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ABSTRACT

The Andean Puna is an arid, high-elevation plateau in which plants such as grasses experience high abiotic stress and distinctive environmental conditions. We assessed colonization by arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) in the roots of 20 native grass species and examined the relationship between root-associated fungi (AMF and DSE) as a function of the elevation of study sites, the photosynthetic pathways of the grass hosts, and the hosts' life cycles. In general, grasses were co-colonized by AMF and DSE and the colonization by AMF and DSE was not extensive. The extension of colonization of AMF and that of DSE were positively correlated, as were number of arbuscules and DSE colonization extension. The extension of AMF colonization differed among sites with different elevations, but DSE colonization was similar across sites. Overall, AMF and DSE patterns shifted as a function of elevation in most grass species, with no general trends observed with respect to host photosynthetic pathway or life cycle. In general, our observations differ from previous studies in the Northern Hemisphere. Variation among sites in AMF and DSE colonization was greater than variation that could be explained by the other factors considered here, suggesting a strong influence of environmental factors. We predict that both AMF and DSE may have established synergistic and beneficial associations with grasses in these distinctive and harsh ecosystems.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are ubiquitous root symbionts in the Glomeromycotina that form a mostly obligate symbiosis with the roots of most land plants in nearly every ecosystem around the globe (Schüßler et al. 2001; Hempel et al. 2007; Spatafora et al. 2016). AMF receive plant-derived carbon (C) from host plants and, in exchange, provide plants with limiting nutrients such as nitrogen (N) and phosphorus (P), enhance host access to minerals and water, and provide protection to plants against pathogens and drought (Smith and Read 2008). However, plants greatly differ in their reliance upon mycorrhizal symbiosis (Hartnett and Wilson 2002). For example, mycotrophy of Poaceae differs as a function of the plant photosynthetic pathway (C_3 vs. C_4) and life cycle (annual vs. perennial). For example, C_3 grasses are typically facultative mycotrophs, whereas C_4 are typically obligate (Hetrick et al. 1990). Additionally, annual species are frequently nonmycorrhizal, whereas perennial species are predominantly mycorrhizal (Trappe 1987; Hartnett and Wilson 2002).

Dark septate endophytes (DSE) also inhabit the root tissues of more than 600 plant species (Jumpponen and Trappe 1998). DSE associations are formed by a polyphyletic group of pigmented anamorphic fungi, including both Ascomycota and Basidiomycota (Jumpponen and Trappe 1998; Smith and Read 2008; Knapp et al. 2012). DSE are thought to be better suited to physiological extremes than AMF because they are more widespread in cold and harsh environments at high elevations or latitudes (Read and Haselwandter 1981; Kohn and Stasovski 1990; Newsham et al. 2009), in deserts (Chaudhry et al. 2006), and in waterlogged and

aquatic habitats (Marins et al. 2009). Like AMF, DSE may play multifunctional roles in the host plant by improving N and P uptake, increasing tolerance to biotic and abiotic stress, and maintaining water relations for plant survival (Mandyam and Jumpponen 2005, 2015; Newsham 2011). However, as a whole, DSE have been reported to have both negative and positive effects on their hosts, forming associations that can range from mutualistic to pathogenic (Jumpponen and Trappe 1998; Newsham 2011; Knapp et al. 2012).

Resource acquisition strategies in grasses may differ among species and ecosystems as a function of their fungal symbionts (AMF, DSE, or both), as each fungal group may access different sources of growth-limiting nutrients (Smith and Read 2008). DSE may replace the function of AMF at high elevations or latitudes, such as in the high Arctic tundra (300–1000 m) and in European and North American alpine communities (ca. 1600–3200 and 3200 m, respectively) (Read and Haselwandter 1981; Trappe 1987; Kohn and Stasovski 1990; Gardes and Dahlberg 1996). Such studies have reported a reduction in AMF colonization in fertile areas, in nival zones (where scant vegetation and low availability of host roots hinder AMF spread by root-to-root contact given by their biotrophic nutrition), and in soils with few AMF spores. In turn, DSE colonization typically is improved in these fertile zones or areas with low availability of roots. DSE have the capacity to decompose organic matter in the absence of host plant roots and have a relatively high tolerance to extreme conditions. Moreover, plants such as the Cyperaceae, which host DSE but are not associated with AMF, often are abundant in these areas. Although DSE and AMF can co-occur in roots, there is no consensus on whether these interactions are competitive, facilitative, or amensalistic (Chaudhry et al. 2006; Scervino et al. 2009; Ruotsalainen and Eskelinen 2011).

The reasons for the paucity of AMF in high-latitude and high-elevation environments remain poorly understood (Gardes and Dahlberg 1996). However, Urcelay et al. (2011) suggested that in an environment characterized by aridity, cold temperature, and nutrient-poor soils, the relative colonization by AMF vs. DSE, rather than the total colonization by AMF or DSE per se, better predicts the functional implications of the root-fungal symbiosis. Plants can respond to increasing levels of water and nutrient availability via fine root proliferation (Pregitzer et al. 1993), such that differences in the proportion of fine roots could be site or patch specific and could influence fungal colonization. In species consistently colonized by AMF and DSE, a relatively lower proportion of fine roots would be offset by an increase in DSE with either no change or a mild decrease in AMF colonization. This prediction is generally consistent with the hypothesis

that there is antagonism between AMF and DSE that might have functional implications for the plant. More generally, low AMF colonization at high elevations may reflect many factors, such as a low number of plants and roots, high levels of environmental stress, a short period of growth, the lack of root-to-root contact, and competition (Read and Haselwandter 1981). In contrast, DSE mycelia appear to occur over a wide range of habitats and plant species (Read and Haselwandter 1981; Kohn and Stasovski 1990). DSE could be more tolerant to harsh conditions than AMF due to their melanized hyphae, which are highly resistant to drought and heat stress (Redman et al. 2002; Knapp et al. 2012). Such environmental stressors are the prevailing conditions in the Puna.

