Arbuscular mycorrhizal symbiosis and dark septate endophytes under contrasting grazing modes in the Magellanic steppe of Tierra del Fuego

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A B S T R A C T

The impact of the mode and intensity of sheep grazing on the co-occurrence of AM fungi and DSE in Poa rigidifolia and Deschampsia flexuosa roots and their associations with N and P in plant tissue was studied in a range of degraded grasslands from Tierra del Fuego (Argentina). Under different grazing modes, AM colonization was always higher in Deschampsia (36.8%) than in Poa (24.3%), whereas DSE colonization was similar in both species (28% in Deschampsia and 27% in Poa). High DSE colonization was associated with soils of low fertility and subjected to grazing associated with an intense grassland disturbance. In contrast, AM colonization was associated with more fertile soils and low grassland disturbance. An opposite response of AM and DSE colonization was observed in low or absence of grazing. Nutrients in the plant tissue were always higher in Deschampsia than in Poa. Nitrogen concentration in both plants was positively correlated with AM and negatively with DSE colonization, whereas P concentration showed no correlation with AM or DSE. The highest AM colonization indexes and nutrients in tissue may explain why Deschampsia is much better adapted to the acid, xeric conditions and low fertility of the soils of these grasslands than Poa.

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1. Introduction

Arbuscular mycorrhizal (AM) fungi are obligate biotrophs that live symbiotically in the roots of most plants. They are involved in obtaining and transporting phosphate and other nutrients from the soil to the plant roots and, in turn, the plant provides fixed carbon to the fungal partner (Smith and Read, 2008). In addition, AM fungi are important in the ecology and physiology of plants, in particular those living under biotic (e.g. grazing) or abiotic stresses (e.g. nutrient and/or water deficient environments), because they may improve nutrient uptake and water supply to plants.

Previous studies have reported that some host plants of AM fungi are co-colonized by dark septate endophytic fungi (DSE) (Jumpponen and Trappe, 1998; Ruotsalainen et al., 2002; Li et al., 2005; Lugo et al., 2009). AM fungi and DSE are distributed in a wide range of ecosystems, but DSE are particularly found in stressed environments (Barrow, 2003; Mandyam and Jumpponen, 2005; Schmidt et al., 2008; Newsham et al., 2009). DSE fungi may not only function as pathogens or saprophytes, but also form mutualistic associations similar to those of mycorrhizae (Jumpponen, 2001). Several studies showed that DSE improve plant growth and N and P uptake (Jumpponen et al., 1998; Barrow and Osuna, 2002; Newsham, 2011).

The interactions among climatic conditions, soil water content, nutrient availability and intensity of grazing are determinants of plant growth and may affect the associations with AM fungi and/or DSE. Gehring and Whitham (2002) hypothesized that high herbivory intensity should have a more negative impact on AM colonization in low-productivity habitats with harsh environmental conditions than in more benign environments. Until now no consistent pattern of AM root colonization in response to grazing has emerged (Lugo et al., 2003; Menoyo et al., 2009), and the effects of grazing on dual colonization pattern of AM fungi and DSE are even less known (Medina-Roldán et al., 2008; Ruotsalainen and Eskelinen, 2011).

In Northern Tierra del Fuego (Argentina), the climate is cold, windy and cloudy. The region is a rare example of cold temperate oceanic grassland in South America (Collantes et al., 1999). The soils are characterized by a low nutrient availability, strong acidity, low base saturation, and high values of the carbon/nitrogen ratio (Collantes et al., 1999; Mendoza et al., 2002). Sheep rearing for wool production in the region has been the dominant activity for more than a century and sheep grazing has, in turn, transformed many grass communities into a spectrum of degraded grasslands. Sheep are preferentially selective in their grazing, they choose more palatable and nutritious grasses, such as Deschampsia flexuosa and Poa rigidifolia, which account for 45% of their diet (Posse et al., 1996; Lin...
et al., 2010). These two grasses are mycotrophic and the spore density in their rhizospheric soil has been positively correlated with the nutrient status in the soil (Mendoza et al., 2002). In addition, it has reported that in a range of grazed grasslands, the nature of the AM fungal spore family in the rhizospheric soil of *Poa* and *Deschampsia* is associated with specific properties and fertility in the soil, which, in turn, are influenced by the mode and intensity of sheep grazing being practiced (Mendoza et al., 2011). Likely, in this stressed environment, a high nutrient status in the soil or in the plant tissue may not necessarily imply low levels of AM fungal colonization in roots because the abundance of mycorrhizae may depend on other factors such as the mode and intensity of grazing. Then, it would be important to study the relationship between the soil properties, the plant nutritional status (especially N and P) and the capacity of these grasses to associate with AM fungi and DSE. The study of the behavior of symbiotic organisms associated with palatable plants in connection with plant performance may contribute to the development of a strategy to improve sheep grazing management to prevent grassland degradation in these cold and xeric environments.

