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RESEARCH ARTICLE

Arbuscular mycorrhizal and dark septate fungi colonization in an invasive plant from Patagonian wetlands

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Abstract

Arbuscular mycorrhizal and dark septate fungi are common plant symbionts, but their role in promoting host plant fitness depends on environmental variables. Particularly in wetland plants, these associations are less understood. We analysed the role of arbuscular mycorrhizal fungi (AMF) and dark septate fungi (DSF) in the roots of *Potentilla anserina* (Rosaceae), an invasive species of Patagonia, widely distributed in wetlands. We tested three hypotheses: that fungi colonization varies according to soil moisture and nutrient content (nitrogen and phosphorus), that they enhance *P. anserina* nutrient content, and benefit plant growth. We measured the percentage of colonization in plants from five wetlands across a moisture gradient with different nutrient content, and performed a growth experiment with soil from these wetlands to evaluate changes in mycorrhizal and endophytic fungal colonization, aerial nutrient content and biomass production. In the field, root colonization by AMF was high in all sites (~90%), whereas DSF was less abundant (~20%), positively related to soil organic matter, and negatively related to soil phosphorus. In the experiment, DSF colonization was inversely related to increasing tissue N and P content. *Potentilla anserina* grew similarly in all the treatments, but biomass was positively related to DSF colonization. Our results provide evidence that DSF, rather than AMF, confer to this invasive species the ability to grow in soils with different water and nutrient content and may help to explain the wide distribution of this alien species in Patagonian wetlands.

KEYWORDS
arbuscular mycorrhizal and dark septate fungi, invasive plant, Patagonian wetlands, *Potentilla anserina*, soil nutrients

INTRODUCTION

Almost every plant form symbiotic associations, depending on the plant species involved. The most common mycorrhizal association is between plant roots and arbuscular mycorrhizal fungi (AMF), belonging to the division Glomeromycota, present in more than 80% of plant species, both in terrestrial and aquatic environments (Brundrett & Tedersoo, 2018; Cuassolo et al., 2012; Helgason & Fitter, 2009; Hu et al., 2020; Smith & Read, 2010; Wang et al., 2018). Although the percentage of colonization in aquatic environments may be low (<25%; Marins & Carrenho, 2017; Wang et al., 2018), in wetlands the intensity of colonization depends on the duration of the hydroperiod (Dolinar et al., 2016; Fusconi & Mucciarelli, 2018; Gaberščik et al., 2017) and oxygen availability (Fougnies et al., 2007; Miller, 2000).

The mycorrhizal interaction plays an important role in plant competition for nutrients, especially phosphorus (P; Javaid, 2009; Khan & Belik, 1995; Mei et al., 2019; Smith & Read, 2010), but also nitrogen (N; Johansen

et al., 1996; Nouri et al., 2014), promoting plant productivity and changes in community diversity and structure (Collins & Foster, 2009; Guo & Gong, 2014). However, mycorrhizal associations do not always promote plant growth. Abiotic factors in the rhizosphere (such as soil moisture and nutrient content) and biotic factors (microbial community composition) mediate mycorrhizal functioning determining the mutualistic or parasitic interaction (Johnson et al., 2015). Root colonization usually decreases with soil moisture (Lekberg & Koide, 2008), with an optimum of 15%–20% of water content in sediments (Deepika & Kothamasi, 2015). Also, soil nutrient content plays a determinant role in the type of association (Hoeksema et al., 2010; Johnson et al., 2015; Nouri et al., 2014). Mutualistic associations could be expected when there is a deficit of inorganic nutrient in soil (Cornwell et al., 2001; Hoeksema et al., 2010; Saif, 1983; Treseder, 2013), while in nutrient-rich soils, the AMF confer little advantage to the plants; in consequence, depending on the plant species, the host plant may reduce root colonization (Daleo et al., 2008).

The diversity of associated fungi is greater than previously assumed, extending to Mucoromycotina fungi (Hoysted et al., 2023). Another group of fungi that colonizes roots is known as dark septate fungi (DSF), a miscellaneous group of anamorphic Ascomycetes (Jumpponen, 2001; Jumpponen & Trappe, 1998; Menkis et al., 2004). The functions and taxonomic affinities of this group began to be understood in the last decade (Mandyam & Jumpponen, 2014; Newsham, 2011). They are one of the most abundant and widespread groups of plant root colonists (Della Mónica et al., 2015) and often contribute to enhancing photosynthetic efficiency, promoting nutrient and water use, and conferring tolerance to abiotic and biotic stress (Barrow et al., 2008; Bueno de Mesquita et al., 2018; Mandyam & Jumpponen, 2014; Wang et al., 2011). A synergistic relationship was hypothesized between DSF-AMF and P availability and uptake in plants, in which DSF increased the P pool in the rhizosphere while AMF transferred P to the host plant (Della Mónica et al., 2015). Also, DSF was suggested to be related to nitrogen mineralization (Bueno de Mesquita et al., 2018). Nonetheless, as for AMF, DSF associations can either stimulate or reduce host plant growth (Caldwell et al., 2000). Also, the interactions between DSF and AMF were reported as positive (Ranelli et al., 2015; Thangavelu & Raji, 2016), negative (Bueno de Mesquita et al., 2018), and neutral (Huo et al., 2021; Seerangan & Thangavelu, 2014). In a recent review of microbial interactions in soils, Albornoz et al. (2022) found that competition or facilitation among microbial groups largely depends on their mechanisms for carbon and nutrient acquisition.