The objectives of this study were to (i) assess the extension of colonization by AMF and DSE in roots of grasses from the Argentine Puna; (ii) study the relationship between AMF and DSE colonization across a range of elevations; and (iii) analyze fungal colonization as a function of host photosynthetic pathway and host life cycle. We propose four working hypotheses: (i) The extent of AMF and DSE colonization will be negatively correlated, consistent with the prediction that AMF and DSE may compete for C and root space. (ii) AMF colonization will decrease but DSE colonization will increase with increasing elevation, consistent with the prediction that DSE may be more tolerant of the extreme conditions prevailing in the Puna. (iii) The extent of colonization by AMF and DSE will be lower in annual than in perennial grasses, as annuals have shorter periods to establish fungal associations given their shorter life cycles. (iv) The extent of colonization by AMF and DSE will be lower in C_3 than in C_4 grasses, consistent with the proposal by Hetrick et al. (1990) that C_3 grasses are less dependent on mycorrhizal associations.

MATERIALS AND METHODS

Study area.—The arid Puna region is a mountain plateau that extends from the northwest of Argentina to southern Peru. It reaches from the Cordillera Real to the east and from the Andes to the west, with elevations ranging from 2000 to 4400 m above sea level. The climate is cold and dry, with a dry season in winter and annual precipitation of 100–400 mm concentrated in the summer months (Dec–Feb); however, in the study area, rain often occurs at the end of Apr. Mean annual temperature ranges between 8.5 and 9.5 C (Cabrera and Willink 1980). The climate is of a desert type, with high solar radiation, low relative air humidity (10–15%), and large daily temperature amplitude of 16–20 C (Martínez Carretero 1995).

The soils are poor, superficial, and immature, with scarce organic matter, sandy and rocky, with typical paleargid (Aridisol) and torriontente lithic (Entisol) types (Vargas Gil et al. 1990a, 1990b). Floristically, the study area is included in the "Jujeño" or "Puna Seca" district, characterized by the presence of *Mutisia saltensis* (Asteraceae), *Chersodoma argentina* (Asteraceae), *Opuntia* cfr. *tilcarensis* (Cactaceae), *Nototriche sarmentosa* (Malvaceae), and *Stipa arcuata* (Poaceae), among other native grasses (Martínez Carretero 1995).

Sampling design and host collection.—The study area was located in Jujuy and Salta provinces in Argentina. Six sites were chosen at different elevations (TABLE 1) in the Puna highlands. The grass species collected were dominant species at each site. All focal grasses were flowering at the sampling time (Apr 2002). These grass species were chosen on the basis of their life cycles and photosynthetic pathways, which are not structured phylogenetically in the focal species. The samples were obtained from moist soils after rainfall. We sampled a total of 20 grass species: 10 C_3 and 10 C_4 species (TABLE 2). Life cycles of grasses (10 annual and 10 perennial species) were distributed between the C_3 and C_4 photosynthetic pathways across our sites (TABLE 4).

Quantification of root colonization.—Five individuals were collected for each species at each site. Samples were stored in plastic bags and maintained at 4 C until processing. In the laboratory, roots were washed and fixed in FAA (formalin-alcohol-acetic acid, 2:10:1) for subsequent clearing and staining (Grace and Stribley 1991). The finest roots of each individual were cut into 1-cm segments, mixed, and randomly mounted on four to seven semipermanent slides in polyvinyl-lactic acid-glycerol. For each preparation, 100 intersections were quantified under the microscope (McGonigle et al. 1990) at 500× magnification to determine the proportion of root length with arbuscules, hyphae, vesicles, and total root AMF and DSE colonization; this proportion was termed "extension of colonization" or simply "colonization" and it

was denoted in tables and figures as a percentage by cm of root ($\%cm^{-1}$). For AMF quantification, hyphae were considered to represent AMF when at least two of these features were observed: (i) they were attached to vesicles, arbuscules, or coils; (ii) they stained blue; (iii) they showed scarce septa distributed irregularly; and/or (iv) hyphal walls were of variable thickness. In addition, they were observed with anatomical and morphological features described by

Table 2. Poaceae species studied in the Argentine Puna, and their photosynthetic pathways (C_3, C_4) and life cycles (annual and perennial).

Poaceae species	Photosynthetic pathway and life cycle
Bromus catharticus Vahl	Annual C_3
Polypogon interruptus Kunth	
Vulpia myuros (L.) C. C. Gmel.	
Danthonia annableae P. M. Peterson et Rúgolo	Perennial C_3
D. boliviensis Renvoize	
Festuca humilior Nees et Meyen	
Hordeum muticum J. Presl	
Jarava plumosula (Nees ex Steud) F. Rojas	
Koeleria praeandina A. M. Molina	
Poa laetevirens R. E. Fr.	
Aristida adscensionis L.	Annual C_4
Bouteloua barbata Lag.	
B. simplex Lag.	
Eragrostis mexicana subsp. *virescens* (J. Presl.) S. D. Koch et Sánchez Vega	
E. nigricans (Kunth) Steud. var. *nigricans*	
E. nigricans var. *punensis* Nicora	
Microchloa indica (L. f.) P. Beauv.	
Aristida asplundii Henrard	Perennial C_4
Cynodon dactylon Merino	
Muhlenbergia rigida (Kunth) Kunth	

Abbott and Robson (1978, 1979) and Abbott (1982). DSE colonization extension was calculated as the proportion of root length colonized by hyphae that exhibited cortical colonization, were dark, and/or formed microsclerotia (defined here as compact knots of interlaced and looping, thick-walled, and brown-pigmented vegetative hyphae).

