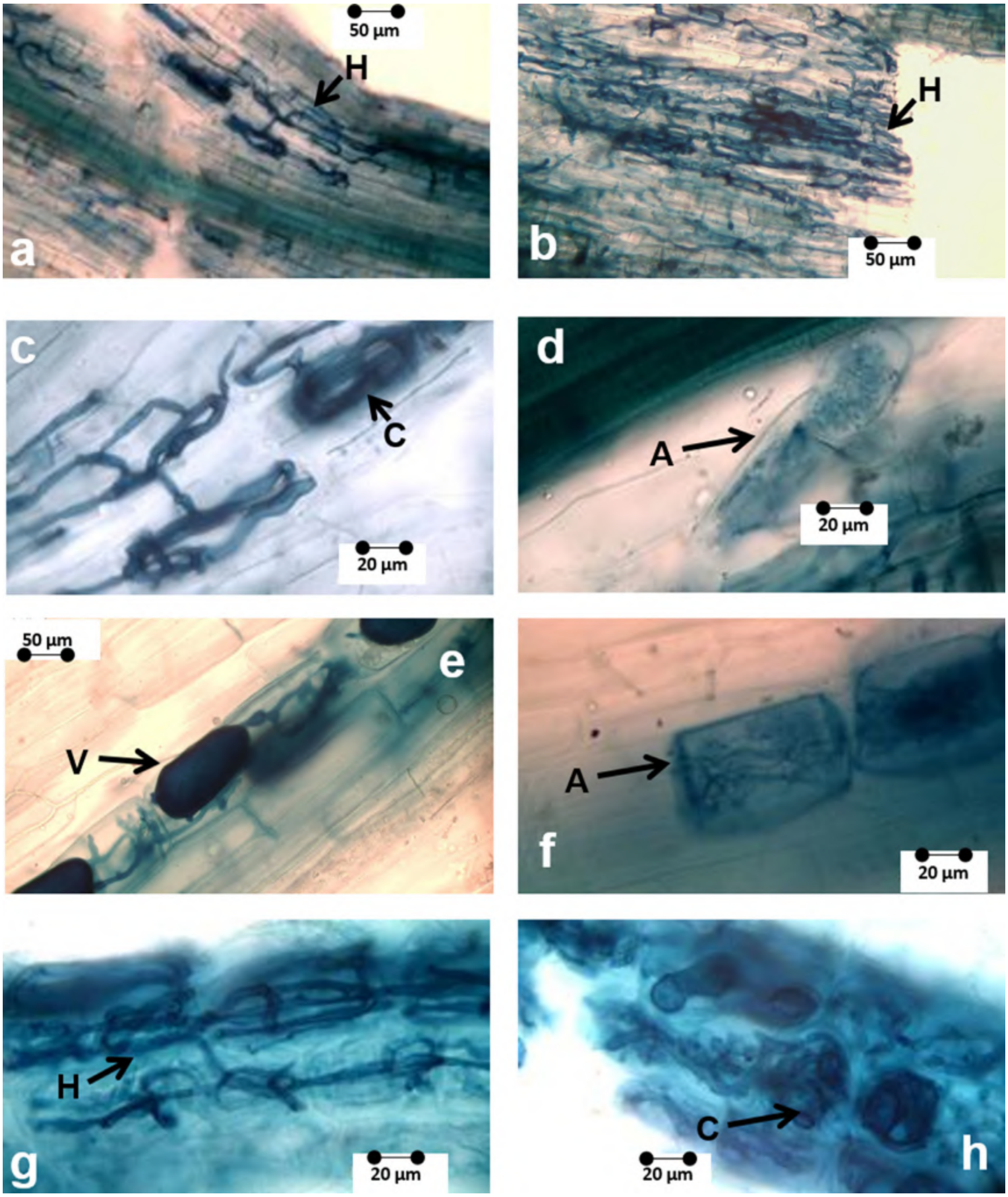

Fig. 2. Different microscopic intraradical structures of arbuscular mycorrhizal (AM) fungi in plants of Southern Patagonia, Argentina: *Mulguraea tridens* (**a, b**); *Poa dusenii* (**c**); *Nardophyllum bryoides* (**d, e**); *Rytidosperma virescens* (**f, g**); and *Festuca gracillima* (**h**). The structures of AM fungi are indicated with *arrows* as *(H)* intraradical hyphae, *(C)* coils, *(V)* vesicles, and *(A)* arbuscules, with scales of 50 μm (**a, b, e**) and 20 μm (**c, d, f, g, h**).

tridens was dominant in MMT (150 mm•yr^{-1} rainfall); the dwarf-shrub *N. bryoides* was in MMT and DMS; while in the HMS (255 mm•yr^{-1}) there was a dominance of herbaceous grass species *P. dusenii, R. virescens,* and *F. gracillima* (see Table 4).