The role of AMF in invasive plant colonization has been recently addressed (Callaway et al., 2001; Policelli et al., 2019; Simberloff, 2006). In some cases, the invasive alien species only invade after the introduction of their symbionts (Richardson et al., 2000). In salt-marshes, the invasive *Spartina alternifolia* showed a high infection rate of AMF which promoted the invasion success, while it is non-mycorrhizal in its native habitat (Eberl, 2011). In North-west Patagonia, invasive species amount to 15.35% in terrestrial environments (Speziale & Ezcurra, 2011), while in temporary wetlands may reach up to 50% (Cuassolo & Diaz-Villanueva, 2019). Among the numerous exotic species in Patagonian aquatic environments, the perennial hemicriptophyte *Potentilla anserina*, originally native to the Northern Hemisphere, is widely distributed (Correa, 1984; Ezcurra & Brion, 2005), both in forest and steppe meadows and also in urban wetlands (Cuassolo & Diaz-Villanueva, 2019). A recent study found that this species was positively associated with temporary wetlands and with the presence of cattle (Cuassolo & Diaz-Villanueva, 2022). In one of these wetlands, the

presence of AMF in *P. anserina* roots was higher than in two native macrophytes (*Eleocharis pachycarpa* and *Carex aematorrhyncha*), although the native species were more colonized by DSF than the invasive species (Cuassolo et al., 2012).

This study aimed to analyse the colonization of AMF and DSF in the roots of *P. anserina* grown in soil from temporary wetlands along a moisture gradient and with different nutrient content (nitrogen and phosphorus). In Patagonia, we found this moisture gradient because there is a steep decline in annual precipitations from west to east, which determines the water tables in wetlands (Paruelo et al., 1998). So, our first hypothesis (H1) was that the variation in soil moisture and nutrient content influences the occurrence and frequency of colonization of roots by AMF and DSF. Also, we experimentally assessed the relation between soil nutrient content, root colonization, and plant nutrient content (nitrogen and phosphorus) and hypothesized (H2) that the frequency of colonization of AMF and DSF enhances plant nutrient acquisition. In this experiment, we also measure plant growth (measured as biomass) and hypothesized (H3) that the invasive plants growth depends on the frequency of colonization by AMF and DSF.

METHODS

Study sites

This study was performed in the Nahuel Huapi National Park (NHHP), North-West Patagonia, Argentina (40°20′ S–41°35′ S; 71°02′ W–71°56′ W, 750 m a.s.l). The climate of the region is cold and temperate, with rainfalls and snow concentrated from April to September (autumn and winter) and ranging from about 2000 mm per year in the west to 200 mm per year in the east (Paruelo et al., 1998). This precipitation gradient generates a concomitant gradient of vegetation, from temperate forests to shrublands and steppe (Dimitri, 1977; Ezcurra & Brion, 2005). The soils are of volcanic origin (andisols) with a low degree of development and high capacity to stabilize organic matter, store water, buffer pH and retain phosphorus (Mazzarino et al., 1998; Satti et al., 2003).

We chose five temporary wetlands in a 34 km West–East-transect which represent a gradient of precipitations. The wetlands differed in the duration of the water presence (hydroperiod) from 4 months in dry years at the East of the transect to 8 months in wet years at the West of the transect (Table 1; Cuassolo & Diaz-Villanueva, 2019). The dry season begins in late spring and last until the beginning of autumn.

Field study

For the field study, the percentage of colonization by AMF and DSF in *P. anserina*'s roots was analysed. Twenty plants were collected in the same growing season, when the wetlands are dry or drying (November 2018) from each of the five wetlands. They were stored in hermetic plastic bags and carried to the laboratory. Random pools of roots were preserved in ethanol 50% to quantify AMF and DSF colonization. From each wetland, we collected five samples of surface soil (5–10 cm depth, ~50 g) with a shovel, from sites of *P. anserina* patches, stored them in hermetic plastic bags, and immediately carried them to the laboratory for organic matter, water and nutrient content determinations.

TABLE 1 Location and relevant characteristics of the five studied wetlands, taken from Cuassolo and Diaz-Villanueva (2019).

Wetland	Llao-Llao	Fantasma	Serena	Teleferico	Bernal
Location	41°03′ S–71°33′ W	41°05′ S–71°26′ W	41°06′ S–77°26′ W	41°07′ S–71°22′ W	41°08′ S–71°10′ W
Wetland area (ha)	2.2	1.1	17.8	0.4	1.5
Potentilla anserina coverage (%)	5	47	8	3	9
Hydroperiod (months)	6–8	5–7	5–6	5–7	4–6
Surrounding biome	*Nothofagus pumilio* forest	Mix forest	Mix forest	Mix forest	Steppe
Land use	None	Peri urban	Peri urban	Urban	Recreation

Experimental design

The greenhouse experiment was performed to quantify changes in the colonization of AMF and DSF and changes in plant nutrient content and growth, after a season of growth (4 months of growing) in the different soils studied. The growth of *P. anserina* in the different soils was measured as aerial biomass production at the end of the experiment. We used soil from the five sites described above. As a control treatment, we used an inorganic substrate, agricultural pearl (gravel; Lombriquen$^{(MR)}$), to avoid nutrient and organic matter supply. Soil samples from the five wetlands were collected (~10 kg) near the sites where *P. anserina* was observed and carried to the greenhouse. The samples were dried at room temperature for a week and then homogenized with a sieve of 4 mm. Small plants of *P. anserina* were collected from one of the wetlands (Fantasma, where the abundance of *P. anserina* is the highest) and carried to the laboratory. All plants were chosen from the same wetland to presume similar initial fungal colonization and be able to test the response to environmental variables. Aerial parts of the plants were removed and the roots were cleaned with tap water. Part of the roots collected were stained (as we described below) to measure the initial percentages of root colonization. Then, 1 g (fresh weight) of each root was planted in pots with 1 kg of sieved soil (10 pots per treatment). The experiment was carried out for 4 months, during the growing season (December 2018–April 2019, austral summer-autumn). All the treatments were irrigated daily and received the same amount of water so that the factor of soil moisture was avoided and only soil nutrient content was considered. The daily temperature ranged from 9.0°C to 27.2°C.

