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Influence of Habitat and Climate Variables on Arbuscular Mycorrhizal Fungus Community Distribution, as Revealed by a Case Study of Facultative Plant Epiphytism under Semiarid Conditions

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In semiarid Mediterranean ecosystems, epiphytic plant species are practically absent, and only some species of palm trees can support epiphytes growing in their lower crown area, such as *Phoenix dactylifera* L. (date palm). In this study, we focused on *Sonchus tenerrimus* L. plants growing as facultative epiphytes in *P. dactylifera* and its terrestrial forms growing in adjacent soils. Our aim was to determine the possible presence of arbuscular mycorrhizal fungi (AMF) in these peculiar habitats and to relate AMF communities with climatic variations. We investigated the AMF community composition of epiphytic and terrestrial *S. tenerrimus* plants along a temperature and precipitation gradient across 12 localities. Epiphytic roots were colonized by AMF, as determined by microscopic observation; all of the epiphytic and terrestrial samples analyzed showed AMF sequences from taxa belonging to the phylum *Glomeromycota*, which were grouped in 30 AMF operational taxonomic units. The AMF community composition was clearly different between epiphytic and terrestrial root samples, and this could be attributable to dispersal constraints and/or the contrasting environmental and ecophysiological conditions prevailing in each habitat. Across sites, the richness and diversity of terrestrial AMF communities was positively correlated with rainfall amount during the most recent growing season. In contrast, there was no significant correlation between climate variables and AMF richness and diversity for epiphytic AMF communities, which suggests that the composition of AMF communities in epiphytic habitats appears to be largely determined by the availability and dispersion of fungal propagules from adjacent terrestrial habitats.

Epiphytism is a characteristic life form of humid and perhumid tropical forest, especially those at middle and higher elevations denominated montane cloud forest. In Mediterranean ecosystems, epiphytic vegetation is restricted to some mesic habitats in temperate regions where ferns, lichens, and bryophytes are common as epiphytes on the trunks of living trees. In semiarid Mediterranean ecosystems, characterized by low irregular annual rainfall and high temperatures (mild winter temperatures and hot summer), epiphytic plant species are practically absent. Actually, under these semiarid environmental conditions, only some species of palm trees can support epiphytes growing in their lower crown area, such as *Phoenix canariensis* (1), which is restricted to gardens, or *Phoenix dactylifera* (date palm), which is widely cultivated and frequently naturalized (2). In date palms the armpits of cut leafs form a good mechanical support place for epiphyte establishment and growth and also constitute an enabling environment for the accumulation of water and organic matter. Although several plant species can eventually be found as facultative epiphytes (epiphytes that can inhabit the canopy or ground interchangeably (3), the most common species and almost always present growing on date palm trunks is *Sonchus tenerrimus* L., a widespread terrestrial herb in semiarid areas.

Epiphytic habitats have generally been considered a nutrient-poor environment for plant development. Some epiphytes have evolved adaptations that provide efficient access to and retention of nutrients, such as litter-trapping leaf arrangements, slow growth rates, absorbent trichomes, and mycorrhizas (3, 4). The results of previous studies suggest that many plant species that are commonly mycorrhizal when they grow terrestrially are inconsistently mycorrhizal when they grow epiphytically (5, 6). However, Rains et al. (4) found abundant mycorrhizal structures on epiphytic roots indicating a significant mycorrhizal presence (arbuscular and ericoid mycorrhizas) in the canopy of a lower montane cloud forest in Costa Rica. In these previous studies, the morphology of structures in or around the root was used to characterize plants as mycorrhizal or nonmycorrhizal; however, morphology provides a limited resolution to the question of whether or not a plant species is mycorrhizal. Thereby, Rowe and Pringle (7) assessed the mycorrhizal status of a variety of epiphytic bromeliad species from the forest canopy in Costa Rica, by identifying both morphological fungal structures of arbuscular mycorrhizal fungi (AMF) and by using a PCR-based identification, to confirm the mycorrhizal status of bromeliad roots. With this molecular approach, these researchers identified AMF associations in one of the three species of epiphytic bromeliad targeted, but they only recognized sequences from members of the genus *Glomus*.

The role of AMF in epiphytism has been poorly studied, and very little is known of this symbiotic association in semiarid Mediterranean conditions. We hypothesize that because of the physiological importance of mycorrhizas in nutrient-limited habitats, it would be expected for vascular epiphytes to be mycorrhizal. Furthermore, due to the extreme differences in habitat and the prob-

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able dispersal limitations of AMF propagules, the AMF communities colonizing epiphytic plants should differ from those inhabiting conspecific terrestrial plants.