AM fungi colonization values varied according to plant species from 5% to 50%, except *C. argentina*, which was nonmycorrhizal (see Table 4). All plant species presented the different fungal structures characteristic of AM colonization, such as intracellular hyphae, vesicles, coils, and arbuscules (Fig. 2).

General analysis of AM

AM colonization in the roots of evaluated plant species in Southern Patagonia ecosystems showed an overall mean of ~30% (Table 5). There were significant differences in AM colonization between the different ecological areas ($P = 0.0023$), with an increasing gradient DMS > MMT > HMS. DMS is significantly different compared with HMS. AM colonization for all sites and plant species analyzed showed no significant differences between sampling yr 2016 and 2017 and between moderate and high sheep stocking rates (see Table 5). There were significant interactions between ecological areas and grazing intensities, ecological areas and plant species, and between plant species and grazing intensities (see Table 5).

The results of the principal components analysis (Fig. 3), including soil, climate, and AM colonization variables, determined that the first three axes explained 88% of the total variance of the samples (55.3%, 22.4%, and 10.3% for PC1, PC2, and PC3, respectively). In the analysis of axes 1 and 2 of the PCA, there was a clear separation between MMT and HMS. The MMT area was explained by

Table 5
General analysis of Southern Patagonia ecosystems considering the arbuscular mycorrhizae according to the ecological areas, yr, and contrasting long-term (> 50 yr) sheep livestock; analyzed with Kruskal-Wallis. Means values and standard deviations (±) are shown. Different letters indicate statistical significance with a $P < 0.05$ test used for all factors.

Factors analyzed	No. of total individuals (Plant species[1])	AM colonization (%)	H	*P* Value
Ecological areas[2]			12.18	0.0023
MMT	126 (Pd, Fg, Nb, Mt)	28 ± 18 ab		
DMS	144 (Pd, Rb, Fg, Nb)	33 ± 20 b		
HMS	108 (Pd, Rb, Fg)	24 ± 15 a		
Yr			1.05	0.3047
2016	180 (Pd, Fg, Rb, Nb, Mt)	29 ± 18 a		
2017	180 (Pd, Fg, Rb, Nb, Mt)	28 ± 19 a		
Grazing intensities			3.35	0.0671
Moderate	189 (Pd, Fg, Rb, Nb, Mt)	30 ± 18 a		
High	189 (Pd, Fg, Rb, Nb, Mt)	27 ± 19 a		
Interactions				
Ecological areas • Grazing intensities			16.69	0.0051
Ecological areas • Plant species			306.55	< 0.0001
Grazing intensities • Plant species			264.30	< 0.0001

[1] Plant species present in each ecological area. Mt indicates *Mulguraea tridens;* Pd, *Poa dusenii;* Nb, *Nardophyllum bryoides;* Rb, *Rytidosperma virescens;* Fg, *Festuca gracillima.*
[2] MMT indicates Mata Negra Thicket; DMS, Dry Magellanic Steppe; HMS, Humid Magellanic Steppe.

