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Fungal root symbionts and their relationship with fine root proportion in native plants from the Bolivian Andean highlands above 3,700 m elevation

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Abstract Here, we examined the colonization by fungal root symbionts in the cultivated Andean grain *Chenopodium quinoa* and in 12 species that dominate plant communities in the Bolivian Altiplano above 3,700 m elevation and explore for the possible relationships between fungal colonization and fine root proportion. The 12 most abundant species in the study area were consistently colonized by AMF and DSE. In contrast, the annual Andean grain *C. quinoa* showed negligible or absence of mycorrhizal fungi colonizing roots. On the other hand, *C. quinoa*, *Junelia seriphioides* and *Chersodoma jodopappa* were infected to a varying degree by the root pathogen *Olpidium* sp. We observed no relationship between AMF and DSE colonization and proportion of fine roots in the root system, but instead, the ratio between DSE and AMF colonization (ratio DSE/AMF) negatively related with proportion of fine roots. Our findings support the hypothesis regarding the importance of DSE at high altitudes and suggest a functional relationship between the rate of DSE/AMF and proportion of fine roots. The colonization by the root pathogen *Olpidium* sp. in *C. quinoa* deserves further study since this Andean grain is increasingly important for the local economy in these marginal areas.

Keywords Arbuscular mycorrhizas · Dark septate endophytes · Root traits · *Olpidium* sp. · High altitude plant communities · Quinoa · Highlands · Altiplano · Andes · Bolivia

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Introduction

Most terrestrial plant species harbor fungal symbionts in their roots. These symbionts range from mutualists to parasites, and among them, mycorrhizal fungi are the most widely distributed partners (Smith and Read 2008). Among other functions, mycorrhizal fungi are generally associated with the provision of limiting soil nutrients to plants in exchange for carbon from photosynthesis (Smith and Read 2008). Read (1993) depicted the global patterns of mycorrhizal distribution among major biomes of the world and suggested that different mycorrhizal types dominate in different biomes. Under this scheme, arbuscular mycorrhizal colonization, the most widely distributed type of mycorrhizal symbiosis, decreases with increasing latitude and altitude being replaced by other type of mycorrhizas. Moreover, it has been proposed that above 3,000 m elevation ecosystems are dominated by species that do not establish mycorrhizal symbiosis. Recent studies, however, showed that some plant species occurring above 3,500 m elevation are indeed colonized by mycorrhizal fungi and that they might depend on these symbionts for nutrient uptake (Barnola and Montilla 1997; Schmidt et al. 2008). In the Peruvian Andes, at elevation ranging from 4,700 to

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5,400 m, Schmidt et al. (2008) observed that among 18 plant species, eight of them were colonized by AMF and dark septate endphytes (DSE), three only by AMF, three by DSE, and four by no fungi. In the Venezuelan páramo at 3,800 m elevation, Barnola and Montilla (1997) also observed the presence of both AMF and DSE in certain herbaceous species. Dark septate endophytes are a widespread group of root symbionts (Mandyam and Jumpponen 2005) generally belonging to Helotiales (Ascomycota) (Upson et al. 2009) and Pleosporales (Ascomycota) (Jumpponen and Trappe 1998) that range from mutualism to parasitism (Jumpponen 2001; Mandyam and Jumpponen 2005). Whether they should be considered mycorrhizal or not, still remains controversial (Smith and Read 2008). Nevertheless, it has been shown that this type of symbionts might be nutritionally important to plants in adverse environments (Haselwandter and Read 1982; Newsham 1999; Barrow and Osuna 2002). Moreover, it has been suggested that DSE replace the function of AMF with increasing altitude and latitude (Haselwandter and Read 1980; Treu et al. 1996).

The mycorrhizal status of plants depends on evolutionary and ecological constraints (Brundrett 2002). Some plant lineages occur in adverse environments such as saline soils, arid habitats or very cold climate in which they are not associated with mycorrhizal fungi (Brundrett 2009). In these habitats that are generally limited in soil resources, plants have developed particular adaptative traits. Because the acquisition of essential resources from the soil volume is performed by small absorbing roots, the so-called "fine roots" (Jackson et al. 1997), it has been suggested that plants with high proportion of fine roots would be less dependent on mycorrhizal fungi to effectively access limiting soil resources (Brundrett 1991). However, studies examining the possible relationship between root traits and AMF and/or DSE in adverse environments are still scarce. This could perhaps be attributed to the fact that ecosystems subjected to strong abiotic constraints are believed to be dominated by non-mycorrhizal species (Brundrett 2009; Newsham et al. 2009). This is the case of the Andean Bolivian highlands known as Altiplano in which most of the extension is characterized by harsh environments. In particular, the area surrounding the Uyuni's basin combines high elevation (above 3,700 m elevation) with aridity and frost (Geerts et al. 2006). Furthermore, the soils present severe limitations of nitrogen and phosphorous (Cardenás and Choque 2008).