The aim of this work was to examine the impact of the mode and intensity of sheep grazing in a wide range of degraded grasslands on the co-occurrence of AM fungi and DSE in *P. rigidifolia* and *D. flexuosa* roots, and to associate the morphology of the colonization of the two symbionts with the status of N and P in plant tissue. We specifically aimed to test the following hypotheses: (1) the mode and intensity of grazing affect the length of the root colonized by AM fungi and DSE in *Poa* and *Deschampsia*, (2) the fraction of root colonized by mycorrhizae is negatively associated with the fraction of root colonized by DSE, (3) the status of N and P in plant tissue is associated with the different proportion of the root colonized by AM fungi and DSE.

2. Materials and methods

2.1. Climate and study area

The research was performed in a complex of moraine deposits and glacial-outwash plains in northern Tierra del Fuego (Argentina). The study area covers 1500 km² extending from the Strait of Magellan to 53°49'S. The mean temperature is about 0°C in July (winter) and reaches up to 1°C in January (summer; Fuerza Aérea Argentina, 1986). The annual rainfall ranges from about 300 mm to 400 mm along a northeast–southwest gradient and is evenly distributed throughout the year, except for a small precipitation peak in December. However, a high water deficit in soil occurs due to high potential evaporation rates causes mainly by the strong winds from the West that increases the dryness in the region (Cingolani et al., 1998). The steppe that covers the area includes a variety of plant communities dominated by different proportions of *Emetrum rubrum* (Vahl ex Willd) and other prostrate shrubs of very low forage value, tussock grass *Festuca gracillima* (Hooker f.), short grasses such as *P. rigidifolia* (Steudel) and *D. flexuosa* (L.) Trin, or by *Chilitrichum diffusum* (Forster f.) O. Kunze)-a medium-height shrub. More details about plant communities can be found in a work carried out by Mendoza et al. (2011).

2.2. Grazing modes

Twelve sites which represent several combinations of habitat conditions and modes of sheep grazing were selected. The sites were identified by the differences in the proportion of the dominant plant species, as each of them was characteristically associated with particular Landsat-image attributes (Cingolani et al., 2002). The modes of grazing were identified from the information provided by the farm managers on the prior land management within the study area (Mendoza et al., 2011). Four grazing modes were characterized: selective, non-selective, sheep-trail and non-grazed (enclosure). Within each grazing mode, different degrees of intensity of grazing were defined based on the stocking rate, which determines different degrees of soil and/or grassland disturbances (Table 1):

1. “Selective” (S) grazing was defined as that in which the number of animals per hectare was relatively low, and the sheep were able to select and consume the most palatable plant species. This type of grazing has been the traditional long-term grazing mode used in the area for the last 100 years and varied from 0.3 to 1.2 sheep per hectare per year. Within selective (S) mode, three degrees of grazing intensity were identified:
   (a) High (SH), when the number of sheep per hectare varied from 1.0 to 1.2.
   (b) Medium (SM), when the number of sheep per hectare varied from 0.7 to 0.9.
   (c) Low (SL), when the number of sheep per hectare varied from 0.3 to 0.6.

2. “Non-selective” (N) grazing was defined as that in which the number of animals per hectare was high, but only on a short-term basis. Sheep had no chance to select any particular species because both the grazing pressure and the stocking level were quite high. These areas are only used as concentration paddocks for 2–3 weeks in summer on the way to shearing. This type of grazing mode was associated with a high degree (NH) of soil and/or grassland disturbance in a relatively short period of time.

