

Plant functional traits and phylogenetic relatedness explain variation in associations with root fungal endophytes in an extreme arid environment

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Received: 13 January 2014 / Accepted: 20 June 2014
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Abstract Since root endophytes may ameliorate drought stress, understanding which plants associate with endophytes is important, especially in arid ecosystems. Here, the root endophytes were characterized of 42 plants from an arid region of Argentina. Colonization by arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSEs) was related to plant functional type (PFT), family, and phylogenetic relatedness. Overall, three main findings were observed. Firstly, only moderate levels of endophyte associations were found across all taxa (e.g., most Poaceae were not colonized by endophytes despite numerous accounts of colonization by AMF and DSEs). We determined 69 % of plant taxa associated with some form of root endophyte but levels were lower than other regional studies. Secondly, comparisons by PFT and phylogeny were often qualitatively similar (e.g., succulents and Portulacineae consistently lacked AMF; variation occurred among terrestrial vs. epiphytic bromeliads) and often differed from comparisons based on plant family. Thirdly, comparisons by plant family often failed to account for important variation either within families (e.g., Bromeliaceae

and Poaceae) or trait conservatism among related families (i.e., Rosidae consistently lacked DSEs and Portulacineae lacked AMF). This study indicates the value of comparing numerous taxa based on PFTs and phylogenetic similarity. Overall, the results suggest an uncertain benefit of endophytes in extremely arid environments where plant traits like succulence may obviate the need to establish associations.

Keywords Arbuscular mycorrhizal fungi · Dark septate endophytes · Functional traits · Phylogenetic trait conservatism · Phylogenetic trait divergence · Xeric environment

Introduction

It has been estimated that *ca.* 80 % of terrestrial plant families are associated with arbuscular mycorrhizal fungi (AMF) but some families, such as Brassicaceae, Caryophyllaceae, Chenopodiaceae, Juncaceae, Polygonaceae, and Proteaceae, are generally regarded as non-mycorrhizal, albeit some exceptions within them exist (Smith and Read 2008; Brundrett 2009). Colonization levels and functional trade-off of the symbiosis is highly variable among mycorrhizal plants. Some authors have examined whether mycorrhizal colonization is related to plant traits (e.g., Hetrick et al. 1988 1990 1988, 1990, 1992; Lugo et al. 2003, 2012; Roumet et al. 2006; Urcelay and Battistella 2007; Pérez and Urcelay 2009) or to phylogenetic affiliation of plants (Trappe 1987; Wang and Qiu 2006; Reinhart et al. 2012), whether AMF colonization relates to phylogeny or functional traits of plants is still debatable.

Another widespread and important fungal association in roots of a wide spectrum of plants is that formed by the so-called “dark septate endophytes” (DSEs). DSEs comprise a polyphyletic heterogeneous group of fungi mainly belonging to Ascomycota and Basidiomycota that have also been shown

Electronic supplementary material The online version of this article (doi:10.1007/s00572-014-0592-5) contains supplementary material, which is available to authorized users.

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to benefit plants (Porrás-Alfaro et al. 2008; Newsham 2011; Knapp et al. 2012). DSEs may improve nutrient uptake and maintain adequate water relationships for plant survival mainly in arid environments (Barrow and Osuna 2002; Barrow 2003). Despite the widespread occurrence of these fungi in plant roots, there are few studies regarding their relationship with plant traits (Chaudhry et al. 2006; Lingfei et al. 2005; Lugo et al. 2007; Urcelay et al. 2011) and their relationship with host phylogeny has not been examined to date.

The aim of this study was to quantify AMF and DSE colonization in roots of 42 native plants inhabiting a harsh xeric environment in Argentina. Variation in occurrence of root endophytes was then related to host characters including plant functional traits, plant family, and phylogeny. Phylogenetic information was also used to examine patterns of trait conservatism (i.e., phylogenetic signal) and divergence.

Materials and methods

Characteristics of the sampling site

Sierras de las Quijadas National Park is located in the NW of San Luis province, Argentina (32° 20', 32° 47' Lat. S and 67° 10', 66° 58' Long. W), at 800 m above sea level. It is an arid region that comprises 150,000 ha of dry lands between the biogeographic provinces of Monte and Chaco (Cabrera 1976). Rainfall is scarce and irregular, with a mean annual precipitation of 200 mm. Highest values tend to occur in summer with up to 250 mm in the rainy season (December and January) and 0 mm in the dry season (February to November). Mean temperatures range from 27°C in summer to 9°C in winter, with a mean annual thermal amplitude of 13.7°C (Rivarola 1997). During summer, temperatures reach a maximum value of 45°C while in winter they fall to a minimum of -12°C, representing large seasonal, daily, and yearly thermal amplitudes. This climate is typical of continental situations and in this case may be called hilly arid. It is included in the temperate-dry type of weather typical of hills and prairies (Capitanelli 1989). In the Park, water and wind sculpt the clay and sandstone into landscapes of "huayquerías" or badlands. The flora is rich in xerophytic species many of which are shrubs. A total of 45 vascular plant families are represented in the Park (Del Vitto et al. 2001). The area is characterized by silt loam soils with alkaline pH (8.01), electrical conductivity of 0.94 ds/m, and low humidity (7.545 %). Main soil nutrients are present in low concentrations: organic matter (0.81 %), carbon (0.41 %), nitrogen (0.05 %), available phosphorus (4.57 ppm), and a C/N ratio of 9.24 (Lugo et al. unpublished data).

Plants sampled

Forty two native plant species were studied, among which 20 are endemic (<http://www.floraargentina.edu.ar>, <http://www2.darwin.edu.ar/Proyectos/FloraArgentina>). They represent 18 families and eight plant functional types (PFTs): annual and perennial forbs, graminoids, subshrub, shrub, succulent, epiphytic, and terrestrial bromeliads (Table 1).

Sampling methodology and quantification of fungal endophytes

Five to eight individuals were randomly collected per plant species (the number of individuals per species was limited by National Park restrictions) in summer and early autumn when plants and fungi are most active. Roots were separated from the flowering stems and were dried for voucher specimens that have been accessioned at the Vegetal Diversity Herbarium, Ecology Area, University of San Luis. The roots were frozen until they were washed and fixed in Formalin-Acetic acid-Alcohol. The roots from each individual were cut into segments, pooled by species, then cleared, stained (Grace and Stribley 1991), and mounted on semi-permanent slides using polyvinyl alcohol. Each slide contained a random subsample of roots per species sampled. The number of fine root fragments per slide varied because of variation in the quantity of available material among species and samples. Root colonization was quantified using an optical microscope at ×400 (McGonigle et al. 1990). For the quantification of arbuscular mycorrhiza, data were summarized as total colonization of roots (% AM). For DSEs, their frequency was calculated as the percentage of root colonized by DSE (% DSE). Hyphae were classified as DSE when they exhibited cortical colonization, were dark, and/or formed microsclerotia (compact knots of interlaced and looping thick-walled and pigmented vegetative hyphae produced as resting structures).