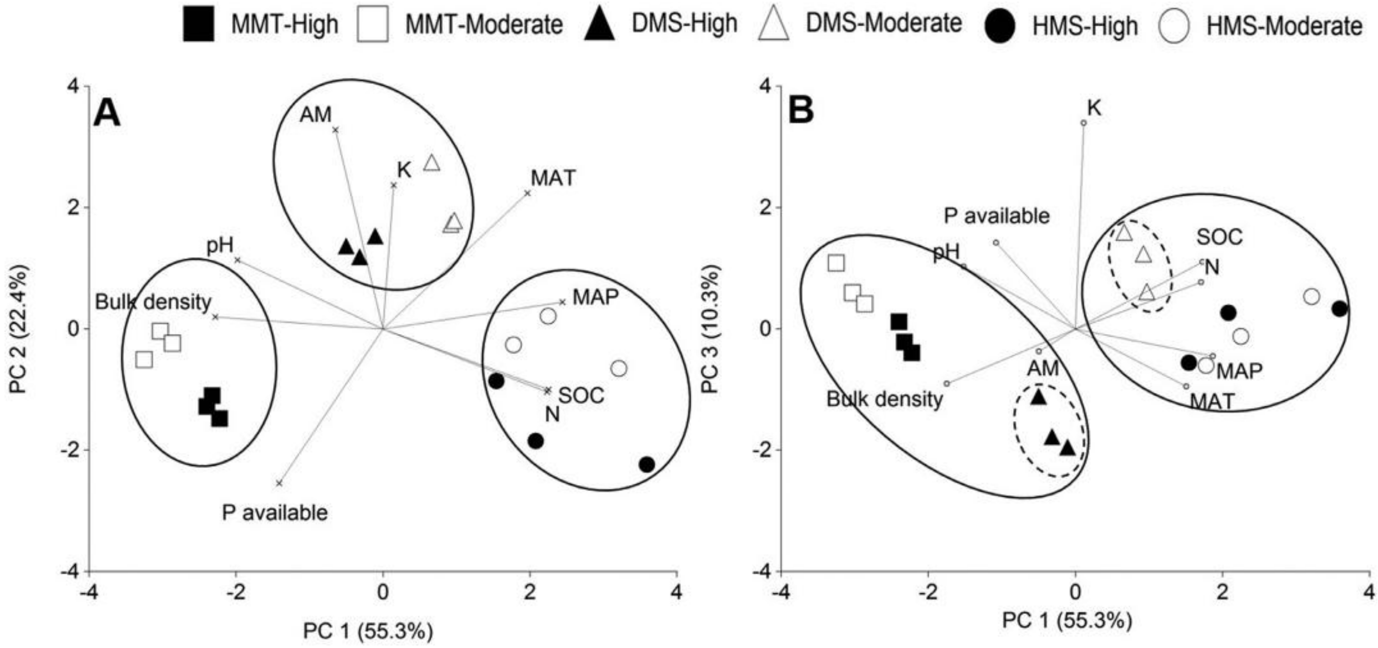

Fig. 3. Principal components analysis including arbuscular mycorrhizal, mean annual precipitation, mean annual temperature, and soil physical-chemical characteristics (*pH, bulk density, SOC [soil organic carbon], N, P,* and *K*) for the ecological areas (MMT indicates Mata Negra Thicket; DMS, Dry Magellanic Steppe; HMS, Humid Magellanic Steppe) of Southern Patagonia, using host plants sampled in 2016 and 2017, discriminating between moderate sheep stocking *(white symbols)* and high sheep stocking *(black symbols)* rates.

higher values of bulk density and pH, while DMS was related to higher AM and K values and HMS revealed higher values of SOC, N, MAT, and MAP (see Fig. 3A). In the axes 1 and 3, the ecological areas were explained by pH, soil bulk density, and in less extent the colonization of AM fungi and opposite to N, SOC, and MAP. In addition, there was a separation between sites of moderate and high grazing intensity in the DMS ecological area (see Fig. 3B).

The results of Pearson's correlation coefficient showed a negative and significant correlation between the colonization of AM fungi and SOC ($r = -0.60$; $P < 0.05$) and soil N ($r = -0.58$; $P < 0.05$). In contrast, pH ($r = 0.57$; $P < 0.05$) and bulk density ($r = 0.45$; $P < 0.06$) had a positive correlation with AM colonization. However, soil P ($r = -0.24$; $P = 0.34$) and K ($r = 0.38$; $P = 0.12$) did not show significant correlation with AM colonization.

Simple linear regression analysis for AM fungal colonization of all host plant species evaluated in Southern Patagonia showed a negative linear relationship ($P < 0.05$) with soil organic carbon (Fig. 4**A**) and soil N (see Fig. 4 B). On the contrary, AM colonization showed a weak and not significant response to the soil P and K content (see Fig. 4C and 4D).

Effect of grazing intensities on AM in the ecological areas and plant species

When the effect of sheep stocking rates (moderate vs. high) on the AM colonization in each of the ecological areas was analyzed separately, the HMS was the only area that showed a significant difference ($P < 0.05$), being lower in high sheep stocking rates sites (Fig. 5).