3. “Sheep-trail” (ST) grazing comprises areas grazed by the traditional long-term selective mode. Additionally, the area was grazed and trampled in summer by large numbers of animals traversing a grassland on their way to shearing. This grazing was also associated with a high degree of soil and grassland disturbance in a relatively short period of time.

4. “Non-grazed” (NG) were defined as those that have been left without any grazing at all for at least the last 15 years (enclosure). The degree of soil disturbance was nil.

The study area and grazing modes were the same as those used previously by Mendoza et al. (2011) and some of the data sets regarding the soil properties and vegetation used in the present study were taken from that work.

2.3. Field sampling

A sampling plot of 40 m × 20 m was placed in each of the 12 sites studied. Each plot was divided into five subplots of 8 m × 20 m to be used as replicates. Ten soil core samples per replicate were taken and mixed homogeneously to form a 3 kg composite sample. Each soil core sample was 15 cm deep. The top 2 cm of the soil core was removed to eliminate part of the top leaf litter. Each soil core contained about 280–320 g of soil. The composite soil samples were thoroughly mixed, kept in a plastic bag at 4°C until processed, and divided into two portions to measure the chemical properties and nitrification and ammonification potential of the soil.

Individuals of *P. rigidifolia* and *D. flexuosa* were collected at each site in March 2009 (late summer). Five replicates of each plant species (adult individuals of similar size) were randomly sampled in each subplot of every site.

Sites were named from 1 to 14 to be consistent with Mendoza et al. (2011). Sites 5 and 6 were not used in this study because *P. rigidifolia* and *D. flexuosa* were absent.

The soils of the study area were classified as Regosol (sites 1–12) and Phaeozem (sites 13 and 14) (FAO, 1990).
2.4. Soil characteristics

Soil pH (soil-solution ratio of 1:2.5 in water); exchangeable Ca and Mg (Ca + Mg) (Jackson, 1964); total C (Ct) (Richter and Von Wistinghaus, 1981); total N (Nt) (Bremner and Mulvaney, 1982) and phosphorus availability (Bray and Kurtz, 1945) were analyzed on a dry soil basis. The percentage of the soil particles was determined by Robinson’s pipette method (Piper, 1964). Soil nitrification and ammonification were additionally assayed by measuring the concentration of NO3– and NH4+ in the laboratory in moistened soil samples incubated in a humid environment at 28 ± 2 °C for 70 days (Marbán, 2005). Nitrification and ammonification are both associated with the potential fertility of the soil.

2.5. Mycorrhizal and DSE analysis

Fresh roots from both plant species were washed, cleared with 10% KOH and 3% H2O2 and stained with 0.05% lactic acid-glycerol Trypan Blue (Phillips and Hayman, 1970). Twenty-five root segments per plant sample were examined under a microscope at a 200× magnification. The fraction of root length colonized (MC), or containing arbuscules (AC), vesicles (VC), and hyphae-only (HO), and the root fraction colonized by DSE were assessed following McConigle et al. (1990). Septate melanized or hyaline hyphae and microsclerotia were classified as DSE (Barrow and Aylton, 2001).

The number of mycorrhizal entry points was measured along root fragments at a magnification of 200× (Amijee et al., 1989) and expressed as the number of entry points (EP) per mm of colonized root.

2.6. Plant nutrition

Oven-dried (70 °C for 48 h) shoot tissue of *P. rigidifolia* and *D. flexuosa* was digested separately in sulfuric acid to determine N concentration by the Kjeldahl method, and in a nitric-perchloric acid mixture to determine P concentration by the molybdenum phosphoric acid method (Jackson, 1964).

2.7. Data analyses

Fungal variables and N and P concentrations in shoot tissue were analyzed through two-way ANOVA with grazing mode and plant species as the first and second factors, respectively. Mean separation was performed by the Tukey test. The normality and homogeneity of variances were previously verified. Non-normal data were transformed to logarithms or arcsine to compare among grazing modes and plant species. A linear regression between the concentration of N or P in shoot tissue (dependent variable) and the root length colonized by AM and DSE in *D. flexuosa* and *P. rigidifolia* (independent variable) was performed for all sites subjected to different grazing modes and intensities. Statgraphic 5.0 plus software was used for statistical analyses.