Data analyses.—A generalized linear mixed model (GLMM) was fit to quantify variation in AMF and DSE colonization among sites of different elevations. We included hierarchical sources of variance (host photosynthetic pathway, host life cycle, and replicates).

Table 1. Soil physicochemical characteristics of the study sites of the Puna.

Site	Geographical coordinates	Elevation (m)	C_f (%)	P (ppm)	EC (mS/cm)	pH	OM (%)	C_{ox} (%)	HH (%)	Texture
1	22°51′35.2″S, 65°13′43.7″W	3320	0.35	1.11	0.275	6.3	0.4	0.22	0.745	Sand, gravel, silty
2	23°00′06.8″S, 65°22′07.3″W	3370	0.32	2.77	0.334	6.8	1	0.50	0.835	Sand, silt, gravel
3	22°59′41.9″S, 65°22′17.1″W	3390	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
4	22°52′14.0″S, 65°14′25.5″W	3520	0.1	6.23	0.295	6.3	1	0.55	0.837	Gravel, sand, silty
5	22°53′34.1″S, 65°16′00.0″W	3700	0.32	6.85	1.081	7.1	2	1.15	1.564	Sand, gravel, silty
6	22°53′23.3″S, 65°14′56.1″W	3870	0.31	1.94	0.334	5.9	1	0.64	1.479	Gravel, sandy

Note. HH = hygroscopic humidity; C_{ox} = oxidable carbon; OM = organic matter; EC = electrical conductivity of extract at 1:2.5 water dilution at 25 C; P = phosphorus; C_f = free carbonates; N/D = no data.

The replicates were included as a source of variance because this inclusion allows collecting the microsite variability. Colonization data were non-normally distributed, heteroscedastic, and autocorrelated, such that the GLMM approach allowed us to manage the data without transformation. The significance of fixed effects was tested using the conditional t-test from the outputs of linear mixed-effects model functions in the NLME package (Pinheiro et al. 2012). Improvements in the models after accounting for random effects were tested with the Bayesian information criterion (BIC), the Akaike information criterion (AIC), and the log-likelihood ratio test (log-link) implemented in linear mixed-effects (LME) models. Outliers were evaluated with the OUTLIERS package, but it was not necessary to remove any data.

Second, we compared the extension of AMF (hypha, arbuscule, vesicle, total AMF) and DSE (DSE) root colonization. The relationships of AMF and DSE root colonization with photosynthetic pathway (C_3, C_4), life cycle (annual, perennial), and site elevation (3320, 3370, 3390, 3520, 3700, 3870 m) were analyzed using Spearman correlations. The site at 3390 m was not included in the analysis of host life cycles and photosynthetic pathways because it contained only one life cycle (annual) and one photosynthetic pathway (C_4). The intersite differences in AMF and DSE root colonization extension were assessed via a Kruskal-Wallis test and post hoc test for multiple comparisons. To explore differences between sites for a single species, grass species present at two sites were analyzed using the Mann-Whitney test, and species occurring at three sites were analyzed using the Kruskal-Wallis test. These nonparametric tests were used because of the non-normal distribution of the residuals. All statistical analyses were performed using R default libraries (R Development Core Team 2012) and two additional R packages: NLME (Pinheiro and Bates 2000; Pinheiro et al. 2012) and OUTLIERS (Komsta 2015).

RESULTS

The relationship between AMF and DSE colonization to elevation was evaluated via a GLMM with different fixed and random effects. For AMF colonization, the model with elevation as a fixed effect was statistically significant. The model behavior showed a highly significant improvement with the inclusion of replicates as a random effect (TABLE 3, model 5) as well as of host life cycle as a random effect (TABLE 3, model 2). In the case of DSE colonization, the models with different fixed effects were not significant and, consequently, DSE colonization was excluded from the analysis.

Table 3. Likelihood-ratio test comparing different structures of the AMF mixed model with mean elevation as fixed effect.

Structure	Model	BIC	AIC	df	Test	Likelihood ratio
fe rep/	5	1061.532	1137.641	6	5 vs. 4	121.7306 ***
fe specie/	4	1257.675	1257.852	5	4 vs. 3	98,779**
fe cycle/	3	1163.263	1210.099	4	3 vs. 2	21.1245**
fe, hpp	2	1174.388	1206.587	3	2 vs. 1	14.5477
fe	1	1178.935	1196.499	2	—	—

Note. BIC = Bayesian information criterion; AIC = Akaike information criterion; df = degree of freedom. Structure with only fixed effects (fe) is progressively expanded by considering random effects on host photosynthetic pathway (hpp), host life cycle within host photosynthetic pathway (cycle/), host species within host life cycle and photosynthetic pathway (specie/), and replicates within host specie, life cycle, and photosynthetic pathway (rep/). Significant differences between models: **$P \le 0.001$; ***$P \le 0.0001$.

AMF-DSE colonization.—All grasses studied were colonized by AMF and DSE at all elevations, except for *Muhlenbergia rigida*, which was exclusively colonized by AMF, and *Cynodon dactylon*, which was colonized by AMF at all elevations but exhibited AMF-DSE dual colonization only at 3520 m (TABLE 4). All grasses exhibited AMF colonization, although mean values of total AMF colonization extension were very low (0–15 %cm^{-1}) in 58.3% of grasses, with a maximum average colonization extension value of 59.5 %cm^{-1} observed in *Bromus catharticus* (TABLE 4). However, mean DSE root colonization extension (6.2 %cm^{-1}) was much lower than mean AMF root colonization extension (17.2 %cm^{-1}). The highest DSE colonization extension (36 %cm^{-1}) was recorded in *Jarava plumosula* (TABLE 4).