Phylogeny construction

Phylogenies were reconstructed for the 42 plant species, mostly angiosperms. Ideally, the reconstruction of a phylogeny for tests of trait conservatism (i.e., phylogenetic signal) is based on DNA data for the focal species and one or more loci (e.g., Kress et al. 2009). Two established and alternative approaches were employed for reconstructing phylogenies and performing tests of trait conservatism (e.g., Cadotte et al. 2009; Ness et al. 2011). Phylogenies were created with the program Phylomatic (Webb and Donoghue 2005) with a branch length adjustment algorithm (Webb et al. 2008), and a second phylogeny was estimated using a Bayesian analysis of molecular data for four loci. Two approaches were used to compare and contrast results since detection of trait conservatism is known to vary among phylogenies derived using these

Table 1 Percent of roots colonized by arbuscular mycorrhizal fungi (% AM) and dark septate endophytes (% DSE) for 42 native species of the Sierra de las Quijadas National Park (Argentina). The plant functional trait (PFT) and family for each species are shown. Means are followed by 95 % confidence intervals

Family/species	PFTs	% AM	% DSE
Amaranthaceae			
<i>Gomphrena colosacana</i> var. <i>andersonii</i>	Subshrub	9.24 (5.47, 14.38)	1.63 (0.34, 4.69)
<i>G. pulchella</i> subsp. <i>rosea</i>	Perennial forb	27.08 (20.02, 35.11)	0.69 (0.02, 3.81)
Asteraceae			
<i>Flaveria haumanii</i>	Annual forb	3.07 (1.54, 5.43)	3.63 (1.95, 6.13)
<i>Parthenium hysterophorus</i>	Annual forb	3.85 (1.06, 9.56)	15.38 (9.06, 23.78)
<i>Senecio hualtaranensis</i>	Subshrub	18.80 (14.01, 24.41)	6.84 (3.96, 10.87)
<i>Thymophylla pentachaeta</i>	Perennial forb	47.00 (41.24, 52.82)	28.33 (23.30, 33.80)
<i>Trixis cacalioides</i>	Shrub	0 (0, 3.24)	0 (0, 3.24)
Boraginaceae			
<i>Ehretia cortesia</i>	Shrub	8.60 (5.26, 13.10)	0 (0, 1.66)
Bromeliaceae			
<i>Bromelia urbaniana</i>	Terrestrial bromeliad	0 (0, 0.92)	0.50 (0.06, 1.79)
<i>Deuterocohnia longipetala</i>	Terrestrial bromeliad	77.25 (74.18, 80.11)	0 (0, 0.46)
<i>Dyckia velascana</i>	Terrestrial bromeliad	100.00 (98.89, 100.00)	0 (0, 1.11)
<i>Tillandsia angulosa</i>	Epiphytic bromeliad	0 (0, 1.48)	34.27 (28.39, 40.54)
<i>T. xiphioides</i>	Epiphytic bromeliad	0 (0, 1.09)	37.69 (32.49, 43.10)
Cactaceae			
<i>Opuntia sulphurea</i>	Succulent	0 (0, 1.83)	2.00 (0.55, 5.04)
<i>Tephrocactus articulatus</i>	Succulent	0 (0, 0.95)	0 (0, 0.95)
Chenopodiaceae			
<i>Atriplex argentina</i>	Subshrub	0 (0, 2.12)	4.65 (2.03, 8.96)
<i>A. lampa</i>	Subshrub	1.74 (0.57, 4.02)	0 (0, 1.28)
<i>A. lithophila</i>	Subshrub	0 (0, 2.13)	0 (0, 2.13)
<i>A. quixadensis</i>	Subshrub	28.50 (22.25, 35.42)	6.22 (3.25, 10.61)
<i>A. spegazzini</i>	Shrub	0 (0, 1.22)	0 (0, 1.22)
Convolvulaceae			
<i>Cressa nudicaulis</i>	Perennial forb	10.33 (7.13, 14.35)	0 (0, 1.22)
<i>Evolvulus arizonicus</i>	Perennial forb	26.67 (21.18, 32.74)	51.67 (45.15, 58.14)
Ephedraceae			
<i>Ephedra</i> sp.	Shrub	17.39 (11.47, 24.76)	0 (0, 2.64)
Fabaceae			
<i>Adesmia cordobensis</i>	Subshrub	39.66 (27.05, 53.36)	3.45 (0.42, 11.91)
<i>A. aff. trijuga</i>	Shrub	2.43 (0.79, 5.57)	0 (0, 1.77)
Halophytaceae			
<i>Halophyton ameghinoi</i>	Annual forb	0 (0, 0.78)	0 (0, 0.78)
Malpighiaceae			
<i>Tricomaria usillo</i>	Shrub	52.56 (44.42, 60.60)	0 (0, 2.34)
Malvaceae			
<i>Sida argentina</i>	Perennial forb	0 (0, 5.96)	0 (0, 5.96)
Nyctaginaceae			
<i>Allionia incarnata</i>	Perennial forb	0 (0, 2.82)	0 (0, 2.82)
<i>Boerhavia pulchella</i>	Perennial forb	14.61 (9.77, 20.67)	1.12 (0.14, 4.00)
Poaceae			
<i>Aristida mendocina</i>	Graminoid	4.41 (1.64, 9.36)	84.56 (77.37, 90.18)
<i>Chloris castilloniana</i>	Graminoid	0 (0, 1.22)	0 (0, 1.22)
<i>Pappophorum caespitosum</i>	Graminoid	0 (0, 1.32)	0 (0, 1.32)

Table 1 (continued)

Family/species	PFTs	% AM	% DSE
<i>Setaria cordobensis</i>	Graminoid	0 (0, 1.22)	0 (0, 1.22)
<i>Sporobolus phleoides</i>	Graminoid	0 (0, 1.22)	0 (0, 1.22)
Polygalaceae			
<i>Monnina dictyocarpa</i>	Perennial forb	19.28 (11.44, 29.41)	0 (0, 4.35)
Portulacaceae			
<i>Grahamia bracteata</i>	Subshrub	0 (0, 1.52)	3.73 (1.72, 6.97)
<i>Portulaca confertifolia</i>	Perennial forb	0 (0, 6.72)	1.89 (0.05, 10.07)
Verbenaceae			
<i>Xeroaloyisia ovatifolia</i>	Subshrub	5.14 (2.76, 8.63)	0 (0, 1.45)
Zygophyllaceae			
<i>Bulnesia retama</i>	Shrub	0 (0, 1.94)	0 (0, 1.94)
<i>Larrea cuneifolia</i>	Shrub	0 (0, 1.96)	0 (0, 1.96)
<i>Plectocarpa tetraantha</i>	Shrub	0.35 (0.01, 1.94)	0 (0, 1.29)

two different approaches (e.g., Kress et al. 2009). Phylomatic organizes previously published phylogenies and generates a phylogeny that is often well resolved at the generic and familiar levels, but unresolved below the genus level. In contrast, phylogenies based on multi-loci molecular data often have better resolution of the terminal branches of the phylogeny (Kress et al. 2009).