At the end of the experiment, aerial parts of *P. anserina* were dried at 60°C for 48 h and weighed to quantify growth (biomass). After this, aerial parts of *P. anserina* were used to measure the final nutrient content. The remaining roots were preserved in ethanol 50% for further analysis of AMF and DSF colonization.

Soil and plant analyses

A fraction of the sediments (without plant fragments, such as leaves or roots) was wet-weighed (WW) and dried at 105°C for 48 h to obtain the dry weight (DW). Soil moisture (M) was calculated as the difference between WW and DW and expressed as the percentage of soil DW. Then, each fraction was combusted at 550°C for 1.5 h to calculate organic matter (OM) as the difference between DW and ash.

For total soil carbon (C), nitrogen (N) and phosphorus (P) content, sieved samples were dried at 60°C. Carbon and N concentrations were

measured with a CN analyser (Thermo Finnigan Flash EA 1112). Total soil P was measured after combustion at 550°C for 1.5 h, followed by the ascorbate-reduced molybdenum method (APHA, 2005). Available P for plants (extractable P) was measured with the Olsen method (Sims, 2000). Soluble C and N were measured in soil leachates. For this, 30 g of soil from each site was mixed with 150 mL of nano-pure water and agitated on a shaker table for 1 h at 200 rev/min for 48 h at 4°C (Gabor et al., 2015). The obtained solution was filtered through a pre-combusted GF/F filter (0.7 μm pore size). Dissolved organic carbon (DOC) and total dissolved N (TDN) were measured using a carbon analyser (TOC–VCS Shimadzu).

To quantify tissue nutrient content (C, N, and P) of aerial parts and roots of *P. anserina*, the parts were dried at 60°C for 48 h and crushed to powder in a clean mortar. Carbon and N contents were measured using a CN analyser (Thermo Finnigan Flash EA 1112). Phosphorus content was measured after the combustion of plant material at 450°C for 1 h, followed by the ascorbate-reduced molybdenum method (APHA, 2005). Molar C:N, C:P, and N:P ratios were calculated based on these results. We compared C:N:P ratios of aerial parts after 4 months of growth.

Fungal analysis

The roots for fungal colonization analysis, both from the field and from the experiment, were cleared in KOH 10% (w/v), acidified with HCl 5% (v/v) and stained with trypan blue (Brundrett et al., 1994). They were then cut into 1 cm pieces and placed on slides. Typical structures of AMF colonization (hyphae, arbuscules and vesicles) and DSF (dark septate hyphae) were quantified according to their presence-absence at 90 fields using a direct microscope Olympus BX50 at 400×. Root segments were analysed according to the gridline-intersect method at 25× magnification (Giovannetti & Mosse, 1980). We quantified root colonization (presence-absence) in a random set of 10 plants per site and in all the treatments where the plants grew. Additionally, when functional structures of AMF (vesicles and arbuscules) were present, they were counted and expressed as the mean number of structures in 30 fields. As vesicles were many, they were classified in ranges (1: 1–25; 2: 25–50; 3: 50–75; 4: 75–100).

Statistical analysis

A principal components analysis (PCA) was performed to associate sites with environmental variables (organic matter, moisture, total C, N, and P, extractable C, N, and P) using the *FactoMiner* package (Lê et al., 2008) and also *factoextra* (Kassambara & Mundt, 2021). Nutrient concentration and endophytic fungi colonization among the treatments were compared using One-Way ANOVA. *Potentilla anserina* growth was compared among sites with an ANOVA on Ranks, due to the lack of normality in the distribution of data. Changes in endophytic fungi colonization (final time vs. initial colonization) in the experiment were compared with ANOVA. The models were fitted using the "*gls*" function from package "*nlme*" (Pinheiro et al., 2022). Multiple post-hoc comparisons with Bonferroni as the P-adjustment method were assessed with the function "*emmeans*" from the "emmeans" package (Lenth, 2022). Qualitative correlograms for the experiment were performed from the *ggplot2* package

(Wickham, 2016) and *corrplot* (Wei & Simko, 2021) from R version 4.2.3 (2022-03-10).

RESULTS

Environmental gradient and endophytic fungi field colonization

The wetlands differed in some of the environmental characteristics (Table 2). There was a significant gradient in soil moisture that coincided with the position in the longitudinal gradient (West–East). This distribution of sites was reflected in the PCA (89.7% explained variance), with Llao Llao, Fantasma, and Serena positively associated with PC1 (58.6% of explained variance), Teleferico to the negative values, and Bernal, the most eastwards wetland, the most negatively associated with PC1 (Figure 1). This distribution was explained not only by moisture but also by total N, C and OM. The PC2 (31.0% of explained variance) segregated sites according to extractable N (positively associated) and extractable P (negatively associated).

We confirmed the presence of AMF (Figure 2a) in all the roots examined, and DSF (Figure 2b) were present in all the sites except in Teleferico. In all the samples AMF were more abundant (intra-radical hyphae colonization >90%) than DSF (colonization <60%; Table 3).

AMF colonization (hyphae) was similar in all the sites (Table 3), and therefore not related to any of the environmental variables (Table 4). However, arbuscules and vesicles differed according to the site (Table 3). While arbuscles were only less abundant in Bernal, vesicles were positively related to total soil C, N, and moisture, though with low correlation coefficients (Figure 3, Table 4). The DSF colonization also differed among sites (Table 3) and was positively related to OM, extractable C, N, and P and negatively correlated to total soil P, with stronger coefficients (Figure 3, Table 4).