When the mycorrhizal colonization was analyzed for each plant species within the ecological area, considering the effect of the livestock stocking rates with its two levels (moderate and high), the following gradient according to the life form was determined: grasses < dwarf-shrubs < shrubs. In general, it was observed that some plant species within each ecological area presented significantly different AM colonization values ($P < 0.05$) depending on

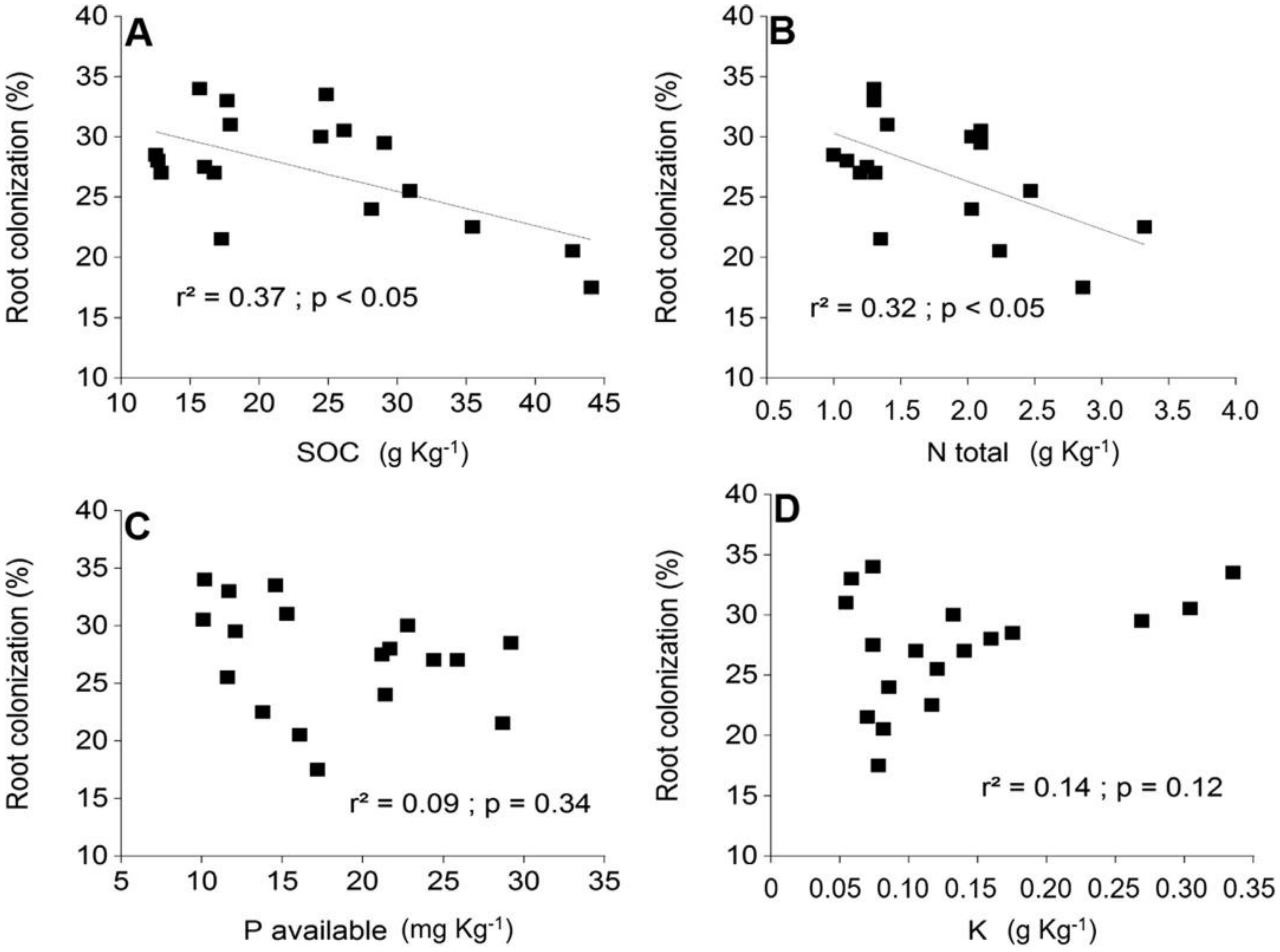

Fig. 4. Relationships between soil nutrient contents and arbuscular mycorrhizal fungal colonization in the ecological areas of Southern Patagonia, Argentina.