In spite of these constraints, this region has been traditionally subjected to the extensive cultivation of the "Quinoa" (*Chenopodium quinoa* Willd.), an Andean grain with exceptional nutritional value (National Research 1989). *Chenopodium quinoa* belongs to Chenopodiaceae, a family consisting mostly of non-mycorrhizal species. The *C. quinoa* fields are immersed in a matrix of semiarid vegetation characterized by shrubby plant communities belonging to the families Fabaceae, Asteraceae, Solanaceae and Verbenaceae together with some Poaceae. Previous studies have shown the presence of arbuscular mycorrhizal spores in soils from some specific areas of the Bolivian Altiplano (Sivila de Cary and Angulo 2006). Nevertheless, studies on mycorrhizal colonization and root traits of native species have not been conducted.

The two objectives of this study were to (1) quantify the colonization by fungal root symbionts and (2) examine the relationships between fungal colonization (AMF, DSE and their ratio) and fine root proportion in the cultivated "quinoa" and in native perennial plant species that dominate the surrounding plant communities.

Materials and methods

Study area

The study was conducted in four sites viz. Jirira Arena (19°51′58.0" S, 67°34′57.2" W, 3,740 m), Jirira Ladera (19°50′13.4" S, 67°33′58.5" W, 3,770 m), Hizo (19°39′11.2" S, 68°19′05.2" W, 3,690 m), and Chacoma (19°41′25.2" S, 68°25′36.0" W, 3,720 m) located on the northern rim of the Salar of Uyuni. The study area is covered by two meteorological stations: Salinas de Garci Mendoza (19°38′S, 67°40′W) and Llica (19°50′ S, 68°15′ W) managed by the SENAMHI (Meteorology and Hydrology National Service, Bolivia). Annual precipitation decreased from the East to the West of the region with 280 mm at Salinas de Garci Mendoza (over 1998–2004 period) and 125 mm at Llica (over 1991–1998) with 90% of the rainfall during the December-March months. The annual average temperature (8.3°C at Salinas de Garci Mendoza) hides daily thermal amplitudes higher than seasonal amplitudes, reaching up until 25°C leading to frost risks throughout the year with 200 days with daily minimum under 0°C. Soils are characterized by sandy textures and a very low content of organic matter (Table 1).

Species selection and collection

The native vegetation of this tropical Andean ecosystem consists of a mountain steppe of herbaceous and shrubby species traditionally used as pastures but progressively encroached by the recent and rapid expansion of quinoa cultivation (*C. quinoa* Willd.). Twelve dominant (i.e., most abundant) perennial species were studied. The species were selected in order to include the most abundant species in each area (see Table 2). In addition, in three of these sites, cultivated *C. quinoa* was also sampled.

Table 1 Soil characteristics of the study sites (n=4)

	Coarse fraction (%) >2 mm	Fine fraction (%)			*N* %	*C* %	Organic matter %
		Clay	Silt	Sand			
Jirira Arena	13.0	14.4	3.2	82.4	0.019	0.232	0.399
Jirira Ladera	11.9	19.3	19.5	61.2	0.026	0.315	0.542
Chacoma	10.1	16.5	5.5	78.0	0.048	0.556	0.956
Hizo	14.7	19.3	12.4	68.3	0.029	0.345	0.593

For each species, four well developed individuals grown in unshaded areas and in relative isolated positions were carefully dug up with a shovel to a depth of 30 cm in the peak of the growing season (i.e., 13–15 January, 2007). Root systems were placed in plastic bags together with surrounding soil. In the laboratory, they were separated from the shoots and cleaned using a fine stream of water in order to remove soil and organic matter particles. Non-attached roots which could have been broken during sampling and processing or which could have come from another individual or species were removed. Root systems were stored in ethanol 50% (volume/volume) at 4°C for later assessment of fungal colonization.