Contrary to our first hypothesis, AMF colonization extension was positively correlated with DSE colonization extension ($R^2 = 0.2341$, $P = 0.0024$). We also found a positive correlation between arbuscule colonization and DSE colonization extension ($R^2 = 0.31201$, $P = 0.00136$); however, we found no correlation between DSE colonization extension and vesicle colonization extension ($R^2 = 0.091$, $P = 0.341$).

AMF-DSE colonization in the study sites.—AMF colonization extension differed significantly among sites ($H = 21.03$, $P = 0.0003$); however, this difference was driven by the site at 3700 m, at which high AMF colonization rates were observed (FIG. 1). In contrast to AMF colonization extension, DSE colonization extension did not differ among study sites ($H = 3$, $P = 0.57$) (FIG. 1). Correlations between the colonization of both symbiont groups at each elevation were also examined. AMF and DSE correlation varied among study sites; positive and significant correlations were found for the sites located at 3370 m ($r_s = 0.72134$, $P = 0.00143$), 3700 m ($r_s = 0.45021$, $P = 0.00437$), and 3870 m ($r_s = 0.69564$, $P = 0.00268$).

Table 4. Mean values of AMF and DSE root colonization extension for 20 Poaceae species at six sites of the Puna (Argentina).

Species	Life cycle	Photosynthetic pathway	DSE (%cm⁻¹)	AMF total (%cm⁻¹)	Vesicle (%cm⁻¹)	Arbuscule (%cm⁻¹)	Hypha (%cm⁻¹)	Site (m)
Bromus catharticus	Annual	C$_3$	2.75 ± 2.06	59.52 ± 11.22a	10.93±3.74a	4.74±3.49	45.11±5.39a	3700
B. catharticus			14.83 ± 8.12	29.39 ± 5.43b	0.00b	2.75 ± 2.22	26.61 ± 5.08b	3870
Polypogon interruptus			0.25 ± 0.50	24.75 ± 6.18	1.75 ± 0.96	3.25 ± 1.71	19.75 ± 5.74	3700
Vulpia myuros			8.50 ± 1.73	0.25 ± 0.50a	0.00	0.25 ± 0.50a	0.00a	3520
V. myuros			6.66 ± 5.95	55.84 ± 8.59b	1.50 ± 2.38	14.40 ± 5.70b	39.94 ± 9.18b	3700
Danthonia annableae	Perennial		15.42 ± 7.56a	2.86 ± 2.77	0.65 ± 0.79	0.00	2.46 ± 2.51	3320
D. annableae			0.75 ± 0.50b	4.25 ± 0.50	1.75 ± 1.26	0.00	2.50 ± 1.29	3870
D. boliviensis			4.10 ± 3.59	5.72 ± 3.45	0.25 ± 0.50	0.00	5.47 ± 3.65	3870
Festuca humillor			8.75 ± 3.20	21.75 ± 5.50	3.50 ± 0.58	0.00	18.25 ± 5.19	3700
Hordeum muticum			3.00 ± 1.83	14.75 ± 1.50	2.00 ± 1.15	1.75 ± 2.06	11.00 ± 2.16	3700
Jarava plumosula			36.00 ± 4.08a	16.25 ± 13.43	3.25 ± 3.40	0.00	13.00 ± 12.81	3370
J. plumosula			1.01 ± 0.03b	3.86 ± 3.19	0.50 ± 0.58	0.26 ± 0.53	3.09 ± 3.11	3520
Koeleria praeandina			17.25 ± 2.63	46.75 ± 6.08	10.50 ± 1.29	11.25 ± 2.22	25.00 ± 4.83	3700
Poa laetevirens			0.50 ± 0.58	9.75 ± 5.50	2.75 ± 1.71	0.25 ± 0.50	6.75 ± 3.77	3700
Aristida adscensionis	Annual	C$_4$	3.49 ± 3.32	41.32 ± 9.82b	3.29 ± 3.76	4.38 ± 4.76	33.64 ± 9.65b	3320
A. adscensionis			1.28 ± 1.33	8.21 ± 5.32a	1.59 ± 2.55	0.25 ± 0.65	6.38 ± 4.78a	3370
A. adscensionis			3.25 ± 1.26	11.50 ± 5.45a	2.50 ± 3.11	0.25 ± 0.50	8.75 ± 4.99a	3520
Bouteloua barbata			5.64 ± 2.83	22.47 ± 9.34b	1.75 ± 1.71	0.25 ± 0.50	20.47 ± 7.47b	3520
B. barbata			4.50 ± 2.38	15.00 ± 1.15a	1.00 ± 0.82	0.50 ± 0.58	13.50 ± 2.38a	3700
B. barbata			2.00 ± 1.41	3.50 ± 3.87b	0.00	0.25 ± 0.50	3.25 ± 3.40ab	3870
Bouteloua simplex			6.25 ± 6.13ab	30.50 ± 7.85	2.00 ± 2.45	7.25 ± 5.68b	21.25 ± 1.71	3320
B. simplex			16.25 ± 10.21a	20.75 ± 12.18	2.83 ± 2.19	0.50 ± 0.58a	17.42 ± 10.47	3370
B. simplex			3.43 ± 2.38b	35.20 ± 8.09	5.88 ± 2.92	0.00ab	29.33 ± 8.11	3520
Eragrostis mexicana subsp. *virescens*			31.64 ± 8.20	6.61 ± 6.24	0.00	0.00	6.61 ± 6.24	3320
Eragrostis nigricans var. *nigricans*			0.98 ± 0.69	5.36 ± 4.50	1.99 ± 1.72	0.00	3.35 ± 2.86	3390
Eragrostis nigricans var. *punensis*			2.03 ± 0.82	0.75 ± 0.96	0.00	0.00	0.75 ± 0.96	3390
Microchloa indica			0.25 ± 0.50	14.00 ± 5.83b	3.00 ± 1.41b	0.00	11.00 ± 5.23b	3320
M. indica			0.75 ± 0.50	4.50 ± 1.73b	0.50 ± 0.58a	0.00	4.00 ± 1.83a	3370
M. indica			8.75 ± 15.50	1.25 ± 0.96ab	0.50 ± 0.58a	0.00	0.75 ± 0.50ab	3520
Aristida asplundii	Perennial		1.46 ± 0.22	33.73 ± 6.79	9.81 ± 5.54	0.32 ± 0.64	23.59 ± 3.48	3320
A. asplundii			2.91 ± 1.52	23.43 ± 4.82	8.22 ± 2.26	0.37 ± 0.74	14.85 ± 5.20	3370
A. asplundii			10.70 ± 10.52	19.66 ± 12.51	5.21 ± 1.59	0.00	14.45 ± 11.52	3520
Cynodon dactylon			0.00a	0.25 ± 0.50a	0.25 ± 0.50	0.00	0.00a	3370
C. dactylon			11.00 ± 6.98b	3.25 ± 1.71b	0.75 ± 0.96	0.00	2.50 ± 1.29b	3520
C. dactylon			0.00a	4.00 ± 2.00b	1.25 ± 0.50	0.00	2.75 ± 1.50b	3700
Muhlenbergia rigida			0.00	0.96 ± 1.25	0.00	0.00	0.96 ± 1.25	3320