Potentilla anserina experimental colonization, nutrient contents, and biomass

The pattern of colonization at the end of the experiment was similar to that in the field: the abundance of AMF was higher than DSF (Figure 4) and there was a negative correlation between them (Table 5, Figure 5). However, the low adjustment indicates that this result should be carefully interpreted. Although initial roots were already colonized by AMF and DSF, after 4 months of growth in the different soils, AMF and DSF colonization decreased in some treatments. In the case of AMF, they decreased in all treatments except in Fantasma (from where the roots were extracted) and Teleferico, while DSF colonization significantly decreased in Serena and Bernal (Table 5, Figure 4).

Regarding nutrient content in plants, both soil N and P were positively related to aerial N:P (Figure 5). However, AMF colonization was not related to plant nutrient content, while DSF colonization was negatively related to aerial N and P, and positively related to C:P (Figure 5).

After 4 months of the experiment, *P. anserina* grew similarly in all the treatments (Table 6); even in control (perlite), plants reached the same biomass as in the other treatments (ANOVA on Ranks, $F = 5.79$, $p = 0.98$), indicating that *P. anserina* growth was independent of nutrient content in

TABLE 2 Soil characteristics of the five studied wetlands used also for the growth experiment.

Wetland	Organic matter (%)	Moisture (%)	C ($mg\,g^{-1}$)	N ($mg\,g^{-1}$)	P ($mg\,g^{-1}$)	N:P ratio	Extractable C ($\mu g\,g^{-1}$)	Extractable N ($\mu g\,g^{-1}$)	Extractable P ($\mu g\,g^{-1}$)
Llao-Llao	26.4 ± 2.6^{a}	74.1 ± 1.0^{a}	150.8 ± 1.3^{a}	11.3 ± 1.6^{a}	0.987 ± 0.006^{a}	25.5 ± 1.2^{a}	283.6 ± 1.9^{ab}	9.6 ± 0.2^{c}	32.4 ± 4.9^{b}
Fantasma	20.8 ± 1.1^{a}	51.6 ± 1.2^{b}	98.1 ± 7.9^{b}	8.5 ± 0.8^{ab}	0.769 ± 0.080^{a}	24.8 ± 2.3^{b}	259.4 ± 1.6^{ab}	7.8 ± 0.2^{d}	44.1 ± 1.9^{a}
Serena	25.4 ± 0.6^{a}	52.9 ± 0.7^{b}	79.3 ± 4.2^{b}	6.3 ± 0.2^{bc}	0.574 ± 0.080^{b}	24.3 ± 1.4^{b}	671 ± 15^{a}	37.8 ± 0.6^{a}	22.7 ± 0.1^{c}
Teleferico	10.6 ± 0.8^{b}	41.6 ± 2.2^{c}	47.3 ± 3.0^{cb}	4.3 ± 0.4^{cd}	1.179 ± 0.023^{c}	8.0 ± 0.9^{c}	153.4 ± 2.2^{b}	25.6 ± 0.4^{b}	38.7 ± 2.8^{b}
Bernal	10.7 ± 0.1^{b}	32.1 ± 3.4^{c}	18.1 ± 1.3^{c}	1.5 ± 0.8^{d}	1.236 ± 0.087^{c}	2.6 ± 0.5^{c}	60.5 ± 0.7^{b}	4.3 ± 0.1^{e}	15.1 ± 1.4^{c}
Perlite	–	–	–	–	–	–	0.43 ± 0	0.04 ± 0	0.5 ± 0.2
p value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
F value	31.4	5.74	23.56	18.48	13.15	7665.4	$H = 20.11$	2005.3	18.9

Note: (Mean ± standard error), letters upper cases indicate significant differences. Bold values emphasize p values < 0.05.

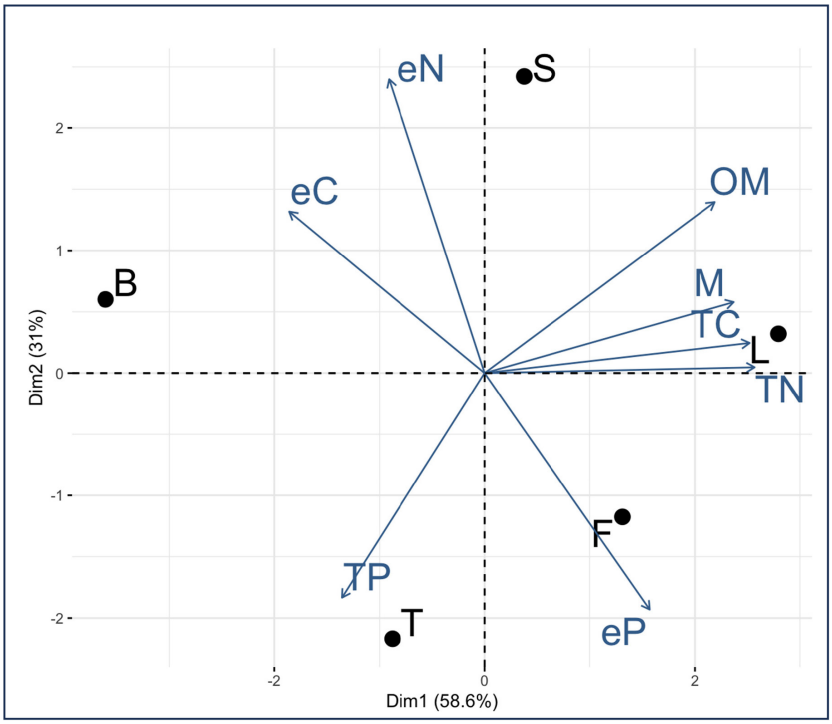

FIGURE 1 Ordination plot resulting from principal component analysis (PCA) for the five study sites (L: Llao-Llao, F: Fantasma, S: Serena, T: Teleferico, B: Bernal) based on environmental variables. The vectors correspond to soil characteristics (eC, eP, and eN, extractable Carbon, Posphorus, and Nitrogen; M, moisture; OM, organic matter; TN, total nitrogen; TP, total phosphorus).