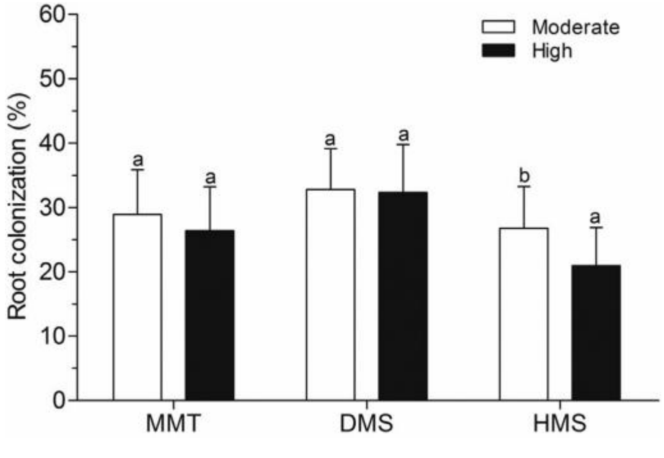

Fig. 5. Influence of grazing intensities (moderate vs. high) on arbuscular mycorrhizal (AM) colonization in each of the ecological areas of Southern Patagonia. Colonization of AM fungi includes all the dominant host species in each ecological area studied. Means and standard deviations are shown. Different letters, in each ecological area, indicate significant statistical differences ($P < 0.05$) for the grazing intensities factor. MMT indicates Mata Negra Thicket; DMS, Dry Magellanic Steppe; HMS, Humid Magellanic Steppe.

sheep stocking rates (Fig. 6). Thus, *F. gracillima* and *N. bryoides* in MMT, *P. dusenii* in DMS, and *P. dusenii* and *R. virescens* in the HMS presented lower AM colonization in sites with high sheep stocking rates compared with sites with moderate grazing (see Fig. 6).

Discussion

Mycorrhizae in Southern Patagonia ecosystems

AM fungi play an important role in ecosystems by maintaining the structure and functionality of plant communities (Rillig 2004; Barea et al. 2011). In our study, we showed that the dominant native plant species preferred by sheep (*P. dusenii, R. virescens, F. gracillima, N.bryoides, M. tridens*) hosted AM (except *C. argentina*). The lack of AM colonization for the genus *Carex* has been previously reported, with the family Cyperaceae generally described as nonmycorrhizal (Tester et al. 1987), or in a few cases as facultative (Smith and Read 1997; Miller et al. 1999; Fontenla et al. 2001).

In general, AM colonization is defined by the interaction among the symbionts (Hoeksema et al. 2010; Davison et al. 2020), plant life forms (Hawkes and Sullivan 2001; Tedersoo et al. 2020), and plant species (Gibson-Roy et al. 2014). The results of the present study of the arid rangelands of Southern Patagonia, Argentina, showed an overall mean value of 30% AM colonization, with values ranging from 5% to 50% depending on the plant species and life form (e.g., *P. dusenii* 5%, *F. gracillima* 28%, *R. virescens* 30%, *M. tridens* 41%, and *N. bryoides* 50%). In similar environments, Cavagnaro et al. (2017) described that the AM colonization of dominant species in