We investigated the AMF community composition of epiphytic and terrestrial *Sonchus tenerrimus* plants growing on date palms trunks and adjacent soil, respectively, in different localities, addressing three specific questions. (i) Is there AMF infection in epiphytic *S. tenerrimus* plants? (ii) If so, are there differences in AMF community composition between epiphytic and terrestrial life forms? (iii) Also, if so, do the fungal associations of epiphytic and terrestrial plants of *S. tenerrimus* vary along a temperature and precipitation gradient? To answer these questions, we collected root samples from *S. tenerrimus*, epiphytic plants growing on the armpits of cut leaves of *Phoenix dactylifera*, and from adjacent terrestrial plants growing around palm trees, across 12 localities with various aridity levels.

MATERIALS AND METHODS

Study area and sampling. In the present study we focused in *Sonchus tenerrimus* L. plants growing as facultative epiphytes in *Phoenix dactylifera* L. and its terrestrial forms growing in adjacent soils. Sampling took place in April and May 2011. A total of 12 locations along an aridity gradient of southeastern Spain were sampled (see Table S1 in the supplemental material). Soils in this area are poorly developed, with organic C, organic N, and available P contents ranging between 1.1 to 2.3 g/100 g, 0.2 to 0.3 g/100 g, and 3 to 8 mg/kg, respectively (8, 9). The climate is semiarid in the whole studied area, the evapotranspiration (ETP) at the locations ranges between 1,076 and 1,490 mm, the annual average rainfall between 250 and 362 mm, and the mean annual temperature averages between 14.7 and 18.6°C, with a pronounced dry season from June to September (Spanish Agency of Meteorology [http://www.aemet.es]). The climate variables considered here are as follows: mean annual temperature, potential ETP, mean annual rainfall, rainfall during the 8 months prior to sampling, and rainfall during the 3 months prior to sampling. The values of environmental variables measured are presented in Table S1 in the supplemental material.

At each location one epiphytic and one terrestrial *S. tenerrimus* per three trees per site were collected. Root systems were placed in polyethylene bags for transport to the laboratory, where fine roots were separated. Roots were then briefly rinsed, quickly dried on paper, and used for molecular analysis.

Root DNA extraction and PCR. DNA extractions from 72 root samples (six root samples from each location) were carried out. The three DNA extractions from each individual *S. tenerrimus* plant, habitat, and location were pooled into one composite sample, resulting in a total of 24 samples. With this procedure we intended to focus on among-site variability rather than within-site variation.

For each sample, 0.1 g of fresh root material was placed into a 2-ml screw-cap propylene tube, together with two tungsten carbide balls (3 mm) and ground (3 min, 13,000 rpm) using a mixer mill (MM 400; Retsch, Haan, Germany). The total DNA was extracted by using a DNeasy plant minikit according to the manufacturer's recommendations (Qiagen). The extracted DNA was suspended in 20 μl of water.

Several dilutions of extracted DNA (1/10, 1/50, and 1/100) were prepared, and 2 μl was used as a template. Partial small subunit (SSU) rRNA gene fragments were amplified using nested PCR with the universal eukaryotic primers NS1 and NS4 (10). PCR was carried out in a final volume of 25 μl using the PuReTaq Ready-To-Go PCR beads (Amersham Pharmacia Biotech), 0.2 μM deoxynucleoside triphosphates (dNTPs), and 0.5 μM concentrations of each primer (PCR conditions: 94°C for 3 min, followed by 30 cycles at 94°C for 30 s, 40°C for 1 min, and 72°C for 1 min, followed by a final extension period at 72°C for 10 min).

Next, 2-μl portions of several dilutions (1/10, 1/20, 1/50, and 1/100) from the first PCR were used as template DNA in a second PCR performed using the specific AM fungi primers AML1 and AML2 (38). PCRs were carried out in a final volume of 25 μl using the PuReTaq Ready-To-Go PCR beads (Amersham Pharmacia Biotech), 0.2 μM dNTPs, and 0.5 μM concentrations of each primer (PCR conditions: 94°C for 3 min, followed by 30 cycles of 1 min of denaturation at 94°C, 1 min of primer annealing at 50°C, and 1 min of extension at 72°C, followed by a final extension period of 10 min at 72°C). Positive and negative controls using PCR positive products and sterile water, respectively, were also included in all amplifications. All of the PCRs were run on a Perkin-Elmer Cetus DNA thermal cycler. Reaction yields were estimated by using a 1.2% agarose gel containing GelRed (Biotium).