The canonical correspondence analysis (CCA) ordination technique by the CANOCO algorithm (Ter Braak, 1987–1992) was used to identify the best linear combinations of soil properties that influence the fungal variables (AM fungi and DSE). Firstly, six fungal variables were included in the main matrix: AM colonization (MC), arbuscular colonization (AC) and DSE colonization of *P. rigidifolia* and *D. flexuosa* roots. The second matrix contained five soil properties (pH, Ca + Mg, C/N, NO3– and NH4+). The Monte Carlo simulation was used to test the hypothesis that there was no correlation between the main (fungal variables) and secondary (soil properties) matrices. The P values were obtained after 1000 simulation runs.
3. Results

3.1. Soil properties

The chemical properties differed among the soil sites (Table 1). Sites 2SH and 3SH showed the lowest pH, highest carbon/nitrogen ratio, and lowest levels of P, Ca + Mg, NO₃⁻, and NH₄⁺ compared to the other sites. At the other end of the soil-fertility scale, sites 9SM, 13SM, 10SL, and 14 NG displayed the highest values of soil pH, Ca + Mg, P, NO₃⁻ and NH₄⁺ (Table 1). Sites 1–4 have a similar soil texture. Clay varied from 19.6 to 25.3%, silt 24.3–31.8% and sand 46.4–51.8% (Table 1). However, these sites are quite different in terms of soil fertility and grazing modes. Similarly, the soils of the sites 7–10 and 13–14 are quite similar in terms of texture, but different in soil fertility and grazing modes (Table 1).

3.2. Fungal colonization

Co-occurrence of AM fungi and DSE was observed in roots of both plant species in all the sites studied (Figs. 1 and 2). AM fungal root length colonization indexes (total fraction of root length colonized, arbuscular colonization and vesicular colonization) were always higher in Deschampsia flexuosa than in P. rigidifolia roots under different modes of sheep grazing (Fig. 1a–c). The mean values over all sites for Deschampsia flexuosa roots were: total colonization 34.51%, arbuscule colonization 10.74%, vesicle colonization 6.47% and hyphae-only colonization 15.76%. For P. rigidifolia roots, the respective values were: 21.81%, 5.77%, 4.27% and 11.15%. The grazing mode and plant species significantly affected the AM fungal colonization indexes (Table 2), but the interaction between grazing mode and plant species on AM colonization indexes was not significant (Table 2). The selective grazing mode showed a decrease in the magnitude of total colonization, as well as of the arbuscular and vesicular colonization indexes with the increase in the degree of soil disturbance (SL, SM and SH) in the two plant species (Fig. 1a–c).

The number of entry points (EP) per millimeter of colonized roots was similar in the roots of both species but the entry points were affected by the grazing mode and the intensity of grazing (Table 2). The average number of EP for Poa and Deschampsia was higher in the non-selective site at high intensity (NH) and in the non-grazed site (NG) (Fig. 1d). The dominance of Arum-type AM colonization was observed in all the sites studied in Poa and Deschampsia roots under different grazing modes, reaching 93.70% of the total root length colonized.

The root lengths of the two plant species were similarly colonized by DSE (Fig. 2). The overall mean of root length colonization was 27.03% in Poa roots and 27.98% in Deschampsia. The mode and intensity of grazing significantly affected the values of DSE colonization (Table 2). The fraction of roots colonized by DSE increased in the selective mode as the intensity of grazing increased from SL to SH with intermediate values for SM (Fig. 2). At the non-grazed site (NG), DSE colonization was lowest for both plants (Fig. 2).
Table 2
Results of two-way ANOVA for the effects of grazing mode (GM) and plant species (Sp) on fungal variables and P and N plant concentrations. Degrees of freedom for each source of variation are between parentheses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>GM</th>
<th>F&lt;sub&gt;(1,108)&lt;/sub&gt;</th>
<th>P</th>
<th>Sp</th>
<th>F&lt;sub&gt;(1,108)&lt;/sub&gt;</th>
<th>P</th>
<th>GM = Sp</th>
<th>F&lt;sub&gt;(3,108)&lt;/sub&gt;</th>
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<td></td>
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<tr>
<td>MC</td>
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<td></td>
<td>23.31</td>
<td>0.0000</td>
<td></td>
<td>0.71</td>
<td>0.6172</td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>6.09</td>
<td>0.0001</td>
<td></td>
<td>20.62</td>
<td>0.0000</td>
<td></td>
<td>0.21</td>
<td>0.9568</td>
<td></td>
</tr>
<tr>
<td>VC</td>
<td>5.95</td>
<td>0.0001</td>
<td></td>
<td>14.80</td>
<td>0.0002</td>
<td></td>
<td>1.43</td>
<td>0.2168</td>
<td></td>
</tr>
<tr>
<td>EP</td>
<td>5.02</td>
<td>0.0004</td>
<td></td>
<td>0.23</td>
<td>0.6304</td>
<td></td>
<td>1.18</td>
<td>0.3244</td>
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<td>DSE (ln)</td>
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<td>0.08</td>
<td>0.7773</td>
<td></td>
<td>0.40</td>
<td>0.8461</td>
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<td></td>
<td></td>
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<tr>
<td>N (ln)</td>
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<td>0.0009</td>
<td></td>
<td>30.08</td>
<td>0.0000</td>
<td></td>
<td>4.30</td>
<td>0.0013</td>
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<tr>
<td>P (ln)</td>
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<td>0.2731</td>
<td></td>
<td>5.51</td>
<td>0.0208</td>
<td></td>
<td>1.67</td>
<td>0.1476</td>
<td></td>
</tr>
</tbody>
</table>