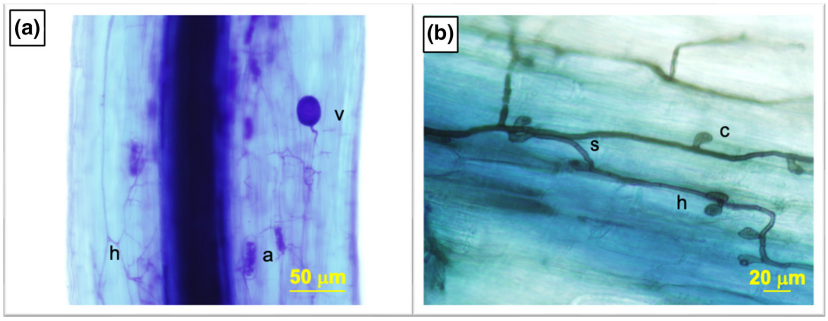

FIGURE 2 Microscope photograph of typical morphological structures of arbuscular mycorrhizal fungi (AMF) and dark septate fungi (DSF) in *Potentilla anserina* roots. (a): hyphae (h), vesicles (v) and arbuscules (a) of AMF; (b): melanized and septate hyphae (s: septum) and conidium (c) of DSF.

soils. Biomass was not related to AMF colonization (Figure 6a); however, a positive correlation was observed with DSF colonization (Figure 6b).

DISCUSSION

Our results showed that both AMF and DSF colonized the roots of *P. anserina* but responded differently to soil characteristics. Our first hypothesis was not confirmed for AMF, since AMF colonization was always high (~90%), independently of soil characteristics, but it was confirmed for DSF, which was positively related to soil OM and negatively related to total soil P. The presence of AMF in the roots of wetland plants is common (Cuassolo

TABLE 3 Field colonization in each wetland of both types of endomycorrhiza (AMF and DSF) and structures of AMF (arbuscules and vesicles; Mean ± standard error).

	AMF	Arbuscules	Vesicles	DSF
Llao-Llao	96.6 ± 6.7	3.1 ± 2.2^{b}	1.5 ± 0.3^{a}	15.0 ± 8.8^{b}
Fantasma	97.3 ± 4.3	10.8 ± 5.9^{ab}	1.1 ± 0.1^{ab}	23.9 ± 7.1^{b}
Serena	92.9 ± 6.7	15.2 ± 1.6^{a}	0.6 ± 0.3^{b}	57.8 ± 10.6^{a}
Teleferico	97.8 ± 3.8	8.1 ± 5.2^{ab}	0.8 ± 0.1^{b}	0.0 ± 0^{b}
Bernal	98.9 ± 1.9	0.6 ± 0.1^{b}	0.6 ± 0.1^{b}	4.4 ± 2.2^{b}
p value	0.67	**0.025**	**0.001**	**0.002**
F value	0.58	4.72	9.97	7.86

Note: Letters upper cases indicate significant differences. Bold values emphasize *p* values < 0.05.

TABLE 4 Results of the Pearson correlation analysis between environmental variables and biological variables (arbuscular mycorrhizal colonization, arbuscules and vesicles, and dark septate fungal colonization) in the field study.

	AMF		Arbuscules		Vesicles		DSF	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Total C	−0.122	0.628	−0.100	0.692	**0.590**	**0.010**	0.205	0.415
Total N	−0.116	0.647	−0.048	0.850	**0.532**	**0.023**	0.214	0.395
Total P	0.315	0.203	−0.133	0.600	0.133	0.599	**−0.782**	**<0.001**
ext-C	0.084	0.739	−0.295	0.234	−0.158	0.531	**0.800**	**0.001**
ext-N	−0.226	0.368	−0.151	0.550	−0.245	0.327	**0.520**	**0.027**
ext-P	0.147	0.561	0.292	0.240	0.021	0.933	0.040	0.880
OM	−0.270	0.279	−0.100	0.693	0.385	0.114	**0.571**	**0.013**
Moisture	−0.139	0.582	−0.175	0.487	**0.661**	**0.003**	0.194	0.441

Bold values emphasize r and *p* values < 0.05.

et al., 2012; Hu et al., 2020; Li et al., 2010; Marins & Carrenho, 2017; Ramirez-Viga et al., 2018; Wang et al., 2018), although the percentage of colonization may be low (<25%; Wang et al., 2018). Moreover, fungal colonization in wetlands had been assumed as unimportant before, since anoxic sediments were considered likely to result in the exclusion of fungal symbionts (Daleo et al., 2008). However, in our field results and a previous study (Cuassolo et al., 2012), root colonization of *P. anserina* by the AMF was high (80%–90% approx). One reason for this may be that *P. anserina* grows in temporary environments during the dry period (Cuassolo & Diaz-Villanueva, 2022) becoming senescent while it remains underwater (Cuassolo et al., 2012). The occurrence of AMF was found negatively correlated with water depth and duration of the flooding period (Dolinar et al., 2016; Fusconi & Mucciarelli, 2018; Gaberščik et al., 2017). Thus, *P. anserina* life trait (growing during the dry period) might confer the capability of bearing high AMF abundance.