Cloning and sequencing. The PCR products were purified using a gel extraction kit (Qiagen) cloned into pGEM-T Easy (Promega) and transformed into *Escherichia coli* (XL1-Blue). Thirty-two positive transformants were screened in each resulting SSU rRNA gene library, using 0.7 U of RedTaq DNA polymerase (Sigma) and a reamplification with AML1 and AML2 primers under the conditions described above. Product quality and size were checked in agarose gels as described above. All clones with inserts of the correct size in each library were sequenced using the universal primers SP6 and T7 by Laboratory of Sistemas Genómicos (Valencia, Spain).

Phylogenetic analysis. Sequence editing was done using the program FinchTV 1.4.0 (Geospiza, Inc., Seattle, WA). A search for sequences similar to the ones from the present study was conducted with the BLAST tool (11) provided by GenBank. Phylogenetic analysis was carried out for the sequences obtained in this study and for sequences corresponding to the closest matches from GenBank, as well as sequences from cultured AMF taxa, including representatives of the major taxonomic groups described by Schüssler and Walker (12). All of the sequences were aligned by using a multiple sequence comparison program, MAFFT (version 7.0 [http://align.bmr.kyushu-u.ac.jp/mafft/software]), and the alignment was adjusted manually using BioEdit software, version 7.0.4.1 (13).

Maximum-likelihood phylogenetic analyses were performed with MEGA v.5.05 software (14). Nucleotide data files were first tested to find the best DNA evolution model. The general time reversible model with a discrete gamma distribution showed the lowest BIC (Bayesian information criterion) scores and was deemed to best describe the nucleotide substitution pattern. Initial tree(s) for the heuristic search were obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using the maximum-composite-likelihood approach. The robustness of all trees obtained was evaluated by 1,000 bootstrap replications. *Endogone pisiformis* Link and *Mortierella polycephala* Coem were used as the outgroups.

Statistical analysis. The Shannon-Weaver (H') index was calculated as a measure of diversity, since it combines two components of diversity, i.e., species richness and evenness. It is calculated from the equation $H' = -\Sigma pi(\ln pi)$, where pi is the proportion of individuals found in the ith species. H' values were corrected by rarefaction and randomized by 100 replications using the software EstimateS 8.00 (15). Diversity and richness values were subjected to a t test for paired samples (epiphytic versus terrestrial within localities) to ascertain significant differences.

The number of sequences and observed operational taxonomic units (OTU) in each sample was used to construct the sampling effort curves (with 95% confidence intervals) using the software EstimateS 8.00 (15). The sample order was randomized by 100 replications.

To investigate the influence of habitat and locality on the distribution of the AMF OTU in the root samples, ordination analyzes were conducted in CANOCO for Windows, version 4.5 (16), using the clone abundance data for each root sample. Monte Carlo permutation tests were conducted using 499 random permutations. With this analysis, climatic variables were not significantly related to community composition. Consequently, a principal component analysis (PCA) was used to better understand how relative abundance of different AMF OTU found in *Sonchus tenerrimus* roots growing in soil or in *Phoenix dactylifera* in 12 different locations were related to each other (distributed) using SPSS 19.0 for Windows (SPSS, Inc., Chicago, IL). As a forward procedure, the REGR factor score values obtained for each community in the PCA were subjected to a Student t test for paired samples (epiphytic versus terrestrial within localities)

to ascertain significant differences between epiphytic and terrestrial AMF communities across localities.

The evenness Pielou index measures equitability and allows comparison of Shannon-Weaver index with the distribution of the observed species. It is calculated from the equation $J = H'/\log(S)$, where H is the Shannon-Weaver index and S is the total number of observed species (OTU) in the community. The index measures how equal a community is numerically. The index can have values ranging from 0 to 1. Correlation analysis between all of the climatic data, and the AMF richness, the Pielou evenness index, and the Shannon Index was carried out using SPSS Statistics v.19 with Pearson correlation coefficients.

Nucleotide sequence accession numbers. Unique sequences of the clones generated in the present study have been deposited in GenBank (http://www.ncbi.nlm.nih.gov) under accession numbers HG004448 to HG004537.