ns = non-significant.

* P<0.05.

3.3. Concentrations of N and P in plant tissue

Nitrogen concentration in shoot tissue differed significantly between plants and grazing modes (Table 2 and Fig. 3a). Except for the ST grazing mode, where Poa showed a higher value than Deschampsia, the concentration of N in shoot was always higher in Deschampsia than in Poa (Fig. 3a). Hence, there was a significant interaction between the plant species and the grazing mode with respect to N concentration in shoot (Table 2 and Fig. 3a). The difference between the two plants was highest in the NG site (enclosure), with 1.08% of N in shoots of Deschampsia and 0.60% in shoots of Poa.

Phosphorus concentration in shoot tissue was also significantly higher in Deschampsia than in Poa (Table 2). However, the differences between plant species were less marked than for N under different grazing modes and intensities (Fig. 3a and b). Hence, there were no significant differences between grazing modes and intensities (Fig. 3b).

Nitrogen concentration in shoots of both plants was significantly and positively correlated with AM colonization (P=0.037) but negatively correlated (P=0.029) with DSE colonization (Table 3). In addition, the difference between the two slopes was highly significant (P=0.002). However, phosphorus concentration in shoot of both plants did not correlate with mycorrhizal (P=0.194) or DSE colonization (P=0.234).

3.4. Implications of soil properties on fungal colonization

Together, the first two axes of the CCA analysis explained 56.8% of the total variance accounted for, axis I at 53.1% and axis II at 3.7% (Fig. 4). The Pearson correlation coefficient between the scores of the first two axes derived from the AM fungal data sets combined with the soil properties was 0.81 for axis I and 0.69 for axis II. The randomization test showed that the overall effect of the soil properties included in the second matrix and the first two canonical axes were statistically significant at a probability (P) of 0.07 and 0.25 for axes I and II, respectively. The four selective grazing sites subjected to high intensity (SH) were grouped on the positive scale of axis I and associated with DSE colonization in Poa and Deschampsia roots (2SH, 3SH, 7SH and 11SH). The sheep trail (ST) and non-selective high (NH) modes were displaced also to the positive scales of axis I (Fig. 4). The other selective grazing modes at low and medium intensity (SL, SM) together with the enclosure site (NG) were segregated to the negative scale of axis I and associated with the AM colonization (MC and AC) indexes of both plant species. In addition, the AM colonization indexes of Poa roots (PAC, PMC) were displaced to the top left quadrant, and the AM colonization indexes of Deschampsia (DAC, DMC) to the bottom left quadrant of the plot. Fig. 4a also shows that DSE colonization indexes of both plant species were situated opposite with respect to the AM properties.

![Fig. 3](image-url)

Concentrations of N (a) and P (b) in shoots of Poa rigidifolia and Deschampsia flexuosa under different modes of sheep grazing. Bars denote standard errors of the mean for selected sites disaggregated by the grazing mode as indicated in Table 1.
indexes. While the AM indexes of Poa (PMC, PAC) are located in the upper left quadrant, the DSE index is located at lower right quadrant. Similarly, the AM indexes of Deschampsia (DMC, DAC) are in the lower left quadrant and the DSE index in the upper right one (Fig. 4).