Note. The photosynthetic pathway (C$_3$, C$_4$) and life cycle (annual and perennial) for each species are indicated. Data are mean ± standard deviation. Different letters indicate significant differences (α = 0.05) between sites of variables analyzed using the Kruskal-Wallis or Mann–Whitney nonparametric tests.
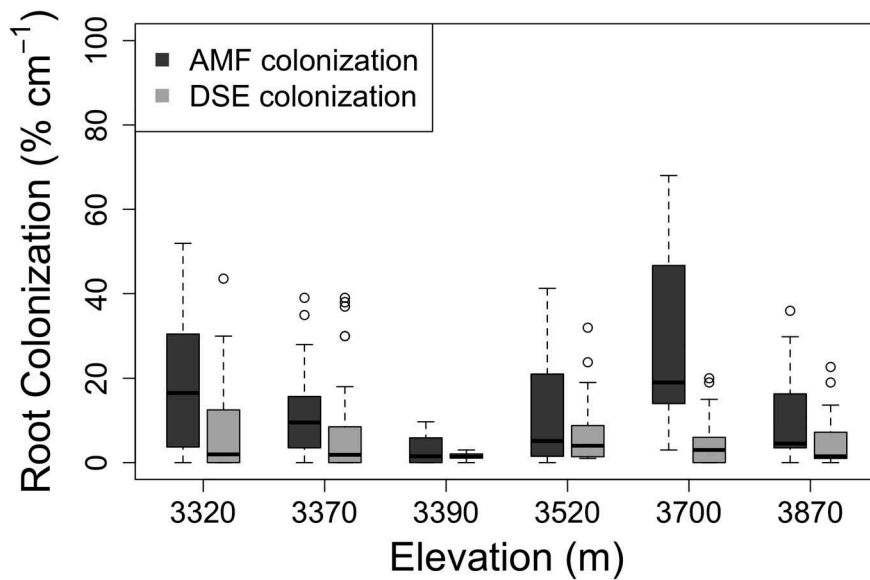
Figure 1. AMF and DSE root colonization extension (in $\%cm^{-1}$) in native grasses of the Puna across elevation sites. Boxes display median values and 1st and 3rd quantiles; whiskers represent $1.5\times$ interquartile range.

AMF-DSE colonization in different photosynthetic pathways and life cycles.—Mean root colonization extension of C_3 grasses across sites was slightly higher than that of C_4 for both AMF and DSE; however, only DSE colonization extension was significantly different between plant types. Thus, AMF colonization extension was 21.1 $\%cm^{-1}$ in C_3 and 14.8 $\%cm^{-1}$ in C_4 grasses ($U = 4265.2$, $P = 0.1325$), and DSE colonization extension was 8.6 $\%cm^{-1}$ in C_3 and 5.5 $\%cm^{-1}$ in C_4 grasses ($U = 4398.2$, $P = 0.0421$) (FIG. 2). Furthermore, AMF colonization extension was higher in annual than in perennial grasses (20.8 and 13.2 $\%cm^{-1}$, respectively; $U = 3956.6$, $P = 0.0167$), although DSE colonization extension did not differ

significantly as a function of grass life cycle (7.1 and 6.5 $\%cm^{-1}$, respectively; $U = 4378.6$, $P = 0.5305$) (FIG. 3). AMF colonization extension was positively and significantly correlated with DSE colonization extension in perennial grasses ($R^2 = 0.3369$, $P = 0.0026$) and in C_4 grasses ($R^2 = 0.53789$, $P = 0.00002$), but not in annual ($R^2 = 0.02805$, $P = 0.79861$) or C_3 grasses ($R^2 = 0.13$, 215, $P = 0.3767$).