Assessment of fungal colonization in roots

We assessed fungal colonization in four individuals of each plant species in each site with the exception of *Nasella pubiflora* of which one individual was examined. Roots were separated and washed with water. All dead and damaged roots were discarded. All thin roots (<2 mm without apparent suberin) that could be potentially colonized were cleared and stained following Grace and Stribley (1991). They were then mounted on semi-permanent slides in polyvinyl-lactic acid-glycerol. Root endophyte quantification was made by the magnified intersection method (McGonigle et al. 1990) using a compound microscope (*Nikon* Eclipse E200), magnification ×200. Eighty to 100 intersections per slide (i.e., *per* individual) were scored depending on sample size. Percentage of AMF colonization (total, vesicle, coil and arbuscule) and DSE colonization (brown septate hyphae and sclerotia) was assessed as the proportion of total root intersections that were colonized and was calculated as follows:

Fungal colonization=100 × (number of intersections with fungal structure/total number of intersections counted)

During endophyte quantification we observed several resting sporangia of *Olpidium* sp. (Chytridiomycota) in certain species. They were also quantified in the same manner described above.

Assessment of fine roots

Additional root clusters for certain species were collected using the same procedure (carefully dug up with a shovel to a depth of 30 cm) for *Fabiana densa (*Jirira ladera, Jirira arena and Chacoma sites), *Baccharis incarum* (Jirira ladera

Table 2 Cover of perennial species in the study sites

Family	Species	Jirira Arena	Jirira Ladera	Chacoma	Hizo
Fabaceae	***Adesmia spinosissima***	9.7	1.1	0.3	0.1
	A miraflorensis			0.1	
Asteraceae	***Baccharis incarum***	2.9	1.3		
	B. boliviensis		0.8		
	C. jodopappa		0.8		
	Chuquiraga atacamensis			0.1	
	Parastrephia lepidophylla		0.1		16.4
	P. quadrangularis			4.7	3.3
	Senecio graveolens			0.1	
Solanaceae	***F. densa***	26.5	33.2	3.5	
Verbenaceae	***J. seriphioides***	0.9			
	L. castellani			10.3	0.7
Poaceae	***Nassella publifora***		0.1		
	S. plumosa			0.1	
	S. leptostachya	0.1	0.1		
Frankeniaceae	*Frankenia triandra*				0.2
Total		40.1	37.5	19.2	20.7

Data are expressed in percent of ground cover. The species studied here are in bold

and Jirira arena), *Lampaya castellani* (Chacoma and Hizo), *Junellia seriphioides* (Jirira arena), S*tipa leptostachia* (Jirira arena), *Parastrephia lepidophylla* (Hizo) and *Parastrephia quadrangularis* (Chacoma). For each species, two root clusters were placed in plastic bags together with surrounding soil and stored at 4°C before processing.

Roots were stained and prepared according to Roumet et al. (2006) before scanning on a flatbed scanner at a resolution of 600 dpi (Acer Scan 300F, 6684 03A). Total root length and root length by diameter classes was determined using the software DELTA - T SCAN (Delta-T Devices, Burwell, Cambridge, UK). The percentage of fine roots was calculated as the ratio of root length with a diameter of less than 0.2 mm to the total root length.

Statistical analysis

We calculated the mean and standard error for each fungal variable in each species in each site. For species occurring in two or three sites, we compared colonization rates between sites by using *T* test and ANOVA respectively. We also compared the effect of family and sites and their interaction, with species nested within family, by using two-way ANOVA. Data showed non-normal distribution that could not be corrected by log-transformation, therefore they were rank-transformed and the analyses were run on the rank data (Zar 1999). In all the cases non-parametric analyses yielded the same conclusions as parametric ANOVAs run on the untransformed data, suggesting that it had sufficient power (Zar 1999). We also performed regression analyses between AMF and DSE colonization means and proportion of fine roots in certain species. In these analyses the variables reached the assumptions of normal distribution and homogeneity of variance. All analyses were carried out with the *Infostat* Statistical Package (Di Rienzo et al. 2008).

Results

The twelve most abundant species in the study area were consistently colonized by AMF and DSE (Fig. 1a–c). In contrast, the annual Andean grain *C. quinoa* showed negligible or no mycorrhizal fungi colonizing roots.

On the other hand, *C. quinoa*, *J. seriphioides* and *Chersodoma jodopappa* were infected to a varying degree by the root pathogen *Olpidium* sp. (Chytridiomycota) (Fig. 1d) (Table 3).