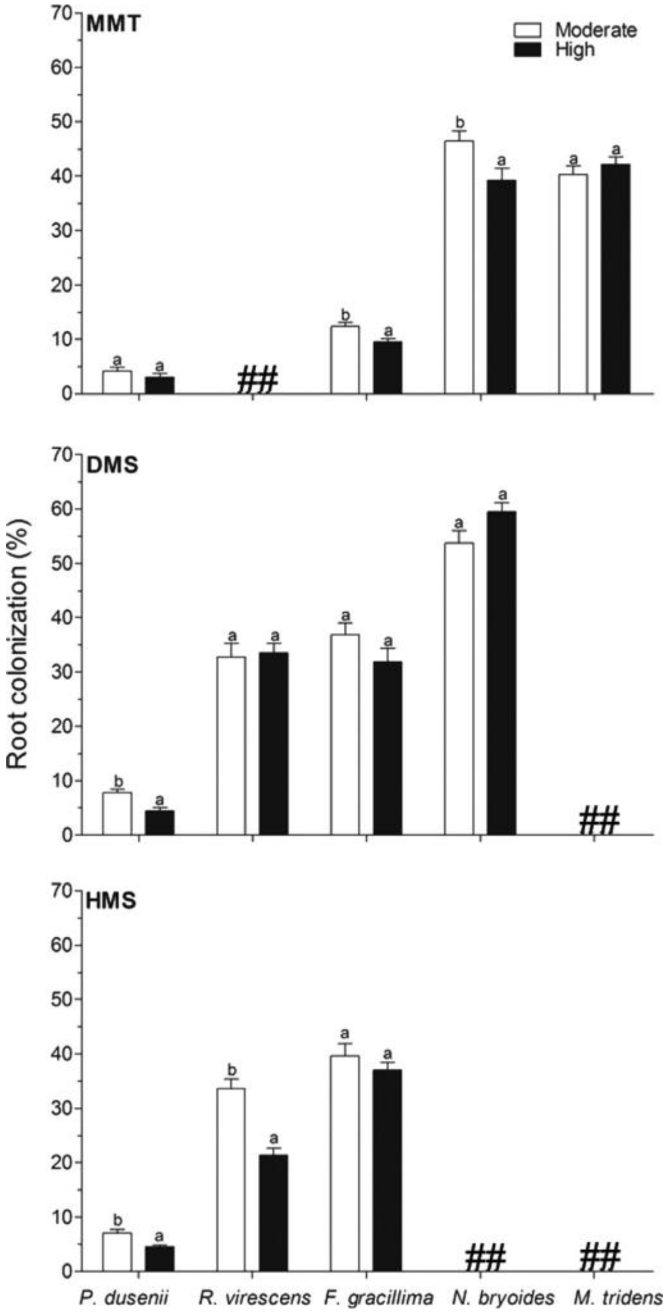

Fig. 6. Arbuscular mycorrhizal colonization in the native plant species (of preferred consumption by sheep) located in each of the ecological areas of Southern Patagonia, in sites with contrasting sheep stocking rates (moderate vs. high). Means and standard deviations are shown. Different letters indicate significant differences ($P < 0.05$) between the sheep stocking rates for each plant species within each ecological area. MMT indicates Mata Negra Thicket; DMS, Dry Magellanic Steppe; HMS, Humid Magellanic Steppe. ## indicates plant species not found and/or analyzed on site because had < 10% soil land cover.

the Northern Patagonian steppe varied between 10% and 70% according to the host species (e.g., 65% *Bromus pictus,* 5% *Poa ligularis,* and 17% *Pappostipa speciosa*). In addition, differences of root depth and age between key plant functional types in Patagonia cold arid ecosystems (Soriano and Sala 1984; Hodge et al. 2009; Reyes and Aguiar 2017) could affect AM colonization between evaluated plant species. This may be important for plant interactions in the ecosystem because the water and nutrient uptake also relies on mycorrhizal fungi with mycelium reaching deeper soil layers than root systems (Brundrett 2002; Smith and Smith 2011). According to Tedersoo et al. (2020), complex networks of mycorrhizal hyphae connect the root systems of plants and regulate nutrient and water flow between and within plant species, ultimately influencing ecosystem functionality.

However, Fan et al. (2019b) highlighted that the proportion of roots colonized needs to be considered in relation to the size of the root system. In our study, *R. virescens* and *F. gracillima* had similar AM colonization percentage (30%) but strongly differed in their root biomass (*R. virescens* = 0.60 g plant^{-1}, *F. gracillima* = 4.68 g plant^{-1}) and *P. dusenii* with 5% AM colonization and 0.34 g root biomass plant^{-1} (Peri and Lasagno 2010; Bjerring et al. 2020). Thus, both data are complementary measurements to explain the real impact of complex root–AM fungal on soil biological fertility in ecosystems (Fan et al. 2019b).

It was also cited that AM colonization values depend on several factors including plant communities and environmental characteristics (Rillig 2004; Deveautour et al. 2018). In our study we determined significant differences in AM colonization between different ecological areas, with DMS > MMT > HMS (see Table 5 and Fig. 3). The distribution of plant life forms with different AM colonization values (grass < dwarf-shrubs and shrubs) could explain the variation of mycorrhizal responses at the landscape level. Thus, while in HMS there was a predominance of grass species (*P. dusenii, R. virescens,* and *F. gracillima*) with the lowest values of AM colonization, dwarf-shrubs and dominant shrubs (*N. bryoides* and *M. tridens*) with a high degree of AM colonization occurred in DMS and MMT. This is consistent with Smith and Read (2008) and Brundrett and Tedersoo (2018), who reported that AM had a characteristic pattern of diminished colonization on herbaceous plants and a major colonization on dwarf-shrubs and shrub species. Another factor to consider is the interaction between soil type and the plant species roots. Brundrett (2002) and Del Mar Alguacil et al. (2016) described how the interaction between roots and the soil influences root morphology and architecture, where mycorrhizal colonization occurs. This becomes particularly important in the Patagonian steppe with low vegetation cover (< 50%), which is integrated by multispecies (perennial grass, dwarf-shrubs, and shrubs) where the root systems of the different species coexist in the same volume of soil (Reyes and Aguiar 2017). Consequently, the interaction between neighboring roots and arbuscular mycorrhizal hyphae of plant species favors the flow of water and nutrients (Davison et al. 2020; Tedersoo et al. 2020).