The GenBank database was searched for four gene sequences often used in published phylogenies: *matK*, *rbcL*, *ITS1*, and *5.8 s* (e.g., Cadotte et al. 2009; Reinhart et al. 2012). Multiple gene sequences are necessary to differentiate plant species because some sequences represent conservative coding regions (e.g., *rbcL*) and others represent more rapidly evolving portions (e.g., *matK*) (e.g., Reinhart et al. 2012). Sequences available for species of the same genus were used when a sequence was not available for a marker for exactly the same species, and when possible of species also native to the Americas (e.g., Cadotte et al. 2009). Forty of the 42 species had at least one of these four genes represented for it or a congeneric relative in GenBank (<http://www.ncbi.nlm.nih.gov>). A common circumboreal fern (*Pteridium aquilinum*) was included as an outgroup to root the molecular phylogeny. Sequences of these 40 species were aligned using MUSCLE version 3.7 (Edgar 2004). The alignment was edited using MEGA version 4. The files were reformatted from FASTA to NEXUS using the program Concatenator version 1. The best-fit maximum likelihood models of nucleotide substitution for each gene were identified using Akaike Information Criterion using MrModeltest version 2.3 (Nylander 2004). The aligned sequences for the four gene sequences were concatenated using Concatenator version 1.

Using the concatenated sequence alignments, a partitioned Bayesian Inference was performed, estimating the posterior probability distribution of all possible phylogenies using a Markov Chain Monte Carlo algorithm (i.e., Metropolis

algorithm) implemented in MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001). Two independent Markov Chains were run, each with four heated chains for 5 million generations. The final average standard deviation of split frequencies was 0.006, indicating good convergence of the two independent runs. Runs were sampled every 500 generations and used a burning of 5,000 trees to generate a majority rule consensus tree (i.e., phylogram). Three species (*Atriplex lithophila*, *A. quixandensis*, and *A. spegazzinii*) which were not included in the consensus tree were added as polytomies with the average branch length of the representative congeners (*sensu* Reinhart et al. 2012). The resulting phylogram was transformed with nonparametric rate smoothing into a chronogram using APE version 2.5 in R.

Statistical analysis

We aimed to determine whether plant functional traits and/or plant family explained variation in endophytes colonization of plants. However, the study design was unbalanced among comparison groups (i.e., PFT and family); some groups had small sample sizes ($n \geq 1$), and others contained only zeros and had a variance of zero (e.g., shrubs with no DSE). Even after data transformations, the data had heterogeneous variances and were often not normally distributed. Therefore, we cautiously interpret differences among groups based on comparisons of group means and 95 % confidence intervals adjusted for proportions data (Zar 1999).

Analyses were performed to assess the degree to which phylogeny explains the ecological similarity of species (i.e., trait conservatism). Since many plants failed to associate with endophytes, the dataset contained a large proportion of zeros. This motivated us to transform the data into binary data and quantify phylogenetic signal with the D-statistic (Fritz and Purvis 2010) which describes tree-wide patterns in trait

conservatism for binary data. In addition, trait divergence (D) of the continuous data was measured which describes the extent to which individual nodal divergences within the phylogeny (i.e., chronogram) contribute to trait variation (Moles et al. 2005). Here, trait conservation refers to whether more trait variation is explained by ancestral than recent divergences in the chronogram, i.e., do closely related plants have similar endophyte associations or not. The phylogenetic signal was quantified using the D -statistic (Fritz and Purvis 2010) implemented in the R package Caper (Orme et al. 2013). This measure of phylogenetic signal estimates phylogenetic conservatism for binary traits that can be compared to both a random shuffle of trait values at the tips of a phylogeny and a Brownian threshold model (Fritz and Purvis 2010). If D -statistic=1, then traits are randomly distributed at the tips of the phylogeny; D -statistic=0 corresponds to a Brownian threshold model; D -statistic <0 when traits are highly conserved, whereas D -statistic >1 is indicative of phylogenetic overdispersion (Fritz and Purvis 2010).

Divergence (D) and contribution indices (CIs) were used to describe the degree to which individual nodal divergences along the chronogram contribute to trait variation (Moles et al. 2005). Divergences and CIs were calculated for each node in the chronogram with AOTF function in PHYLOCOM version 4.1 (Webb et al. 2008). Divergence calculates the root mean square deviation in trait values between daughter nodes at each node, and the CIs estimate the contribution of these nodal divergences to overall variation. The statistical significance of D was determined against a distribution of 2,000 simulated estimates derived from the randomization of trait values across the tree tips (Moles et al. 2005).

Results

Endophyte variation across plant species, functional traits, and families

Among the 42 native plant species studied, 69 % were associated with root fungal endophytes (26 % only with AMF, 17 % only with DSEs, and 26 % dual associated with AMF and DSEs [Table 1]).

Colonization of roots by AMF varied depending on plant functional type (Fig. 1a) and family (Fig. 2a). Comparisons of the overlap of 95 % CIs indicated that terrestrial bromeliads had the greatest level of AMF colonization. Intermediate AMF colonization was observed for perennial forbs, subshrubs, and shrubs (Fig. 1a). Two plant families (Bromeliaceae and Malpighiaceae) had relatively high levels of AMF colonization (Fig. 2a). Intermediate levels were observed for six other families including Amaranthaceae, Asteraceae, Convolvulaceae, Ephedraceae, Fabaceae, and Polygalaceae. Colonization by