The biplots show the ordination of the soil properties that are significantly ($p < 0.05$) correlated with the axes (Fig. 4). The carbon/nitrogen (CN) arrow points to the sites of highest DSE colonization values in the roots of both plants species (Fig. 2) subjected to selective grazing at high intensity (SH and ST). The pH, Ca + Mg, NO$_3^-$ and NH$_4^+$ arrows point to the negative scale of axis I associated with low or moderate selective grazing intensity (SL and SM) or non-grazed (NG) sites. The non-selective grazing site at high intensity (NH) was displaced to the lower right of the plot in between the AM and DSE indexes and between the sites of low and high grazing intensity (Fig. 4).

4. Discussion

The roots of *P. rigidifolia* and *D. flexuosa* were co-colonized by AM fungi and DSE in a range of xeric and degraded grasslands of northern Tierra del Fuego, but the responses of each fungal group exhibited marked differences between them, associated with the sheep grazing modes, soil fertility and plant nutritional status. The prevalence of dual colonization seems to be common in harsh environmental conditions, suggesting an eventual role in such ecosystems (Mandyam and Jumpponen, 2005; Schmidt et al., 2008; Newsham et al., 2009).

This study showed that an increase in the intensity of the selective grazing mode (SL, SM, SH) decreased AM colonization and increased DSE colonization in the roots of both plant species. In addition, opposite responses between AM and DSE colonization were observed in the absence of grazing (NG), thus suggesting that low grazing intensity and/or enclosure favor AM colonization and decrease the colonization by DSE. These results indicate that a specific grazing mode and intensity might either increase or decrease the fraction of roots colonized by AM fungi and DSE in *Poa* and *Deschampsia*, which is in agreement with hypothesis 1.

This study also showed that AM colonization in roots of *D. flexuosa* (36.8%) was always higher than in roots of *P. rigidifolia* (24.5%). We propose two possible explanations to justify these results. First, the differences in the length of colonized roots between *D. flexuosa* and *P. rigidifolia* may be ascribed to the interactions between the affinity of the fungus by the host plant and the fungal ability to colonize new roots, the growth rate of the AM fungi within the roots, and the growth rate of the roots within the soil. The second explanation is related to the fact that both plant species differ in their tolerance to acidity, and the presence of *D. flexuosa* is an indicator of soil acidity (Larcher, 1975). The low pH characteristic of the soils of the Magellanic steppe may explain why *Deschampsia* – which is better adapted to acidity – showed a higher fraction of colonized roots than *Poa*. The number of entry points per millimeter of AM colonized roots was similar in the roots of both species but was affected by the grazing mode. We have previously reported that AM fungal spore density in the rhizospheric soil of *Poa* was higher than that found in soils of *Deschampsia* (Mendoza et al., 2011). In the present study, we found no relationship between AM root colonization and spore density in the soil in all the sites studied (data not shown). However, a similar number of entry points in the roots of both species determined a higher length of root colonized in *Deschampsia* than in *Poa*, regardless of the number of spores present in their rhizospheric soils. The highest root colonization in *Deschampsia* may contribute to improving the acquisition of water and nutrients from the rhizosphere and to tolerating soil acidity, favoring the persistence and distribution of this species in grasslands of northern Tierra del Fuego.