Moreover, other biotic and abiotic factors such as soil and climate have been reported to influence mycorrhizal symbiosis (Mohammad et al. 2003). Our results from both Pearson's correlation and PCA analysis were consistent with Hoeksema et al. (2010), who reported in a meta-analysis the influence on mycorrhizal symbiosis by physical and chemical soil characteristics interacting with the environment. The lowest AM colonization in the HMS ecological area presented better soil nutrient contents (SOC and N), water availability (by higher rainfall), and grass species dominance. Similarly, Tuomi et al. (2001) proposed that a lower AM colonization occurs in environments with higher amounts of soil nutrients. In contrast, AM colonization in MMT and DMS areas was related to less favorable conditions for plants (soil and climatic characteristics).

In these harsh environments, soil N is generally scarce (Gherardi et al. 2013; Peri et al. 2019). Nouri et al. (2014) observed that AM symbiosis was stimulated under N limitation, independently of soil P content. However, Blanke et al. (2005) found increased AM colonization under N limitations in soils with high P content. Our study indicated different responses to AM colonization when considering the N and P soil contents. Although AM colonization increased in DMS (site with low values of P soil and medium values of N soil) and MMT (site with low values of N soil and high values of P soil), in HMS the AM colonization decreased (site with high values of N soil and medium values of P soil). Interestingly, our results suggest that plants promote a higher root AM colonization as long as at least one of the nutrients (N or P) is scarce. Additionally, precipitation in the ecological areas described in the gradient HMS (255 mm) > DMS (231 mm) > MMT (150 mm) (see Table 2 and Fig. 3) could lead to low soil moisture content that limits the availability of nutrients and, thus, probably stimulates AM colonization in DMS and MMT (Miller et al. 2012; Deepika and Kothamasi 2015; Li et al. 2015).

AM symbiosis in Southern Patagonia could be a survival strategy that determines the coexistence of both symbionts (Smith and Smith 2011; Deveautour et al. 2018). Thus, AM fungi colonization in arid ecosystems with adverse conditions (periods of drought, extreme temperatures, and soils with low nutrients availability) is a beneficial strategy for plant development by facilitating water and soil nutrients uptake, which are later transferred to plants (Smith and Read 2008; Barea et al. 2011; Hodge and Storer 2015).

Mycorrhizae and grazing intensity

Livestock grazing strongly affects the structure and function of natural grassland ecosystems worldwide (Eldridge et al. 2016). The effects of grazing on mycorrhizal symbiosis showed variable responses, with some studies reporting that animal grazing decreased the AM colonization and other reporting that grazing increased AM colonization (Bethlenfalvay et al. 1985; Grigera and Oesterheld 2004; Hokka et al. 2004; Kula et al. 2005). In contrast, other studies described that grazing did not modify AM colonization (Lugo et al. 2003; Van der Heyde et al. 2017). In the present work, we found at a landscape level (considering three ecological areas) that AM fungi colonization in the natural rangelands of Southern Patagonia was not affected by long-term sheep stocking rates under continuous grazing management. However, the effect of grazing intensity in AM depends largely on environmental conditions of each ecological area.