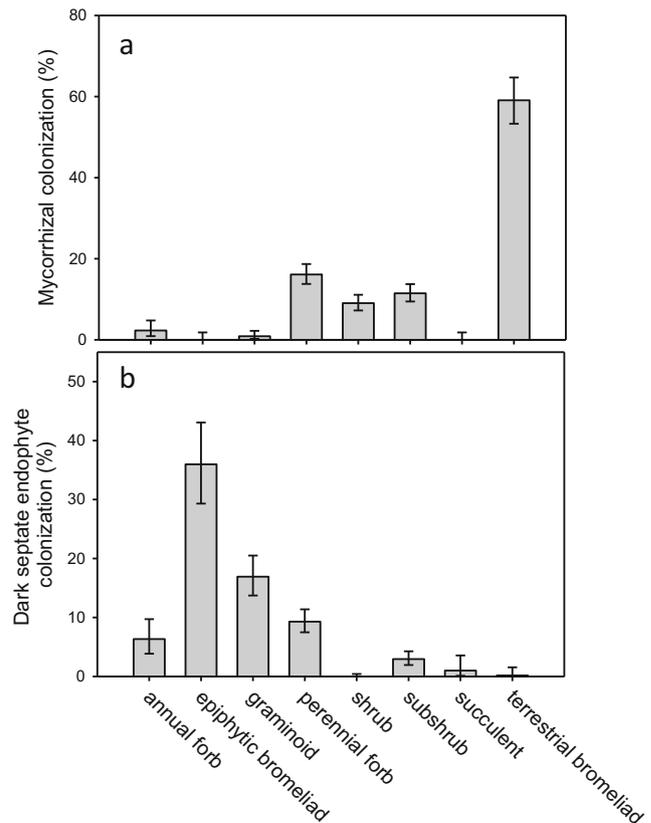


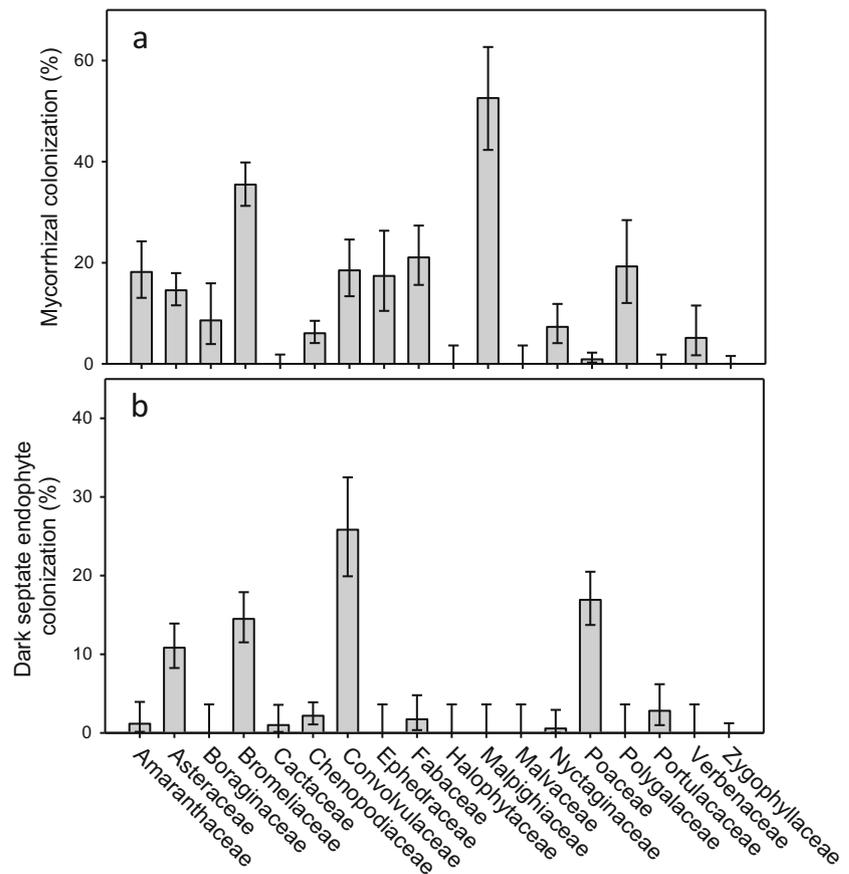
Fig. 1 Variation in root colonization by arbuscular mycorrhizal fungi (a) and dark septate endophytes (b) of plant species grouped by plant functional type. Bars are means with 95 % CI. The number of species per plant functional type is ranged from two to nine

DSEs also varied depending on plant functional type (Fig. 1b) and plant family (Fig. 2b). Epiphytic bromeliads had the greatest level of DSE colonization (Fig. 1b). Over all families, the Bromeliaceae had only the third greatest level of DSE colonization (Fig. 2b). Two other families (Convolvulaceae and Poaceae) had greater levels of DSE.

Endophyte variation across plants phylogeny

The phylogenetic signal for type of endophyte association (AMF and DSE, transformed to binary data) was evaluated across the entire plant community directly using the D -statistic on chronograms based on multi-loci molecular data and Phylomatic (see material and methods). Variation in AMF colonization showed a significant phylogenetic pattern, regardless of the tree used (D -statistic_{multi-locus tree}=0.33, P =0.003 and D -statistic_{Phylomatic tree}=0.33, P =0.02), and did not differ from Brownian expectations (P ≥0.16 for the multi-locus and Phylomatic trees). In contrast, variation in DSE colonization did not exhibit a phylogenetic pattern (D -statistic_{multi-locus tree}=0.78, P =0.15 and D -statistic_{Phylomatic tree}=0.88, P =0.30), and differed from Brownian expectations (P ≤0.02 for the multi-locus and Phylomatic trees).

Fig. 2 Variation in root colonization by arbuscular mycorrhizal fungi (a) and dark septate endophytes (b) of plant species grouped by family. Bars are means with 95 % CI. The number of species per plant family is ranged from one to five



Important areas of divergence (D) were detected within the phylogeny based on multi-loci molecular data. For root colonization by AMF, node 1 representing the Bromeliaceae and node 2 representing a subset of Bromeliaceae (Fig. 3) exhibited greater levels of D in mycorrhizal colonization than expected by chance (Fig. 3). A comparison of the contribution index values indicates that node 2 ($CI=0.43$) contributed more to overall tree-wide levels of divergence than node 1 ($CI=0.11$). Within node 2 (a subset of the Bromeliaceae), *Dyckia velascana* and *Deuterocohnia longipetala* were heavily colonized by AMF while the two *Tillandsia* species exhibited no root colonization. A similar pattern was observed for the phylogeny created using the program Phylomatic. However, this analysis detected only a single case of divergence which was for the node representing the Bromeliaceae ($CI=0.48$, Fig. S1). Despite this subtle variation across results, these findings suggest a recent divergence in mycorrhizal associations among taxa of this family. In contrast, one node exhibited lower levels of D (i.e., trait conservatism) in associations with mycorrhizal fungi than could be expected by chance (Fig. 3). Specifically, node 3 representing the suborder Portulacineae including taxa of Halophytaceae (*Halophyton ameghinoi*), Portulacaceae (*Grahamia bracteata*, *Portulaca confertifolia*), and Cactaceae (*Opuntia sulphurea* and *Tephrocactus articulatus*) exhibited lower levels of variation in mycorrhizal colonization than

expected by chance (Fig. 3). For these taxa, roots were consistently uncolonized by mycorrhizal fungi (Fig. 3). Separate analyses with the phylogeny created using Phylomatic also detected low levels of divergence among taxa of the suborder Portulacineae including Cactaceae (*O. sulphurea* and *T. articulatus*), Halophytaceae (*H. ameghinoi*), and Portulacaceae (*G. bracteata*, *P. confertifolia*) (Fig. S1).

Within the phylogeny, colonization of roots by DSEs varied across the phylogeny based on multi-loci molecular data and exhibited cases of both trait conservation and divergence (Fig. 4). Specifically, a node was detected for 10 taxa from the order Poales (Bromeliaceae and Poaceae) that exhibited higher levels of divergence in associations with DSEs than other taxa within the phylogeny (Fig. 4). Separate analyses with the phylogeny created using Phylomatic failed to detect significant cases of divergence in associations with DSEs (Fig. S2). Using the phylogeny based on multi-loci molecular data, a node representing eight taxa in the subclass Rosidae (Fabaceae, Malpighiaceae, Malvaceae, Polygalaceae, and Zygophyllaceae) exhibited lower levels of D in associations (i.e., trait conservatism) with DSE than could be expected by chance (Fig. 4). These eight taxa were consistently uncolonized by DSEs. When using the phylomatic tree, there was no evidence for trait conservatism in associations with DSE (Fig. S2).