When comparing root colonization in those species that occur in more than one site, only *Baccharis incarum* showed significant differences: DSE colonization in *B. incarum* from Jirira Arena was significantly higher than in Jirira Ladera (Table 3, P=0.0168). In contrast, *L. castellani*, *Stipa leptostachya and F. densa* showed no differences in the rates of colonization for all the variables with the only exception of colonization by arbuscules in *L. castellani* (P= 0.0024) although the values were very low (Table 3).

The analyses on the effects of sites and family on fungal colonization rates revealed that there were differences between families for all variables, except for AMF coil

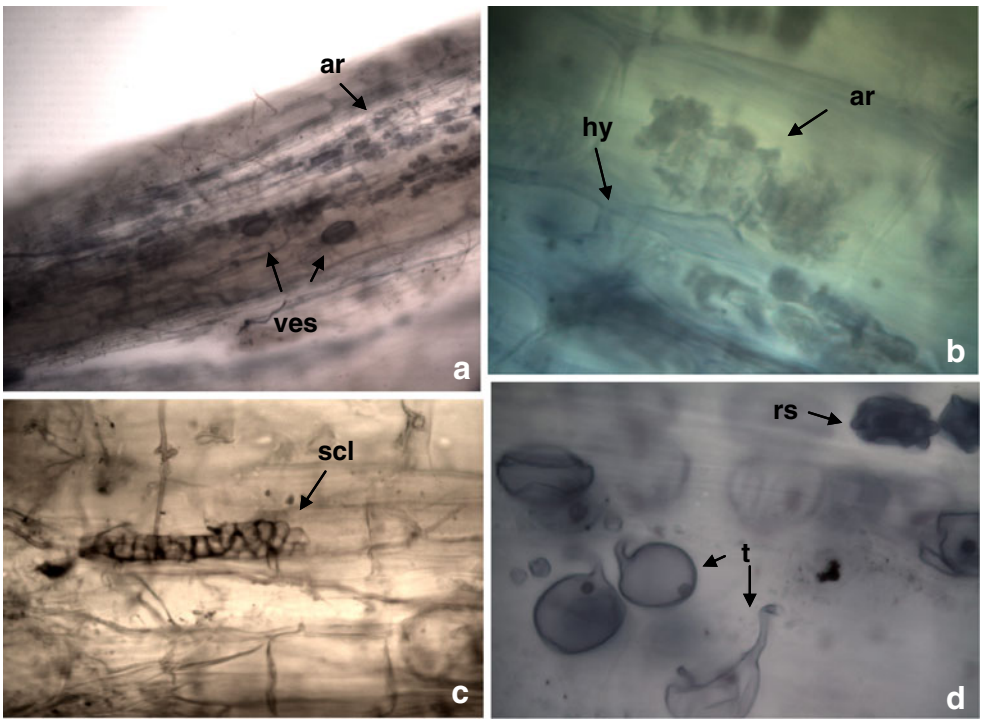

Fig. 1 Fungal root symbionts in native plants from the Bolivian Altiplano. **a** Vesicles (*ves*) and arbuscules (*ar*) in *Parastrephia lepidophylla*; **b** detail of arbuscule and hypha (*hy*) in *Parastrephia lepidophylla*; **c** sclerotia (*scl*) of dark septate endophytes in *Chersodoma jodopapa*; and **d** resting spores (*rs*) and thallus (*t*) of *Olpidium* sp. in *C. quinoa*

Table 3 Fungal colonization (percent root length) of native perennial species and *C. quinoa* annual cultivated species (n=4)