Colonization in different photosynthetic pathways and life cycles as a function of elevation.—AMF colonization extension differed as a function of elevation for all annual grasses, regardless of their
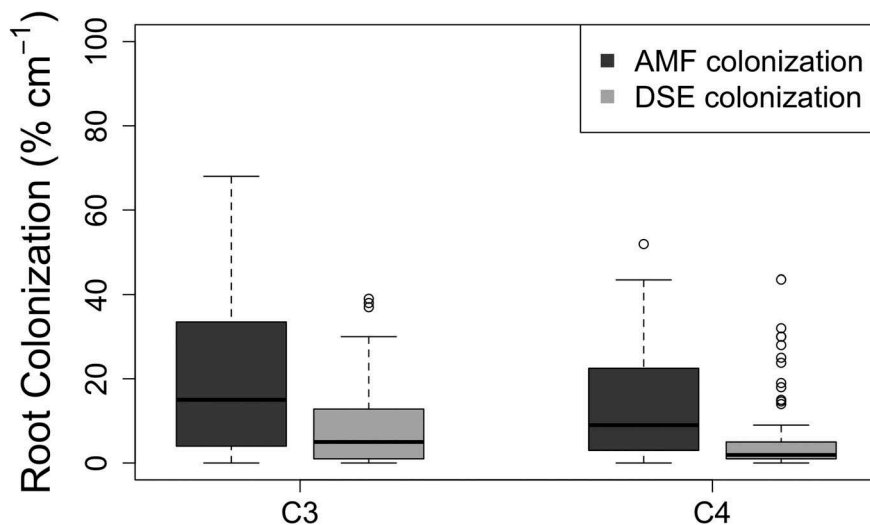
Figure 2. AMF and DSE root colonization extension (in $\%cm^{-1}$) in native grasses of the Puna as a function of photosynthetic pathway. Boxes display median values and 1st and 3rd quantiles; whiskers represent $1.5\times$ interquartile range.
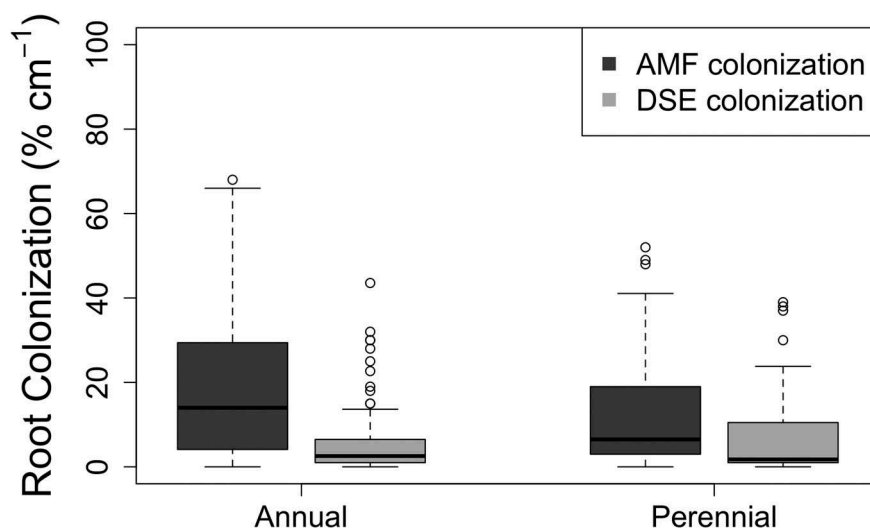
Figure 3. AMF and DSE root colonization extension (in %cm^{-1}) in native grasses of the Puna as a function of life cycle. Boxes display median values and 1st and 3rd quantiles; whiskers represent 1.5× interquartile range.

photosynthetic pattern (TABLES 4, 5). Colonization of most C$_4$ annual grasses decreased with increasing elevation (TABLE 4); however, colonization patterns of C$_3$ annual grasses differed among species (e.g., AMF colonization in *Bromus catharticus* decreased by 50.6% with increasing elevation, whereas in *Vulpia myuros* root colonization extension increased by 99.5% with elevation) (TABLE 4). Arbuscule extension differed significantly between photosynthetic pathways only in two species of annual grasses (TABLES 4, 5). However, the C$_3$ species *Vulpia myuros* had a 98.3% increase in arbuscules extension with elevation, in contrast to a 59% reduction in the C$_4$ species *Bouteloua simplex*. Colonization by DSE in C$_3$ perennials decreased with elevation in species that occurred across at least two sites (TABLES 4, 5). However, in C$_4$ grass species of both life cycles (annual and perennial), DSE colonization extension fluctuated among sites (TABLE 4), without showing an elevational trend.

DISCUSSION

We examined root-associated fungi in native grasses along an elevational gradient in the Puna of Argentina, including C$_3$ and C$_4$ species with perennial and annual life histories. To understand the relationships of the abundance of these fungi with elevation, host photosynthetic pathway, and life cycle of host grasses, we used two complementary statistical approaches. First, we modeled the responses of AMF and DSE colonization along an elevation gradient using a hierarchical approach that allowed us to attribute the variance to different explanatory variables in a progressive way. Second, we conducted correlation analyses to identify the specific relevance of each factor on the enhancement of model behavior. For DSE colonization, none of the models (with different fixed and random effects) were significant, suggesting that there were no differences as a function of elevation, photosynthetic pathway, and/or life cycle. These results were confirmed by the

Table 5. Site effects on AMF and DSE root colonization extension among grasses of the Puna.

Species	Life cycle	Photosynthetic pathway	DSE (%cm^{-1})	AMF (%cm^{-1})	Vesicles (%cm^{-1})	Arbuscules (%cm^{-1})	Hyphae (%cm^{-1})
Bromus catharticus	Annual	C$_3$	—	U = 26.00*	U = 26.00*	—	U = 26.00*
Vulpia myuros			—	U = 10.00*	—	U = 10.00*	U = 10.00*
Danthonia annableae	Perennial		U = 26.00*	—	—	—	—
Jarava plumosula			U = 26.00*	—	—	—	—
Aristida adscensionis	Annual	C$_4$	—	H = 8.74*	—	—	H = 8.74*
Bouteloua barbata			—	H = 8.00**	—	—	H = 8.35**
B. simplex			H = 6.62*	—	—	H = 7.27 *	—
Microchloa indica			—	H = 9.85**	H = 7.38*	—	H = 9.55**
Aristida asplundii	Perennial		—	—	—	—	—
Cynodon dactylon			H = 7.38*	H = 6.95*	—	—	H = 7.39*

Note. Grass species represented at two sites were analyzed using the Mann-Whitney test, and species at three sites were analyzed using the Kruskal-Wallis test (α = 0.05). U = statistic of Mann-Whitney test; H = statistic of Kruskal-Wallis test.
*P ≤ 0.05; **P ≤ 0.005.

analysis of the correlation between DSE colonization and elevation.