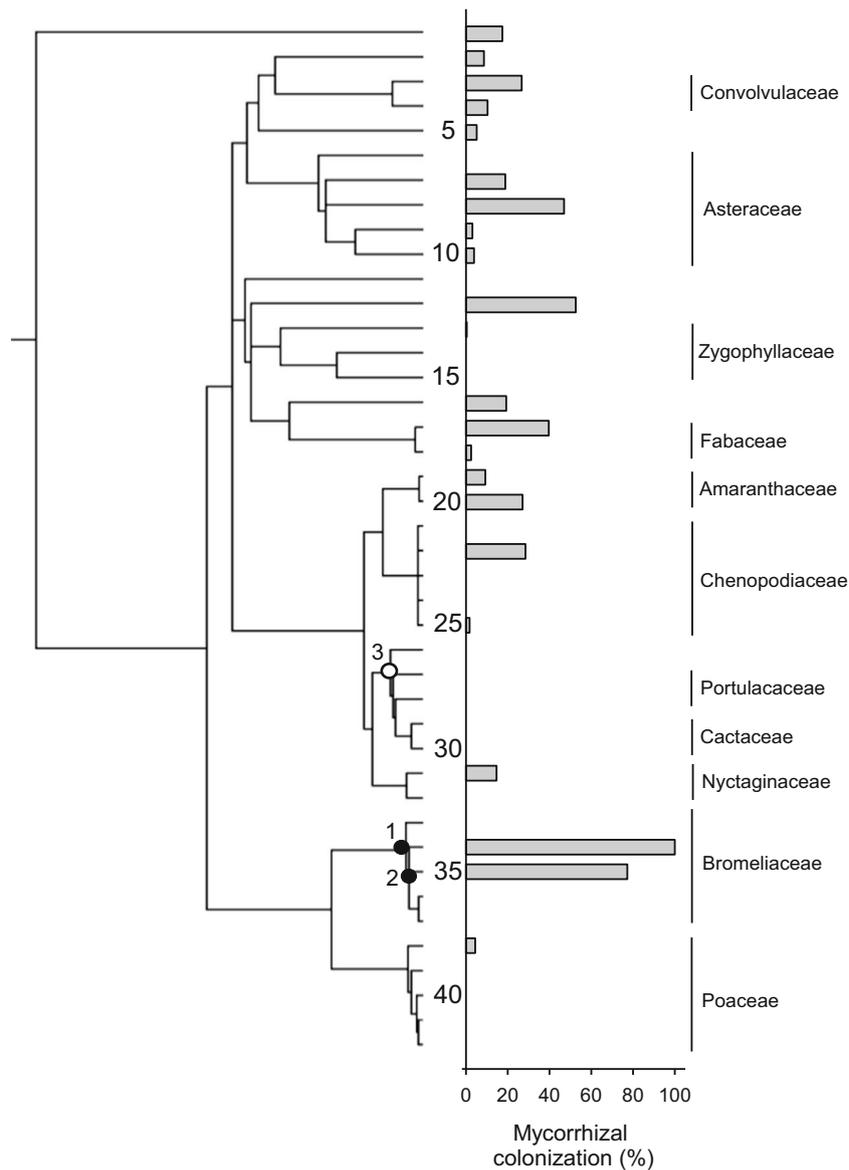


Fig. 3 Molecular phylogeny (chronogram) of 42 plant species from Sierras de las Quijadas National Park (*on the left*) and percent arbuscular mycorrhizal root colonization. *Numbered circles* indicate nodes with greater (*black circle*) or lower levels (*white circle*) of divergence than expected by chance (2-tailed test of significance, $P \leq 0.05$). Families with two or more representative taxa are illustrated by *vertical lines* and *family names*. Branch tip numbers correspond to: 1 *Ephedra* sp., 2 *Ehretia cortesia*, 3 *Evolvulus arizonicus*, 4 *Cressa nudicaulis*, 5 *Xeroaloyisia ovatifolia*, 6 *Trixis cacalioides*, 7 *Senecio hualtaranensis*, 8 *Thymophylla pentachaeta*, 9 *Flavelia haumanii*, 10 *Parthenium hysterophorus*, 11 *Sida argentina*, 12 *Tricomaria usillo*, 13 *Plectrocarpa tetracantha*, 14

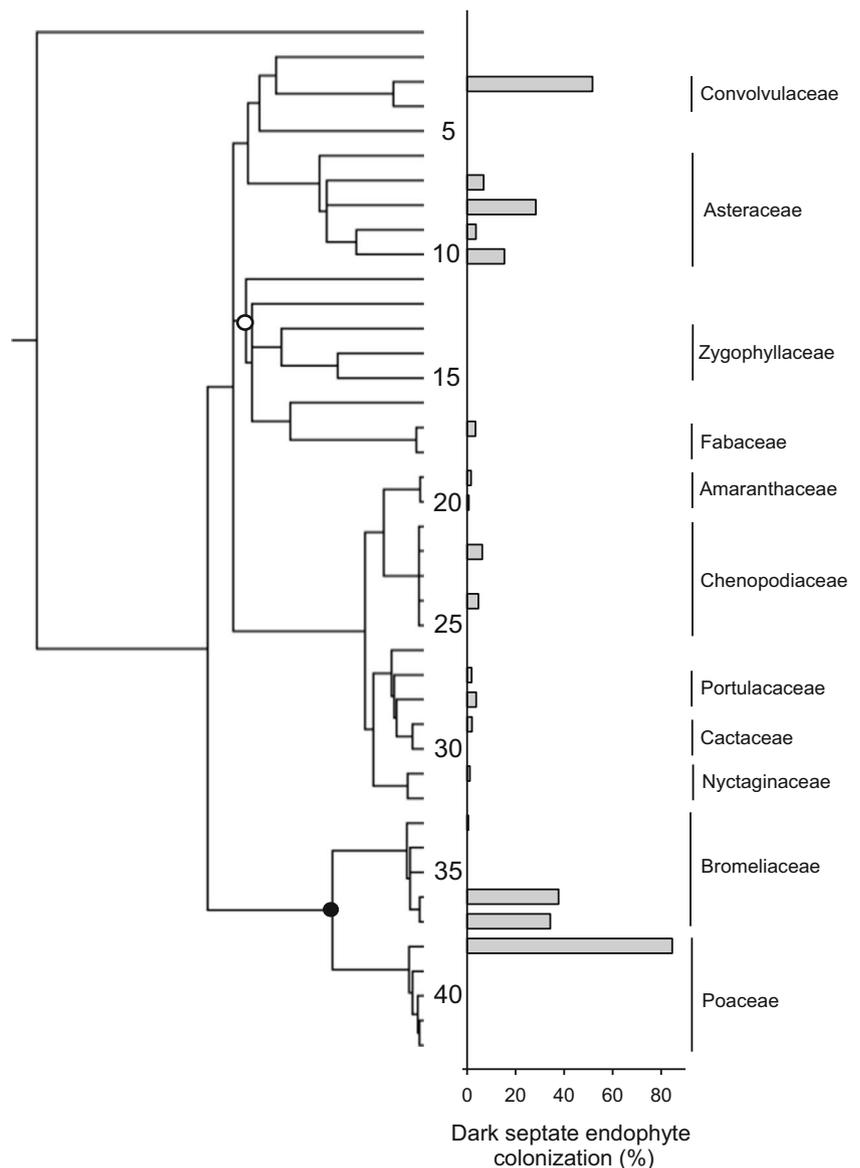
Bulnesia retama, 15 *Larrea cuneifolia*, 16 *Monnina dictyocarpa*, 17 *Adesmia cordobensis*, 18 *A. aff. trijuga*, 19 *Gomphrena colosacana* var. *andersonii*, 20 *Gomphrena pulchella* subsp. *rosea*, 21 *Atriplex spigazzinii*, 22 *A. quixadensis*, 23 *A. lithophila*, 24 *A. argentina*, 25 *A. lampa*, 26 *Halophyton ameghinoi*, 27 *Portulaca confertifolia*, 28 *Grahamia bracteata*, 29 *Opuntia sulphurea*, 30 *Tephrocactus articulatus*, 31 *Boerhavia pulchella*, 32 *Allionia incarnata*, 33 *Bromelia urbaniana*, 34 *Dyckia velascana*, 35 *Deuterocohnia longipetala*, 36 *Tillandsia xiphioides*, 37 *T. angulosa*, 38 *Aristida mendocina*, 39 *Setaria cordobensis*, 40 *Sporobolus phleoides*, 41 *Chloris castilloniana*, 42 *Pappophorum caespitosum*