However, although DSF was found as the dominant group in some species (Cuassolo et al., 2012; de Marins et al., 2009; Santillán-Manjarrez et al., 2019), they are usually less abundant than AMF (Bueno de Mesquita et al., 2018; Seerangan & Thangavelu, 2014), as confirmed by our field study and experiment. However, the role of DSF in plant fitness is poorly understood. Bueno de Mesquita et al. (2018) suggested a role in mineralizing inorganic N, but the interactions between DSF and AMF remain uncertain. While some authors found positive interactions among them (Ranelli et al., 2015; Scervino et al., 2009; Thangavelu & Raji, 2016),

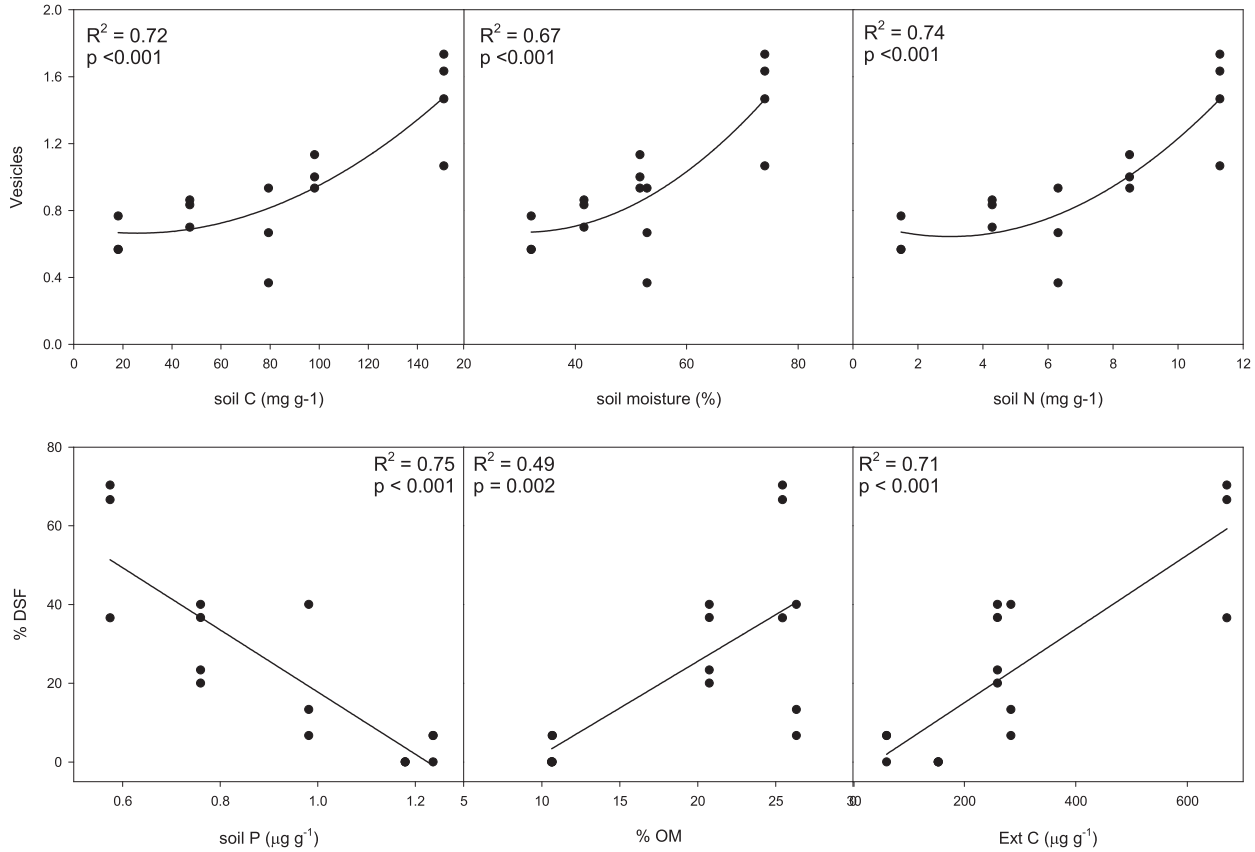

FIGURE 3 Relationships between the frequency of arbuscular mycorrhizal fungi (AMF) vesicles (above panels) and dark septate fungi colonization (below panels) and correlated environmental variables.

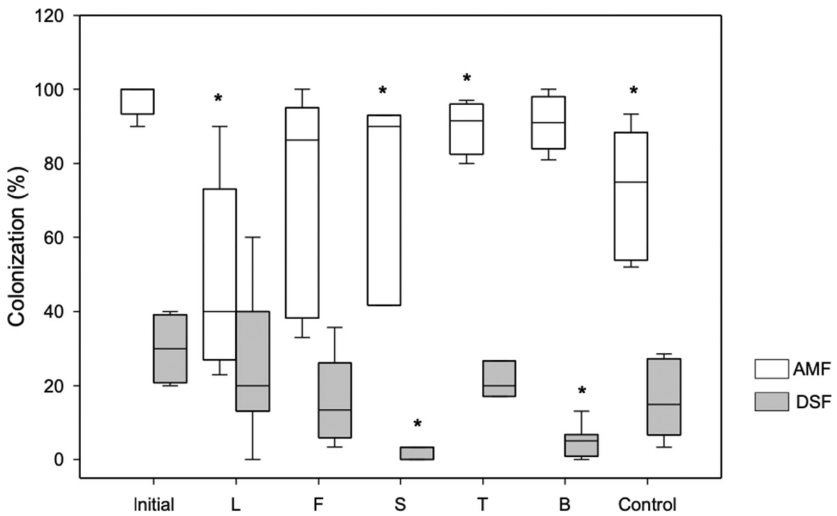

FIGURE 4 Initial and final experimental colonization percentages of each type of symbiont fungi (arbuscular mycorrhizal fungi [AMF] and dark septate fungi [DSF]), in the six treatments. References: L: Llao-Llao, F: Fantasma, S: Bahía Serena, T: Teleferico, B: Bernal. The (*) mean significant differences ($p < 0.05$). Medians and standard deviations are shown.

others found that AMF and DSF responded differently to the same environmental factors (Bueno de Mesquita et al., 2018; Gooden et al., 2019; Huo et al., 2021; Jones & French, 2021). The different patterns of colonization may be attributed to the fact that AMF are obligate symbionts. Although

TABLE 5 Colonization of both types of endomycorrhiza (AMF and DSF) and structures of AMF (arbuscules and vesicles) in the growth experiment, in the five treatments, and in control (perlite) after 4 months of growth (t_f; Mean ± standard error), and statistical results (ANOVA and ANOVA on ranks).