The model for AMF and elevation as a fixed effect was statistically significant for all the included parameters (data not shown). The model behavior was highly significantly improved when replicates were included as a random effect, suggesting a significant variation in environmental conditions of the sampling sites (TABLE 3, model 5). This is not surprising given the large variation in microsite characteristics (e.g., soil physicochemical and textural properties, air temperature) within and among sampling sites and the known effect of such factors on AMF colonization (Ruotsalainen et al. 2004; Zubek et al. 2009, 2016; Ranelli et al. 2015).

Site effects on root colonization.

—AMF colonization was found at all sites up to 3870 m, although high elevation has been suggested to be nonoptimal for mycorrhizas (see Trappe 1987). AMF colonization has been recorded at 3700 m and higher in highlands around the world, particularly in North and South America (Schmidt et al. 2008; Urcelay et al. 2011; Lugo et al. 2012). AMF colonization generally decreases with increasing elevation in the highlands and alpine environments of the Northern Hemisphere (elevations of 1245–1300 m; Ruotsalainen et al. 2004) with some exceptions, and intermediate trends were observed along lower elevational gradients (Read and Haselwandter 1981). AMF colonization in the roots of other grasses from the Puna decreased with increasing elevation at 21 sites from 3320 to 4314 m (Lugo et al. 2012).

We found a decrease of DSE colonization in C_3 grasses with increasing elevation and differences in AMF root colonization between sites. However, we did not identify a general relationship between AMF and DSE colonization patterns and elevation. This could be due to large differences at the microsite level and/or the relatively small differences (550 m) in elevation between sites.

AMF-DSE colonization in annual vs. perennial grasses.

—To our knowledge, the present work is the first to compare DSE colonization extension between grasses with different life cycles (annual vs. perennial). Inclusion of host life cycle as a random effect significantly improved model behavior for AMF with elevation as a fixed effect (TABLE 3, model 2).

AMF colonization extension in annual grasses was more extended than in perennial grasses. This contrasts with reports indicating that annuals are less likely to associate with AMF than perennials (Trappe 1987). Higher root colonization in annual grasses has been previously reported for native grass species from the Puna (Lugo et al. 2012).

The differential rate of AMF colonization in relation to host life cycle may be influenced by habitat type, differential efficiency in nutrition processes in host-plant interactions, and non-nutritional or indirect nutritional effects of mycorrhizal association, i.e., host protection against plant pathogens affected by P levels (Newsham et al. 1995; Newsham and Watkinson 1998). In contrast to AMF colonization, DSE colonization did not differ significantly between grass life cycles. This absence of pattern could be attributed to the fact that DSE is a heterogeneous and polyphyletic group of fungi with different nutritional forms, ranging from saprophytic to facultatively biotrophic (Jumpponen and Trappe 1998; Knapp et al. 2012; Mandyam and Jumpponen 2015).

AMF-DSE colonization in C_3 vs. C_4 grasses.

—Mean colonization of C_3 grasses was higher than that of C_4 for AMF and DSE at three of the five sampling sites (3390, 3520, 3700 m). These results are in contrast to our hypothesis, which was based on previous reports for grasses associated with AMF in different ecosystems, such as native tallgrass prairie (Hetrick et al. 1990) and Pampa de Achala (Lugo et al. 2003), and extended to both (AMF-DSE) associations in grasses of the Puna. Those studies suggest that C_4 species would be obligate mycotrophs with higher mycorrhizal dependence, whereas C_3 plants are facultative mycotrophs (Hetrick et al. 1988, 1990). In the Puna, this was not the case. The lower AMF and DSE colonization in C_4 than in C_3 grasses may be due to the high elevations and low temperatures of the sampling sites, favoring root colonization of cool season (C_3) grasses, whose mycorrhizal dependency may remain unchanged, whereas C_4 grasses are less able to interact with AMF and DSE under the stressful conditions of the Puna. Finally, the trend to a higher mean AMF and DSE root colonization of C_3 than C_4 grasses also changed with the life cycle of grasses at different elevations, which could reinforce the site effect on AMF and DSE associations.

AMF-DSE dual colonization.

—At most sites in the Puna highlands, grass species were colonized by both AMF and DSE. Co-colonization of AMF and DSE has been reported for a broad range of environments and hosts (Mandyam and Jumpponen 2005; Scervino et al. 2009; Massenssini et al. 2014). Among Poaceae, AMF-DSE associations have been found in European and Asian environments, including the arid and semiarid plains (Knapp et al. 2012), low alpine (Ruotsalainen et al. 2002), taiga and tundra (Ruotsalainen et al. 2004), mountains (Zubek et al. 2009, 2016), deserts (Chaudhry et al. 2006), grasslands (Li et al. 2005), North American

prairies and montane meadows (Pérez Naranjo 2009; Ranelli et al. 2015), and South American highlands (Urcelay et al. 2011). However, these studies did not find clear trends in dual-colonization patterns in grasses in relation to elevation or other environmental factors. This may reflect environmental heterogeneity across these systems (Mandyam and Jumpponen 2015). Furthermore, most dual-colonization studies have been conducted in northern environments and little is known for South American ecosystems, especially in environments as extreme and distinctive as the Puna.