Family	Species	Site	Total mycorrhizal colonization (%)		Vesicular colonization (%)		Coil colonization (%)		Arbuscular colonization (%)		Dark septate endophyte colonization (%)		Olpidium colonization (%)	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fabaceae	*Adesmia spinosissima*	Jirira Ladera	48.3	6.6	5.5	0.5	0.0	0.0	0.0	0.0	20.7	3.9	0.0	0.0
Asteraceae	*Bacharis incarum*	Jirira Arena	45.6	25.4	5.5	10.3	1.4	1.2	3.0	0.8	68.3	9.1	0.0	0.0
	B. incarum	Jirira Ladera	42.8	13.7	5.8	3.9	0.6	0.7	4.5	5.2	34.2	17.1	0.0	0.0
	C. jodopappa	Jirira Ladera	33.4	19.3	4.4	3.1	0.3	0.5	0.9	1.7	47.1	32.6	0.8	1.5
	Chuquiraga atacamensis	Chacoma	57.1	18.2	6.1	7.6	1.1	1.5	5.5	7.7	40.3	12.5	0.0	0.0
	Parastrephia lepidophylla	Hizo	69.0	9.8	10.0	4.4	1.7	2.7	5.9	4.4	38.5	17.0	0.0	0.0
	P. quadrangularis	Chacoma	70.0	11.9	12.0	7.0	2.0	0.9	4.2	3.1	60.1	19.3	0.0	0.0
Solanaceae	*Fabiana densa*	Chacoma	12.0	11.2	1.5	1.9	0.8	1.0	2.2	2.1	32.9	26.8	0.0	0.0
	F. densa	Jirira Arena	17.0	13.8	2.6	2.1	1.6	1.8	1.3	1.5	23.6	10.2	0.0	0.0
	F. densa	Jirira Ladera	22.3	4.2	1.0	1.2	0.7	1.0	3.3	3.1	57.4	25.4	0.0	0.0
Verbenaceae	*J. seriphioides*	Jirira Arena	35.6	29.0	8.1	12.4	3.0	3.3	2.5	1.7	42.8	25.2	8.7	16.7
	Lampaya castellani	Chacoma	76.6	22.8	21.2	7.3	0.9	1.0	0.0	0.0	65.3	29.5	0.0	0.0
	L. castellani	Hizo	68.6	15.5	14.9	9.3	0.6	1.3	2.2	2.0	44.3	20.5	0.0	0.0
Poaceae	*Nassella publiflora*	Jirira Ladera	13.9	-	1.4	-	0.0	-	0.0	-	52.8	-	0.0	-
	Stipa leptostachya	Jirira Ladera	6.6	0.9	0.0	0.0	0.0	0.0	0.4	0.7	28.3	33.0	0.0	0.0
	S. leptostachya	Jirira Arena	6.5	5.3	0.3	0.6	0.8	1.0	0.0	0.0	9.8	7.4	0.6	0.6
	S. plumosa	Chacoma	16.6	10.8	5.2	6.3	0.0	0.0	0.0	0.0	25.7	30.5	0.0	0.0
Chenopodiaceae	*C. quinoa*	Chacoma	0.7	0.8	0.3	0.6	0.0	0.0	0.0	0.0	0.3	0.6	1.6	1.3
	C. quinoa	Hizo	1.3	1.9	1.0	2.0	0.0	0.0	0.0	0.0	0.3	0.5	30.0	21.3
	C. quinoa	Jirira Ladera	0.3	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.6	7.9

colonization (Table 4). In contrast, there were no differences between sites (Table 4). Verbenaceae, Asteraceae and Fabaceae evidenced the highest rates of total AMF colonization, Solanaceae intermediate, while Poaceae and Chenopodiaceae the lowest rates (Fig. 2a). With regard to DSE colonization, Verbenaceae, Asteraceae, Solanaceae and Poaceae showed the highest values, followed by Fabaceae which had intermediate levels. In Chenopodiaceae DSE were almost absent (Fig. 2b).

Regressions analyses between AMF and DSE colonization and the percentage of fine roots were not significant (R=0.09, P=0.3923 and R=0.09, P=0.4090 respectively). However, the ratio between DSE and AMF colonization (ratio DSE/AMF) negatively related with fine roots (R=0.54, P=0.0104) (Fig. 3). In some cases, differences in proportion of fine roots were greater within species occurring in different sites than between different species (Fig. 3, Supplementary material S1).