The CCA indicated that AM colonization took values opposite to those reached by DSE colonization in both plant species under different modes of sheep grazing, as proposed in hypothesis 2. DSE that differ in strategy and morphology but may function similarly to AM fungi may be better adapted to the harsh conditions of xeric and cold environments than AM fungi (Mandyam and Jumpponen, 2005; Schmidt et al., 2008). In our study, the competition with other fungal endophytes for root occupation could limit the distribution of AM fungi in the soil and/or in the root, decreasing AM root colonization and increasing DSE colonization in both plant species. Medina-Roldán et al. (2008) reported that DSE colonization was four times higher than AM colonization in roots of *Bouteloua gracilis*, a dominant grass from semi-arid grasslands of Mexico, suggesting that competition for resources may control the symbiont proportion under contrasting grazing managements. In accordance with hypothesis 2, the intensity of the grassland disturbance generated by the grazing modes decreased the fraction of roots colonized by mycorrhizae and increased the fraction of root colonized by DSE. Within the selective grazing mode, a livestock reduction from SH to SL decreases soil degradation, increases arbuscules, vesicles and hyphae-only, but decreases DSE colonization in roots of the grasses studied. The results of the CCA indicate that high DSE colonization in *P. rigidifolia* and *D. flexuosa* roots was associated with soils of low fertility (high acidity, high C/N ratio, low NO$_3^-$ and NH$_4^+$) subjected to selective high (SH), non-selective high (NH) and sheep-trail (ST) grazing modes, all of which are characterized by a relatively high intensity of grassland disturbance. In contrast, arbuscular colonization, which is an indicator of AM symbiosis functionality, was associated with more fertile soils under the selective grazing mode at low or medium intensities (SL and SM). Our results are in accordance with those of Gehring and Whitham (2002), who hypothesized that high herbivory intensity should have a more negative impact on mycorrhizal colonization in low-productivity habitats with low resource availability than in environments that are more benign. In the present study, the number of sheep per hectare was relatively low in the selective grazing mode at high intensity (SH). The sheep were able to consume the most palatable grasses in the SH grazing mode, continuously removing the
aboveground biomass. In these grazed plants, the carbon balance is altered in such a way that plant carbon is preferentially allocated to shoots as a physiological adjustment in order to recover the leaf area lost (Gill, 2007). Therefore, defoliation by grazing, which influences the photosynthetic capacity of plants, may affect the AM symbiosis (Barto and Rillig, 2010). Then, the higher proportion of removed biomass in Poa and Deschampsia may decrease the carbon resources that lead to shoot regrowth and the mycorrhizal symbiont may function as a competing sink for the limited carbon reserves by decreasing the root colonization in SH sites. On the other hand, DSE colonization in both plants was highest in SH sites, all of them characterized by low soil fertility (see Table 1). This may evidence a complementary role of the two fungal symbionts in the tolerance to the loss of aboveground biomass and low nutrient availability in xeric environmental conditions. A recent meta-analysis supports the hypothesis that DSE may enhance plant growth by mineralizing organic N compounds in the rhizosphere (Newsham, 2011). The prevalence of organic N in cold and xeric environments, caused by the slow decomposition of organic matter at low temperature (Schmidt et al., 1999), increases the likelihood that DSE will have positive effects on plant growth in grasslands of Tierra del Fuego.

The concentrations of N and P in shoot tissue were higher in Deschampsia than in Poa, except in sites 4NH and 12ST (both of a large stocking rate) in a short-time basis, which increases vegetation disturbance and soil compaction, but also leads to enhanced soil fertility through trampling and fecal and urine depositions (Mendoza et al., 2011). As previously observed for AM colonization, Deschampsia and Poa differed in their responses to the mode and intensity of grazing with respect to N and P in plant tissue. The fact that the MC and AC indexes were higher in Deschampsia roots may explain why Deschampsia shows higher concentration of nutrients in tissue and is much better adapted to the xeric and low fertility in the soils of these degraded grasslands than Poa (see Fig. 1a and b, 3a and b, and Table 3). These results are in agreement with hypothesis 3.

In the present study, AM fungi may have a role in plant N nutrition, but not directly related to P uptake. Unlike DSE, AM fungi are not known to have saprotrophic behavior (Smith and Read, 2008). However, a recent study has shown that AM fungi acquire N from decomposing organic matter and use this N principally for their own growth and metabolism (Hodge and Fitter, 2010). Moreover, DSE are more abundant in habitats poor in P (Haselwandter and Read, 1982; Ruotsalainen et al., 2002) (such as the grasslands studied), and it is thought that DSE are metabolizers of P and mediate P uptake for their hosts (Jumpponen et al., 1998; Barown and Osuna, 2002). Then, both fungal associations may collaborate with plant nutrient uptake by increasing the mineralization or mobilization and/or plant use of N and P from decomposing organic matter in the soil of Tierra del Fuego.

This is the first report of dual colonization of AM fungi and DSE in forage grasses of the Magellanic steppe of Tierra del Fuego. It constitutes an important step for future research to understand the role of microorganisms in N and P cycling, plant nutrition and plant tolerance to growth in these cold and xeric environments under different grazing managements.

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