Discussion

There exists varying degrees of evidence suggesting that AMF (Augé 2001; Smith and Read 2008) and DSEs (Kovács and Szigetvári 2002; Knapp et al. 2012) are prevalent in arid environments and may improve the drought tolerance of colonized plants. DSEs have been described as being widespread

in cold and harsh high altitudinal and latitudinal environments, hot deserts, or waterlogged and aquatic habitats (Read and Haselwandter 1981; Kohn and Stasovski 1990; Chaudhry et al. 2006; Sraj-Krzic et al. 2006; Marins et al. 2009; Newsham et al. 2009; Urcelay et al. 2011). In the present study of the association of roots with fungal endophytes (AMF and DSEs) in Sierras de las Quijadas National

Fig. 4 Molecular phylogeny (chronogram) of 42 plant species from Sierras de las Quijadas National Park (*on the left*) and percent root colonization by dark septate endophytes. *Circles* indicate nodes with greater (*black circle*) or lower levels (*white circle*) of divergence than expected by chance (2-tailed test of significance, $P \leq 0.05$). Families with two or more representative taxa are illustrated by *vertical lines* and *family names*. Branch tip numbers are defined in Fig. 3



Park, which is located in a particularly arid region of Argentina, moderate levels of colonization by DSEs (43 %) and AMF (52 %) were detected across all taxa sampled. However, the levels are about half those reported for other regions in South America where root endophytes of 10 or more herbaceous plant species were characterized (mean DSE=84 %, range 70 to 100 %; mean AMF=84 %, range 56 to 100 %, Table S1 [Schmidt et al. 2008; Fracchia et al. 2009; Urcelay et al. 2011; Lugo et al. unpublished data]). Moreover, dual associations with AMF and DSE were relatively rare in the present study (26 % of taxa) relative to other regional studies (mean AMF and DSE=78 %, range 50 to 100 %, Table S1 [Schmidt et al. 2008; Fracchia et al. 2009; Urcelay et al. 2011; Lugo et al. unpublished data]).

One potential concern may be that roots were sampled at a single point in time giving only a limited snapshot of AMF and

DSE colonization. However, sampling during the wet season ensured that plants were biologically active and likely to have associations with functional root endophytes. Also, the decision to characterize the root endophytes of many species made it logistically unfeasible to sample plants at more than one time point. Other studies have indicated temporal variability in AMF communities and root colonization (Lugo et al. 2005; Smith and Read 2008). Characterization of temporal variation is typically performed for either bulk soil or for a small number of plant species per community. Despite the sampling of plants in Sierras de las Quijadas National Park during the wet season, it is possible that roots did not correspond to the time of peak endophyte infection. Thus, the patterns that were reported here should be further substantiated in this and other xeric systems in order to build a robust database on the importance of root endophytes of plants in xeric environments.

In the present study, plant functional type (PFT) classes helped explain relatively large amounts of variation in root endophyte associations. Some interesting variation in endophyte associations was detected among different types of bromeliads. Specifically, terrestrial bromeliads were the PFT with the highest level of AMF colonization but associated with minimal amounts of DSEs. Epiphytic bromeliads were the opposite and were primarily colonized by DSEs. These findings for bromeliads were qualitatively similar to those based on phylogenetic analyses (see below). Graminoids, perennial forbs, and annual forbs were moderately colonized by DSEs, while several other PFTs were associated with moderate levels of colonization by AMF (perennial forbs, shrubs, and subshrubs). To our awareness there is no other study for this arid region that has related PFT to associations with AMF.

The roots of grasses are often colonized by AMF (Wilson and Hartnett 1998; Wang and Qiu 2006) with reports of obligate mycotrophic associations with C_4 grasses and facultative symbioses among C_3 grasses (Hetrick et al. 1988 1990 1988, 1990, 1991). Furthermore, Mandyam et al. (2012) found higher AMF colonization in C_3 and C_4 grasses than in forbs in a mesic tallgrass prairie, albeit at lower colonization rates than DSEs. However, most of the C_4 grasses studied in the harsh environment of Sierras de las Quijadas National Park were not colonized by AMF and only one was colonized at low levels (*Aristida mendocina*). Similar results have been found in other harsh South American environments along 21 sampled sites (Lugo et al. 2012). This suggests that grasses in extremely harsh environments may be less dependent on AMF than C_4 grasses found in more mesic systems.

Results from the present study also explain variation in some endophyte associations based on phylogenetic relationships of the plants. A phylogenetic signal was detected for variation in associations with AMF but not DSEs across the entire host phylogeny, and patterns were consistent across phylogenies. However, there is some limited evidence that results may be sensitive to whether the phylogeny was derived from the popular program Phylomatic versus multi-loci molecular data (but see Kress et al. 2009). The lack of phylogenetic signal for DSE might be related to the fact that these fungi belong to different lineages in the widely diverse phyla Ascomycota and Basidiomycota, and that most of them are supposed to be facultative symbionts (Jumpponen and Trappe 1998; Porras-Alfaro et al. 2008; Knapp et al. 2012). Instead, AMF belong to Glomeromycota, a notably less diverse clade of obligatory fungal symbionts (Smith and Read 2008).

The phylogenetic analyses performed in the present study revealed several important areas within the phylogeny with either trait divergence or trait conservatism. Important areas of the phylogeny often corresponded with comparisons based on PFT but not those based on plant family. Analyses of both phylogenies revealed divergence of traits related to AMF for taxa of Bromeliaceae. Within the Bromeliaceae, two

bromeliads (*D. velascana* and *D. longipetala*) were heavily colonized by AMF (77–100 %) while the other three (*Tillandsia xiphioides*, *Tillandsia angulosa*, and *Bromelia urbaniana*) were uncolonized by AMF. The taxa of the three terrestrial bromeliads (*B. urbaniana*, *D. velascana*, and *D. longipetala*) were from two separate subfamilies of Bromeliaceae which appeared to have variable levels of colonization by AMF. Only taxa from the subfamily Pitcairnioideae (*D. velascana* and *D. longipetala*) associated with AMF while the single taxa (*B. urbaniana*) from the subfamily Bromelioideae was uncolonized by AMF. Epiphytic bromeliads (*Tillandsia xiphioides* and *T. angulosa*, subfamily Tillandsioideae) also did not associate with AMF. These findings, which are consistent with another phylogenetic analysis considering photosynthetic pathways and life form of Bromeliaceae (Crayn et al. 2004), suggest a recent divergence in mycorrhizal associations among taxa that might be related to a combination of phylogenetic affiliation and life habit.