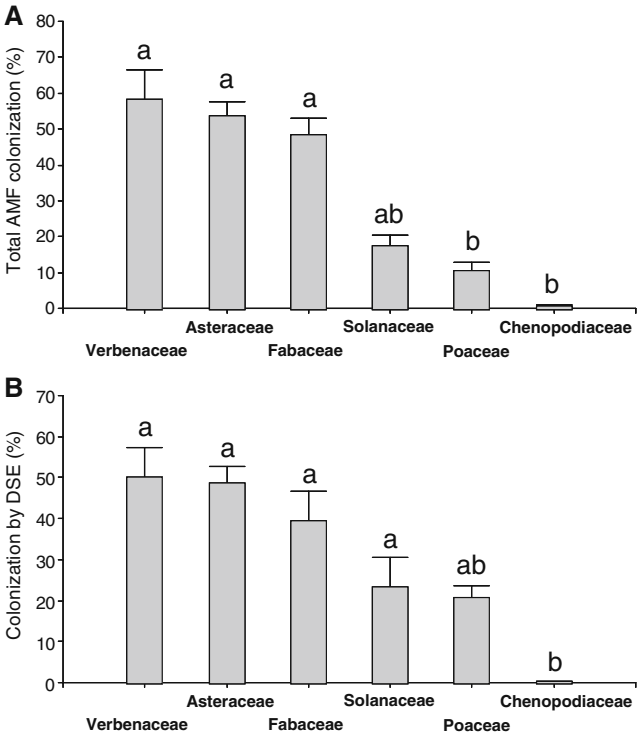

Fig. 2 Colonization by arbuscular mycorrhizal fungi (**a**) and dark septate endophytes (**b**) in plant families occurring in the Bolivian Altiplano. *Bars* with the same letters are not significantly different (Tukey test, P<0.05)

Discussion

In this study we assesed for the first time the mycorrhizal status of twelve dominant perennial species of the Bolivian Altiplano and of the annual cultivated Andean grain *C. quinoa*. With the exception of *C. quinoa*, roots of all the species were consistently colonized by AMF and DSE.

From a phylogenetic point of view, our results are in accordance with previous findings regarding the mycorrhizal status of certain plant lineages. That is, Fabaceae, Asteraceae, Verbenaceae, Solanaceae and Poaceae are reported to be mycorrhizal lineages while Chenopodiaceae has been predominantly reported as non-mycorrhizal (Brundrett 2009). With the exception of *Adesmia spinosissima*, *Nasella publiflora* and *Stipa plumosa*, all arbuscular mycorrhizal species had arbuscules and/or coils in their roots. In addition to the well known wide distribution of AMF, our study supports the suggestion that DSE are also widely distributed (Mandyam and Jumpponen 2005; Newsham et al. 2009). From an ecological point of view however, our results do not support the proposition that above 3,000 m altitude ecosystems are dominated by non-mycorrhizal species. Thus, these results argue against a global pattern of altitudinal distribution of mycorrhizas (Ruotsalainen et al. 2004). Rather, their distribution may depend on the combined effect of altitude and latitude. For example, in the Peruvian tropical Andes, Schmidt et al. (2008) only found roots with no AMF structures above 5,300 m, even in Asteraceae, a well known mycorrhizal family. These plant species were, however, colonized by DSE leading the authors to suggest an important role for these symbionts at this altitude. In addition, in the páramo

Table 4 Results of two-ways ANOVA on the effects of family and site (d.f.=5 and 3, respectively) on fungal colonization (percent root length)

Variable	Source of variation					
	Site		Family		S × F	
	F	*P*	*F*	*P*	*F*	*P*
Total AMF colonization	0.24	0.8706	9.17	0.0056	0.62	0.6510
AMF Vesicle colonization	0.02	0.9954	4.26	0.0425	1.21	0.3166
AMF Coil colonization	1.08	0.3665	2.48	0.1344	0.09	0.9840
AMF Arbuscule colonization	1.22	0.3097	5.24	0.0255	1.56	0.1972
DSE colonization	0.40	0.7561	16.45	0.001	3.41	0.0143