The effect of grazing on AM colonization is masked by interactions with biotic and abiotic factors in each particular environment, thus in the potential grazing-soil-plant-AM relationship (Mendoza et al. 2002; Ruotsalainen and Eskelinen 2011). Therefore, when analyzing each ecological area independently, we found that sheep stocking rates (moderate and high) affected AM fungi colonization depending on site characteristics. In the HMS, the site with higher precipitation and grass dominance, the AM colonization decreased significantly with high sheep livestock compared with moderate grazing sites. However, in the other two ecological areas, DMS and MMT, no changes associated with grazing intensities were observed. The impact of animal grazing on AM colonization could affect directly AM fungi or indirectly through the effects on host plants or the soil-root ecosystem (Ruotsalainen and Eskelinen 2011; Van der Heyde et al. 2017). A direct factor affecting the soil and AM fungi is through animal feces and urine input to the soil ecosystem (Mikola et al. 2009). Other factors are the damage caused by animal trampling associated with soil compaction and deterioration of the aboveground and plant roots (Lezama and Paruelo 2016). Cavagnaro et al. (2017) reported that in the steppe of Northern Patagonia the existence of shrubs allowed herbaceous species to have a greater AM colonization because shrubs act as a "refuge" against grazing. Taking into consideration this statement, it is possible to assume a mitigating effect of dwarf-shrubs and shrub species on AM colonization in DMS and MMT areas. In addition, dwarf-shrubs and shrub species present in the DMS and MMT areas probably developed greater root systems that reached more exploration soil depths (Bertiller et al. 2004, 2005), which minimized the negative effects of animal trampling. According to Giovannetti et al. (2004) and Tedersoo et al. (2020), the existence of networks of hyphae below ground allows the mobility of nutrients and water between individuals of the same species, even between different species and life forms in the ecosystem plant community. In ecosystems dominated by grasses species (HMS), root systems are more superficial and the vegetative parts (leaves and stems) are more likely to be consumed by animals; consequently, grazing would have a negative effect on the AM fungi colonization (Diaz et al. 2007; Yang et al. 2020). An indirect factor suggested by Bethlenfalvay and Dakessian (1984) and Bethlenfalvay et al. (1985) is that grazing affects mycorrhizal symbiosis through the removal of plant biomass, affecting plant-fungi interaction and the energy balance of the symbiosis, since AM fungi are biotrophic microorganisms dependent on plant photosynthates.

Moreover, animals tend to select most palatable species for consumption, along with the intensity and duration of grazing could determine the major frequency of the plants consumed (Barto and Rillig 2010; Eldridge et al. 2016). Grazing may affect the AM fungi colonization depending on plants strategies, which as a result change the pattern of carbon allocation by new tissues growth or by the root exudates, consequently affecting the AM fungi (Hetrick et al. 1990; Barto and Rillig 2010; Van der Heyde et al. 2017; Ren et al. 2018). Additionally, removal of aerial biomass by grazing increases C exudates to the rhizosphere, probably resulting in improvements to root-fungi AM interaction (Hamilton and Frank 2001; Hamilton et al. 2008).

Gehring and Whitham (2002) and Barto and Rillig (2010) have proposed that the type of host plant is a significant moderator on the effects of grazing on AM symbiosis. In the present study, when the effect of sheep grazing intensity on mycorrhizae response was analyzed for each plant species, it was observed that grazing had a differential effect. For example, AM colonization decreased in *P. dusenii* roots in the DMS and HMS areas that present high sheep livestock, as well as *R. virescens* in HMS. This could be attributed to the sheep preference and selectivity on these palatable plant species (Borrelli and Oliva 2001; Andrade et al. 2015).

However, AM colonization in *P. dusenii* roots in the MMT was not affected by sheep stocking rates, while in *F. gracillima* and *N. bryoides* species AM colonization decreased significantly in sites with high livestock stocking rates. Ferraro and Oesterheld (2002) reported some cases in which a reduction in aerial biomass by grazing was not accompanied by a reduction in root biomass and AM colonization. The persistence of active meristems in plants after defoliation permits the development of new photosynthetic tissues, which allows the plant to sustain mycorrhizal symbiosis (Walling and Zabinski 2006).

Our results highlight the importance of moderate grazing in shaping AM fungi and the negative consequences that overgrazing can have on the rangeland ecosystems. Under continuous and intense grazing, AM fungi abundance is reduced and benefits for natural grassland ecosystems, such as improved nutrient and water uptake, soil formation processes, and ecosystem productivity, could be reduced.

In conclusion, AM symbiosis benefits growth and survival of plants when exposed to unfavorable conditions, such as Patagonia cold arid environment. AM colonization was affected by soil characteristics and climatic conditions, where plants with high root AM colonization occurred when nitrogen, SOC, and/or rainfall were limiting. Also, our study determined that continuous high-grazing intensity reduced the plant-AM fungi association compared with moderate grazing sites.

Implications

By increasing our understanding of the impacts of grazing intensity interacting with the environment on belowground ecology, it assists stakeholders in sustainable management practices by maintaining soil ecosystem processes in grazed Patagonian semiarid rangelands. Our findings indicate that moderate grazing allows us to maintain higher AM colonization compared with high grazing sites, enhancing aboveground production of palatable plant species for livestock. Maintenance of AM fungi may increase the positive effects on ecosystem structure and functioning, particularly in degraded rangelands like Patagonian steppes. Also, AM fungi can be used as an indicator of ecosystem deterioration in rangelands of arid environments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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