Phylogenetic analyses revealed one clade of taxa that consistently lacked associations with AMF. Specifically, taxa from the suborder Portulacineae showed lower levels of variation in mycorrhizal colonization than could be expected by chance (i.e., trait conservatism). This finding is qualitatively similar to that based on PFT for succulents. It is worth mentioning that this node includes diverse growth forms (cacti, forbs, and subshrubs). Despite this, the taxa share traits related to conserving water. Specifically, cacti have succulent structures (fleshy photosynthetic stems and spiny leaves) while species from Halophytaceae and Portulacaceae, belonging to forbs and subshrubs, have succulent leaves (i.e., fleshy and pulpy). We cautiously interpret that shared traits (e.g., aboveground structures, dimorphic root system: far-reaching lateral roots and taproots) related to water conservation, due to infrequent and limited rainfall (Bobich and North 2009), may explain the lack of associations with AMF in this clade of plants.

DSEs are widely distributed among terrestrial plants (e.g., Jumpponen and Trappe 1998), particularly in adverse environments (Newsham et al. 2009). Their effects on their hosts are variable, ranging from negative to positive (Newsham 2011). No study has examined whether this widespread plant-fungus association is related to plant phylogeny or functional traits (but see Urcelay et al. 2011 for relationship with fine root production). Some variation by PFT was observed in the plants studied in Sierras de las Quijadas National Park but this was primarily driven by the epiphytic bromeliads which were heavily colonized by DSEs. Some authors have suggested that DSEs are likely to be most common and beneficial in water stressed habitats (Knapp et al. 2012). However, it was found that only 18 of the 42 (43 %) plant species in Sierras de las Quijadas National Park associated with DSEs and colonization levels were often low. One possible interpretation is that associations with DSEs are less frequent and predictable in some arid regions than others.

Associations with DSEs in the studied arid ecosystem exhibited important variation across the phylogeny and exhibited cases of both trait conservatism and divergence. A clade of eight plant taxa (subclass Rosidae) exhibited trait conservatism; DSEs were not detected in roots of these taxa. DSE variability was detected in the order Poales only when using the phylogeny based on multi-loci molecular data and not by Phylomatic phylogeny. Specifically, two epiphytic bromeliads from the subfamily Tillandsioideae (*Tillandsia xiphioides* and *T. angulosa*) and one grass (*A. mendocina*) associated with moderately high levels of DSEs. Furthermore, DSEs were not detected for the two taxa from the subfamily Pitcairnioideae (*D. velascana* and *D. longipetala*) and the other four grass taxa, and only minor levels of DSEs were detected for a single taxon (*B. urbaniana*) of the subfamily Bromelioideae.

It has been reported that AMF show a certain degree of trait conservatism in their relationship with plant roots (Powell et al. 2009). As in another study (Reinhart et al. 2012), evidence for a tree-wide phylogenetic signal for root colonization by AMF was also detected in the present study. Multiple cases of phylogenetic conservatism and divergence were identified in plant associations with AMF and DSEs, which results suggests that the evolutionary constraints on each eukaryotic group (plant and fungi) are complex.

Endophyte associations varied relative to plant functional type and plant phylogeny across all the plant taxa examined in Sierras de las Quijadas National Park. Comparisons by PFT and phylogeny were often qualitatively similar (e.g., succulents and Portulacineae consistently lacked AMF, variation occurred among Bromeliaceae [terrestrial vs. epiphytic bromeliads]). Findings based on plant family, however, were often qualitatively different from those based on PFT and phylogeny. This was because they failed to account for important variation either within families (e.g., Bromeliaceae and Poaceae) or trait conservatism among related families (i.e., Rosidae consistently lacked DSEs and Portulacineae lacked AMF). Taxa from this arid landscape in Argentina appear to defy predictions that plants will frequently associate with AMF and DSEs when challenged with moisture stress. Although the benefit of root associated mutualists may increase with increasing abiotic stress, there may be bounds to this relationship. Plants from systems beyond those bounds may be subject to a more severe environmental filter that root endophytes are unable to help alleviate.

Acknowledgments We are grateful to Brian Anacker for comments on the manuscript. This work was supported by grants from PROICO 2-O203, and PICT 0781-2008. C.U. wish to acknowledge Secyt, Mincyt (Córdoba), and the assistance of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and the Universidad Nacional de Córdoba, both of which have provided facilities used for this study. M.L., C.U. are researchers from Argentine Council (CONICET) and K.R. from Agricultural Research Service (USDA). The authors declare that they have no conflict of interest.

References

- Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* 11:3–42
- Barrow JR (2003) Atypical morphology of dark septate fungal root endophytes of *Bouteloua* in arid southwestern USA rangelands. *Mycorrhiza* 13:239–247
- Barrow JR, Osuna P (2002) Phosphorous solubilization and uptake by dark septate fungi in fourwing saltbush, *Atriplex canescens* (Pursh) Nutt. *J Arid Environ* 51:449–459
- Bobich EG, North GB (2009) Structural implications of succulence: architecture, anatomy, and mechanics of photosynthetic stem, pachycauls, and leaf succulents. In: De la Barrera E, Smith WK (eds) Perspective in biophysical plant ecophysiology: a tribute to Park S. Nobel. UNAM, México, pp 3–37
- Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil* 320:37–77
- Cabrera AL (1976) Territorios fitogeográficos de la República Argentina. Enciclopedia Argentina de Agricultura y Jardinería, 2nd ed, II (1). ACME, Argentina
- Cadotte MW, Cavender-Bares J, Tilman D, Oakley TH (2009) Using phylogenetic, functional and trait diversity to understand patterns of plant community productivity. *PLoS One* 4:e5695
- Capitanelli R (1989) Geografía de San Luis. Gobierno de la Prov. de San Luis. Ministerio de Cultura y Educación, pp 205–216
- Chaudhry MS, Nasim FH, Khan AG (2006) Mycorrhizas in the perennial grasses of Cholistan Desert, Pakistan. *Int J Bot* 2:210–218
- Crayn DM, Winter K, Smith JC (2004) Multiple origins of crassulacean acid metabolism and the epiphytic habit in the Neotropical family Bromeliaceae. *PNAS* 101:3703–3708
- Del Vitto LA, Petenatti EM, Petenatti ME (2001) Catálogo preliminar de la Flora Vasculare, Parque Nacional “Sierra de las Quijadas” San Luis, Argentina. Serie Técnica del Herbario UNSL 8:1–13
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 35:1792–1797
- Fracchia S, Aranda A, Gopar A, Silvani V, Fernández L, Godeas A (2009) Mycorrhizal status of plant species in the Chaco Serrano woodland from central Argentina. *Mycorrhiza* 19:205–214
- Fritz SA, Purvis A (2010) Selectivity in mammalian extinction risk and threat types: a new measure of phylogenetic signal strength in binary traits. *Conserv Biol* 24:1042–1051
- Grace C, Stribley DP (1991) A safer procedure for routine staining of vesicular arbuscular mycorrhizal fungi. *Mycol Res* 95:1160–1162
- Hetrick BAD, Kitt DG, Wilson GWT (1988) Mycorrhizal dependence and growth habit of warm-season and cool-season tallgrass prairie plants. *Can J Bot* 66:1376–1380
- Hetrick BAD, Wilson GWT, Todd TC (1990) Differential responses of C₃ and C₄ grasses to mycorrhizal symbiosis, phosphorus fertilization, and soil microorganisms. *Can J Bot* 68:461–467
- Hetrick BAD, Wilson GWT, Leslie JF (1991) Root architecture to warm- and cool-season grasses: relationship to mycorrhizal dependence. *Can J Bot* 69:112–118
- Hetrick BAD, Wilson GWT, Todd TC (1992) Relationships of mycorrhizal symbiosis, rooting strategy, and phenology among tallgrass prairie forbs. *Can J Bot* 70:1521–1528
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17:754–755
- Jumpponen A, Trappe JM (1998) Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytol* 140:295–310
- Knapp DG, Pintye A, Kovács GM (2012) The dark side is not fastidious—dark septate endophytic fungi of native and invasive plants of semi-arid sandy areas. *PLoS ONE* 7(1–8):e32570