AMF vs. DSE colonization.—Contrary to our hypothesis, we found that AMF colonization extension far exceeded DSE colonization rates along the elevation gradient, with mean DSE root colonization being generally low. By contrast, DSE typically exceed AMF in a variety of habitats and ecosystems, such as semiarid grasslands (Medina-Roldan et al. 2008), monsoon grasslands (Li et al. 2005), tallgrass prairies (Mandyam and Jumpponen 2008), deserts (Chaudhry et al. 2006), Andean highlands (Urcelay et al. 2011), and the alpine tundra (Schmidt et al. 2008). DSE have been considered to be well adapted to extreme environmental conditions and are assumed to be abundant in harsh environments such as Arctic and Antarctic regions, alpine environments, and arid environments (Read and Haselwandter 1981; Barrow 2003; Newsham et al. 2009; Zubek et al. 2009). The very low DSE colonization found in the Puna could be associated with the DSE nutritional mode. In the Puna highlands, unlike in other harsh environments, soil organic matter content was very low (0.4–2%). Consequently, if DSE were dependent upon saprotrophy, the community would be less abundant than in those harsh environments, due to the lack of accumulated substrate in the organic pools of Puna soils.

DSE may replace AMF under harsh alpine environmental conditions, which are disadvantageous for AMF root colonization (Read and Haselwandter 1981; Kohn and Stasovski 1990; Gardes and Dahlberg 1996; Ruotsalainen et al. 2002). Moreover, a wide and diverse range of fungal endophytes were confirmed to be DSE, covering a broad spectrum of not only fungal taxa but also nutritional strategies (Jumpponen and Trappe 1998; Knapp et al. 2012). Therefore, DSE colonization of the roots of Puna grasses may include fungi of different trophic types that may be beneficial to plants even at low levels of root colonization. However, AMF have been described as the dominant root colonizer in Poaceae from Andean Peru (Schmidt et al. 2008) and southern India (Muthukumar et al. 2006), in *Ochthochloa compressa* from Pakistan (Chaudhry et al. 2006), and in all species studied in the Argentine Puna.

Despite the high tolerance of DSE to extreme conditions as those prevailing in Puna, they did not outcompete AMF, suggesting a negative correlation between AMF and DSE colonization; however, we found a positive correlation between DSE and AMF root colonization. These results are consistent with some, but not all, of the other studies examining this relationship. DSE colonization and AMF colonization were negatively correlated in Asian perennial grasses of the Cholistan Desert (Chaudhry et al. 2006) and in a dicot herb in mountain tundra (Ruotsalainen and Eskelinen 2011). Alternatively, Scervino et al. (2009) found that exudates from DSE fungal strain *Dreschlera* sp. colonizing *Lolium multiflorum* stimulated hyphal length and branching of AMF *Gigaspora rosea*, possibly playing a role in facilitating AMF colonization of host roots. Positive correlations between DSE and AMF were also found in the roots of Cyperaceae, Ranunculaceae, and Rosaceae in low alpine environments in subarctic Fennoscandia (Ruotsalainen et al. 2002), in Poales from southwest China, and grasses from Colorado Rocky Mountains (Li et al. 2005; Ranelli et al. 2015).

We also found that the colonization extension of AMF arbuscules (the nutrient exchange organ between fungi and plants) was positively correlated to DSE colonization extension, suggesting an interaction between DSE and arbuscules of AMF in the studied Puna grasses. Ruotsalainen et al. (2002) found a similar positive relationship between AMF and DSE, but also a positive correlation between DSE and AMF vesicles, concluding that this correlation was possibly an expression of the DSE association that occurred in old senescent roots and that it may have a saprotrophic/decomposing function. However, we did not observe any correlation between vesicles and DSE. Therefore, we conclude that in our system, AMF and DSE may function synergistically and that rather than competing against each other, AMF could benefit from DSE decomposition because AMF may use the available substrates released through DSE decomposition, which explains the positive correlation between DSE colonization and AMF arbuscules that we found in Puna. The arbuscules of AMF are the site where P, N, and other substances are exchanged between these fungi and their host cells; accordingly, Della Monica et al. (2015) showed that whereas DSE increase the pool of P in the rhizosphere, AMF are responsible for P transfer to the host, with co-colonization of plants by DSE and AMF showing a synergistic outcome.

Therefore, DSE of Puna could be degrading complex nutrients and releasing simple substances such as C, N, and P, making them available to AMF; these C, N, and P available in the host-AMF interface could stimulate the formation or avoid degradation of arbuscules by AMF (Wang et al. 2017), explaining the positive correlation between DSE colonization and the colonization extension of AMF arbuscules.

We consider that site rather than elevation effect may suggest an environmental modulation of DSE-AMF co-colonization, which would be in agreement with the mutualism-parasitism-continuum model proposed by Mandyam and Jumpponen (2015) for DSE and for AMF context dependence (Hoeksema et al. 2010). In addition, nutrient-poor soils and drought stress prevailing in the Puna are in agreement with the mutualistic response of both root-fungal associations in this model. Therefore, AMF-DSE colonization may function synergistically as a mutualistic symbiosis modulated by the harsh environmental features prevailing in the Puna.

Our study addresses the gap of information about DSE and AMF colonization in South America and provides baseline information for a further analysis of fungal diversity in this type of ecosystems; studies of specific DSE or AMF taxa using molecular methods will be necessary to obtain more thorough and conclusive results. We found a consistent AMF-DSE colonization of native grasses in this harsh, arid highland of the Argentine Puna at 3320–3870 m as well as trends of dual colonization in grasses of different metabolic types and life cycles, and from elevations different from those reported to date. Our results underscore the importance of understanding environmental contexts that drive colonization and co-colonization patterns as a function of elevation, since the patterns we observed are different from those found in the Northern Hemisphere.

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