Data were rank transformed

AMF arbuscular mycorrhizal fungi, *DSE* dark septate endophytes

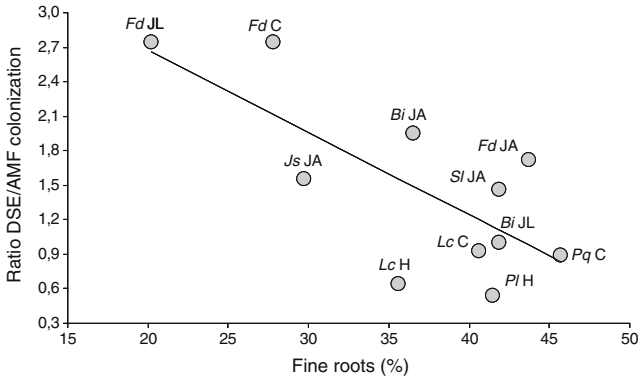

Fig. 3 Relationship between proportion of fine roots and the ratio of DSE/AMF in a set of species in the Uyuni's basin (R=0.54, P= 0.0104). Abbreviations: *Fd JL Fabiana densa* in Jirira ladera, *Fd C Fabiana densa* in Chacoma, *Fd JA Fabiana densa* in Jirira arena, *Bi JL Baccharis incarum* in Jirira ladera, *Bi JA Baccharis incarum* in Jirira arena, *Lc C Lampaya castellani* in Chacoma, *Lc H Lampaya castellani* in Hizo, *Js JA J. seriphioides* in Jirira arena, *Sl JA* S*tipa leptostachia* in Jirira arena, *PL H Parastrephia lepidophylla* in Hizo, *Pq C Parastraphia quadrangularis* in Chacoma

in Venezuela, some species occurring at 3,700 m elevation also harbor AMF in their roots (Barnola and Montilla 1997). Our results also suggest that in the Bolivian tropical Andes the altitudinal limit for AMF is higher than previously proposed. In our study, most of the species occurring between 3,700 and 4,000 m were consistently colonized by both AMF and DSE. Therefore, we asked whether the dual colonization by these fungal symbionts may have some functional implications. As a first approach, we looked for possible relationships with a relevant root-functional trait. We examined the possible relationship between fungal symbionts rates of colonization and the proportion of fine roots in a selected set of species, but we found no significant regression between AMF and DSE and fine roots. However, a significant negative relationship between the DSE/AMF ratio and proportion of fine roots was observed. This interesting finding suggests that in this adverse environment, which combines aridity, cold temperature, and poor nutrient content it is the relationship between the root endophytes rather than the colonization by AMF or DSE per se, that better predicts the functional implications of the fungal-root symbiosis. In other words, in these species, which are consistently colonized by AMF and DSE, a lower proportion of fine roots would be compensated by an important increase in DSE without important changes (or perhaps a decrease) in AMF colonization. Importantly, this set of data suggests that the antagonism between these fungal symbionts might have functional implications for the plant. It is known that plants can respond to increasing levels of water and nutrient availability by fine root proliferation (Pregitzer et al. 1993). Therefore, differences in the proportion of fine roots could be site- or patch-specific and influence fungal colonization. This could be seen not only among species but also within the same species growing in different sites. For example, in *F. densa* growing in different sites, when the proportion of fine roots was high, the colonization of DSE was low (i.e., Jirira arena, see Fig. 3). Inversely, when proportion of fine roots was less than half than in that case, DSE doubled the rates of colonization without important changes in AMF colonization (i.e., Jirira ladera and Chacoma, see Fig. 3). To a lesser degree, this pattern was also observed for *Baccharis incarum*. Whether this pattern extends to other high altitude plant communities and relates to resource acquisition by plants in these stressful ecosystems still remains an open question. Nevertheless, the evidence provided here support the hypothesis regarding the importance of DSE in altitudinal plant communities.

Because Chenopodiaceae is considered a non-mycorrhizal family, it was not surprising to find negligible rates of AMF colonization in *C. quinoa*. In addition, this species also lacked DSE. Instead, the roots of this pseudo-cereal were considerably infected by *Olpidium* sp. (Chytridiomycota) in two of the three studied sites considered here. This was also the case of *J. seriphioides* in the only site examined, albeit less frequently. *Olpidium* spp. are a relatively harmless root pathogens (Agrios 2005; Webster and Weber 2007) but responsible for the transmission of several viruses (Fauquet et al. 2005). Due to the dramatic increase of *C. quinoa* cultivated areas in the southern part of the Bolivian Altiplano (Vassas et al. 2008), further knowledge on the seasonal and spatial dynamics of *Olpidium* is needed. This information, together with an assessment of the effects of infection rates on plant growth, may have important impacts on this Andean grain production and thus on the local economy of the region.

Conclusions

This study provides evidence that fungal colonization of dominant plant species consistently occur above 3,700 m elevation in the Bolivian altiplano. Our results confirm previous observations on the occurrence of AMF colonization above the 3,000 m altitude, specifically at 3,700 m. In addition, our findings support the view regarding the widespread occurrence and the importance of DSE at high altitudes and further suggest the existence of a functional relationship between the rate of DSE/AMF and the proportion of fine roots. Finally, the colonization by the root pathogen *Olpidium* sp. in *C. quinoa* deserves further investigation as this Andean grain become increasingly important for the local economy of these marginal areas.

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