- Kohn LM, Stasovski E (1990) The mycorrhizal status of plants at Alexandra Fiord, Ellesmere Island, Canada, a High Arctic site. *Mycologia* 82:23–35
- Kovács GM, Szigetvári C (2002) Mycorrhizae and other root-associated fungal structures of the plants of a sandy grassland on the Great Hungarian Plain. *Phyton* 42:211–223
- Kress WJ, Erickson DL, Jones FA, Swenson NG, Perez R, Sanjur O (2009) Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. *PNAS* 106:18621–18626
- Lingfei L, Anna Y, Zhiwei A (2005) Seasonality of arbuscular mycorrhizal symbiosis and dark septate endophytes in a grassland site in southwest China. *FEMS Microbiol Ecol* 54:367–373
- Lugo MA, González Maza ME, Cabello MN (2003) Arbuscular mycorrhizal fungi in a mountain grassland II: seasonal variation of colonization studied, along with its relation to grazing and metabolic host type. *Mycologia* 95:407–415
- Lugo MA, Anton AN, Cabello MN (2005) Arbuscular mycorrhizas in the *Larrea divaricata* shrubland at arid “Chaco”, Central Argentina. *J Agric Tech* 1:163–178
- Lugo MA, Menoyo E, Negritto MA, Anton AN (2007) Micorrizas arbusculares *versus* “septados oscuros” en pastos puneños. *Bol Soc Argent Bot* 42(Supl. 2007):210
- Lugo MA, Negritto MA, Jofré M, Anton A, Galetto L (2012) Colonization of native Andean grasses by arbuscular mycorrhizal fungi in Puna: a matter of altitude, host photosynthetic pathway and host life cycles. *FEMS Microbiol Ecol* 81:455–466
- Mandyam K, Fox C, Jumpponen A (2012) Septate endophyte colonization and host responses of grasses and forbs native to a tallgrass prairie. *Mycorrhiza* 22:109–119
- Marins J, Fraccaro DE, Carrenho R, Thomaz SM (2009) Occurrence and coexistence of arbuscular mycorrhizal fungi and dark septate fungi in aquatic macrophytes in a tropical river–floodplain system. *Aquat Bot* 91:13–19
- McGonigle TP, Miller MH, Evans DG, Fairchild DL, Swam JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol* 115:495–501
- Moles AT, Ackerly DD, Webb CO, Tweddle JC, Dickie JB, Westoby M (2005) A brief history of seed size. *Science* 307:576–580
- Ness JH, Rollinson EJ, Whitney KD (2011) Phylogenetic distance predicts susceptibility to attack by natural enemies. *Oikos* 120:1327–1334
- Newsham KK (2011) A meta-analysis of plant responses to dark septate root endophytes. *New Phytol* 190:783–793
- Newsham KK, Upton R, Read DJ (2009) Mycorrhizas and dark septate root endophytes in polar regions. *Fungal Ecol* 2:10–20
- Nylander JA (2004) MrModeltest v2. [2.13]. Program distributed by the author. Evolutionary Biology Centre, Uppsala University
- Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W (2013) Caper: Comparative Analyses of Phylogenetics and Evolution in R. Version 0.5.2. <http://CRAN.R-project.org/package=caper>
- Pérez M, Urcelay C (2009) Differential growth response to arbuscular mycorrhizal fungi and plant density in two wild plants belonging to contrasting functional types. *Mycorrhiza* 19:517–523
- Porras-Alfaro A, Herrera J, Sinsabaugh RL, Odenbach KJ, Lowrey T, Natvig DO (2008) Novel root fungal consortium associated with a dominant desert grass. *Appl Environ Microbiol* 74:2805–2813
- Powell JR, Parrent JL, Hart MM, Klironomos JN, Rillig MC, Maherali H (2009) Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. *Proc R Soc B Biol Sci* 276:4237–4245
- Read DJ, Haselwandter K (1981) Observations on the mycorrhizal status of some alpine plant communities. *New Phytol* 88:341–352
- Reinhart KO, Wilson GWT, Rinella MJ (2012) Predicting plant responses to mycorrhizae: integrating evolutionary history and plant traits. *Ecol Lett* 689–695
- Rivarola D (1997) El Parque Nacional Sierra de las Quijadas y sus recursos naturales. Printers Impresores, Argentina
- Roumet C, Urcelay C, Diaz S (2006) Suites of root traits differ between annual and perennial species growing in the field. *New Phytol* 170:357–368
- Schmidt SK, Sobieniak-Wiseman LC, Kageyama SA, Halloy SRP, Schadt CW (2008) Mycorrhizal and dark-septate fungi in plant roots above 4270 meters elevation in the Andes and Rocky Mountains. *Arct Antarct Alp Res* 40:576–583
- Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic, London
- Sraj-Krzic N, Pongrac P, Klemenc M, Kladnik A, Regvar M, Gaberscik A (2006) Mycorrhizal colonization in plants from intermittent aquatic habitats. *Aquat Bot* 85:331–336
- Trappe JM (1987) Phylogenetic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. In: Safir GR (ed) *Ecophysiology of VA mycorrhizal plants*. CRC Press, Boca Raton, Florida, pp 5–25
- Urcelay C, Battistella R (2007) Colonización micorrícica en distintos tipos funcionales de plantas herbáceas del centro de Argentina. *Ecología Austral* 17:179–188
- Urcelay C, Aho J, Joffe R (2011) Fungal root symbionts and their relationship with fine root proportion in native plants from the Bolivian Andean highlands above 3,700 m elevation. *Mycorrhiza* 21:323–330
- Wang B, Qiu Y-L (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16:299–363
- Webb CO, Donoghue MJ (2005) Phylomatic: tree assembly for applied phylogenetics. *Mol Ecol Notes* 5:181–183
- Webb CO, Ackerly DD, Kembel SW (2008) Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* 24:2098–2100
- Wilson GWT, Hartnett DC (1998) Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. *Am J Bot* 85:1732–1738
- Zar JH (1999) *Biostatistical analysis*. Prentice-Hall, Inc., Upper Saddle River, New Jersey