Treatments	AMF	Arbuscules	Vesicles	DSF
Llao-Llao	47.6 ± 9.6^{a}	0.9 ± 0.4	0.3 ± 0.2	25.7 ± 7.4^{a}
Fantasma	73.2 ± 11.9^{ab}	3.1 ± 2.1	0.3 ± 0.1	15.9 ± 5.1^{ab}
Serena	74.9 ± 16.6^{ab}	2.5 ± 1.5	0.4 ± 0.2	2.2 ± 1.1^{b}
Teleferico	90 ± 3.6^{b}	1.7 ± 1.0	0.5 ± 0.1	16.7 ± 4.9^{b}
Bernal	90.6 ± 2.5^{b}	4.2 ± 0.9	0.7 ± 0.1	4.9 ± 1.5^{b}
Perlite	65.4 ± 9.4^{ab}	2.5 ± 1.7	0.5 ± 0.2	14.8 ± 3.8^{b}
p value	**0.003**	0.17	0.21	**0.004**
F/*H* value	$F = 4.67$	$H = 7.61$	$H = 7.10$	$F = 4.37$

Note: Letters upper cases indicate significant differences. Bold values emphasize *p* values < 0.05.

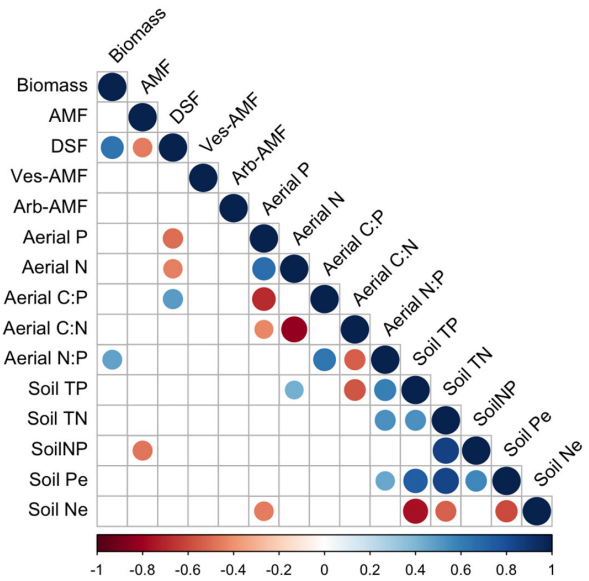

FIGURE 5 Qualitative correlograms with experimental fungi colonization, biomass production, aerial nutrient content, and soil nutrient content. The size of the circles and colour scale portray each correlation coefficient; 1 denotes a perfect positive correlation and –1 is a perfect negative correlation. All correlation coefficients with a *p*-value >0.05 are white boxes (non-significant).

the extraradical mycelium may develop well in soil, AMF usually respond to plant species more than to environmental variables (Ranelli et al., 2015). Our results would suggest that DSF seem to be more dependent on environmental variables. In fact, in our study, DSF was positively related to soil OM while AMF was not, suggesting that DSF may be more dependent on soil OM and AMF on plant sources, at least in the range of OM values found in our sites. In consequence, the results obtained up to now indicated that more research is needed to clarify the ecological interaction between these symbionts. As most research focuses on the effects of AMF on plants, the role of DSF still needs more investigation (Albornoz et al., 2022) as well as the role of these types of mycorrhizae across terrestrial and aquatic environments.

The relations between fungi and soil nutrients were not consistent between the field survey and the experiment. This could be due, on one hand,

TABLE 6 Aerial biomass of *Potentilla anserina* in the five soil treatments and perlite (control) and molar nutrient ratios (C:N, C:P and N:P) of aerial parts after 4 months of growth.

	Biomass (g)	C:N	C:P	N:P
Llao-Llao	0.63 ± 0.43	30.0 ± 4.2^{ab}	667 ± 49.5^{a}	27.7 ± 2.2 ^{ab}
Fantasma	0.51 ± 0.19	29.0 ± 6.8 ^{ab}	406.7 ± 27.7^{b}	15.3 ± 3.4 ^{b}
Serena	0.48 ± 0.25	21.3 ± 2.7 ^{b}	538.3 ± 67.7^{ab}	25.3 ± 0.9 ^{a}
Teleferico	0.80 ± 0.62	29.0 ± 2.5 ^{ab}	662.7 ± 81.9^{a}	23.0 ± 0.6 ^{ab}
Bernal	0.77 ± 0.17	17.0 ± 1.5 ^{b}	345.0 ± 46.6^{b}	20.0 ± 1.5 ^{ab}
Perlite	0.55 ± 0.16	39.0 ± 3.6 ^{a}	666.7 ± 123.5^{a}	17.0 ± 2.5 ^{ab}
F value	5.79	6.14	3.85	3.39
p value	0.98	**0.007**	**0.026**	**0.039**

Note: (Mean ± standard error), and results of the One-Way ANOVA. Letters upper cases indicate significant differences. Bold values emphasize *p* values < 0.05.

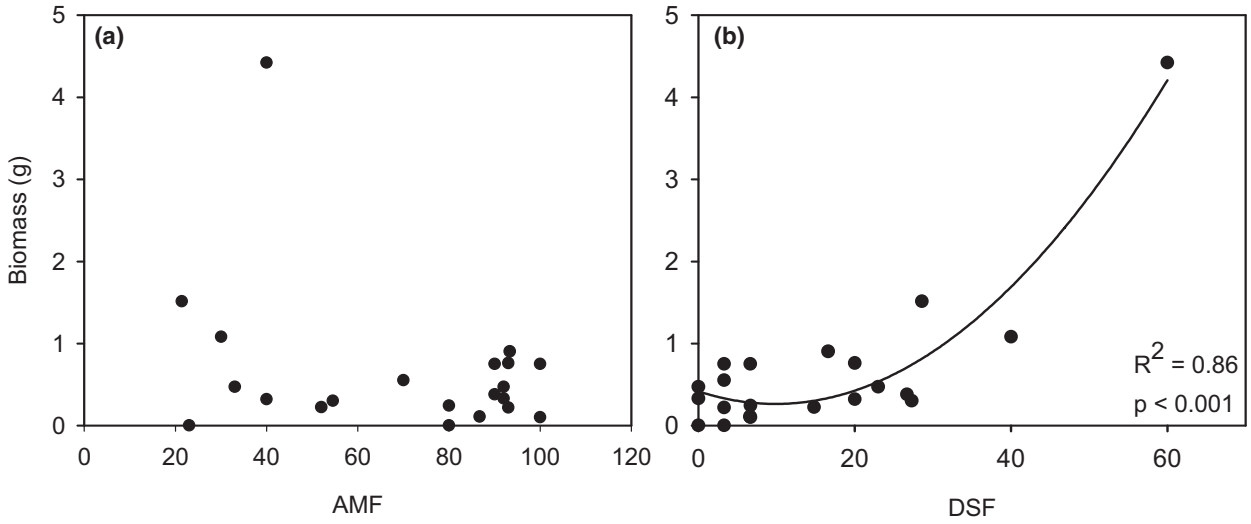

FIGURE 6 Biomass production as a proxy of growth related to arbuscular mycorrhizal fungi colonization (a) and dark septate fungi [DSF] colonization (b).

to differences in environmental conditions between the field and the experiment; for example, soil moisture was similar in all treatments in the experiment, while in the field there was a significant gradient of moisture. The moisture gradient (from 32% to 74%) in the field may have had a crucial impact on the role of AMF in plant development, though there were no differences in AMF root colonization. Recent studies demonstrated that AMF can benefit plant growth and phosphorus uptake mostly under low water availability (Frew, 2023; Hu et al., 2020; Ramirez-Viga et al., 2018). On the other hand, all treatments in the experiment began from roots taken from only one place, to assume that the initial inoculum was similar. The sympatric combination of roots and soil resulted in better growth (in Fantasma), which coincided with previous results (Remke et al., 2021), in which the authors suggested that sympatric pairs (plants-AMF) optimize benefits and minimize the costs of the symbioses.

Also, the experiment allowed testing the effect of AMF and DSF on nutrient acquisition and plant growth in soils with different nutrient content. In this regard, we also rejected the second hypothesis of higher nutrient acquisition in plants with higher AMF and DSF. We found no relation between

AMF and plant nutrients, and even higher aerial N and P content in plants with lower DSF colonization (Figure 6). This result could reflect that when the host plant can obtain nutrients from the soil, DSF colonize less (Daleo et al., 2008; Kiers et al., 2011; Olsson & Tyler, 2003).

The similar growth of *P. anserina* in the treatments, even in the control treatment, suggested a high nutrient reserve allocated in the roots, which was used for growth. In a previous study, Cuassolo et al. (2012) found that when the wetland is flooded, root C:P decreases, and when it dries out, *P. anserina* begins to sprout and the P stored in the roots is sent to the new shoots. This strategy gives this species the advantage of being P enriched when the growing season begins. Many studies demonstrated that plants colonized by AMF grow larger than uncolonized ones (Hoeksema et al., 2010; Sudová, 2009; Treseder, 2013); the lack of difference in plant growth from soil treatments in our experiment could be attributed to the fact that we did not compare the growth of plants with and without mycorrhizae. The majority of vascular plants had high AMF colonization (72%; Brundrett, 2004), and host plants can support high colonization only if they benefit either in growth or in nutrient acquisition (Daleo et al., 2008). So, the high root colonization by AMF would indicate that *P. anserina* benefited from the mutualistic interaction. Besides, DSF was positively related to growth, which may indicate that the presence of this endophyte, though less abundant than AMF, confer the plant with some benefit to grow, or that higher plant growth benefits DSF colonization.

The colonization of AMF and DSF in the roots of a wetland plant is differently related to abiotic factors. While AMF was much more abundant than DSF in *P. anserina* roots, the role of DSF might be more critical in plant growth and nutrient acquisition. However, the interactions between these endophytic fungi still need more investigation. Further studies should also address the importance of these two groups of fungi in the invasion success of *P. anserina* in Patagonian wetlands, as the presence of endophytes may help to explain the wide distribution of this exotic species.

AUTHOR CONTRIBUTIONS

Florencia Cuassolo: Conceptualization (lead); data curation (lead); formal analysis (lead); methodology (lead); writing – original draft (lead). **Verónica Díaz-Villanueva:** Funding acquisition (lead); supervision (lead); writing – review and editing (lead).

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CONFLICT OF INTEREST STATEMENT

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

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author on reasonable request.

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