RESULTS

Molecular analysis of AMF. All of the pooled DNA extractions from *Sonchus tenerrimus* roots growing on soil or on *Phoenix dactylifera* in 12 different locations were amplified successfully by nested PCR with the AML1-AML2 primer combination. The generated PCR products of the expected band of ~795 bp were used for cloning and for creating a clone library. Twenty-four clone libraries were created, and a total of 768 clones were screened by PCR (32 clones were analyzed per sample/library); of these, 671 clones contained inserts of the right length and, subsequently, these clones were sequenced. The BLAST search revealed that 467 sequences had a high degree of similarity (97 to 100% similarity) to sequences from taxa belonging to the phylum *Glomeromycota*, which were grouped in 30 AMF OTU, the rest of the sequences were *Olpidium* (43%), *Rhizoctonia* (12%), *Quitridiomycetes* (15%), *Ascomycetes* (13%), *Basidiomycetes* (5%), and nematodes (2%). Unique sequences of each OTU were submitted to the EMBL database and are included in the phylogenetic tree shown in Fig. S1 in the supplemental material.

All of the samples analyzed from both epiphytic and terrestrial *S. tenerrimus* plants showed AMF sequences. Of 30 AMF OTU detected, 13 grouped to species belonging to *Glomus* genera, one to *Rhizophagus*, three to *Funneliformis*, 11 to *Claroideoglomus*, one to *Diversispora*, and one to *Archaeospora* (see Fig. S1 in the supplemental material). Only six AMF OTU were grouped to sequences of AMF in culture and therefore could be assigned to recognized species. These were Rh1 (*Rhizophagus intraradices-R. irregularis* group), Glo G6 (*Glomus iranicum*), Fu1 (*Funneliformis caledonium*), Fu3 (*Funneliformis mosseae*), Cl9 (*Claroideoglomus viscosum*), and Cl11 (*Claroideoglomus lamellosum*, *claroideum-C. etunicatum* group). The OTU—Cl2, Cl3, Cl4, Cl5, Cl8, Cl10, and Glo G12—were *Claroideoglomus* and *Glomus* species not related to any sequences of AMF in the database. The remaining OTU were grouped with uncultured *Glomeromycota* species available in GenBank. The rarefaction curves indicated that the number of clones sequenced was sufficient to represent the AMF diversity in the majority of samples analyzed in the present study, since the majority of curves reached a plateau (see Fig. S2 in the supplemental material).

AMF community composition and diversity. The most abundant OTU detected was Glo G3, which represents 17.8% of clones and was found in 8 of the 12 locations in both epiphytic and terrestrial root samples. Six OTU were found exclusively in samples from epiphytes (see Table S2 in the supplemental material), representing almost 26% of the clones reported, Glo G2 alone represented 21% of the clones in the epiphytic plants, which might indicate a possible preference for or adaptation to this kind of habitat. Thirteen OTU were found only in samples from *S. tenerrimus* plants growing on soil, representing almost 28% of the clones in the terrestrial habitat. The remaining OTU identified were present both in epiphytic and soil plants (see Table S2 in the supplemental material). There was not a single OTU that was present at all 12 locations, regardless of *S. tenerrimus* root sampling place (soil or plant). Glo G1 was the most ubiquitous, being found in 10 of the 12 locations.

The distribution of AMF OTU was investigated by using a multivariate statistical approach (CCA analysis). In the case of epiphytes, the analysis explained 41% of the cross-site variance, whereas 33.7% of the cross-site variance (see Fig. S3A and B in the supplemental material) was explained for terrestrial plants. However, none of the climate variables had a significant influence on the variance observed.

PCA of the AMF community composition found in the *S. tenerrimus* roots growing in soil or in *P. dactylifera* in 12 different locations identified two components that accounted for 54.81% of the total variance, being explained by axis 1 (29.3%) and by axis 2 (25.5%) (Fig. 1). The AMF community composition was significantly different between epiphytic and terrestrial *S. tenerrimus* roots ($F = 7.237$; $P < 0.05$) according to the Student t test for paired samples calculated with the regression factor scores provided by the PCA. The Student t test did not reveal significant differences for AMF diversity ($F = 0.597$; $P = 0.186$) and richness ($F = 0.971$; $P = 0.074$) mediated by the habitat. In 9 of 12 locations, the AMF communities colonizing the roots of terrestrial plants were both richer and more diverse than those colonizing the roots of adjacent epiphytic plants. Only in 3 of 12 locations the AM fungi communities colonizing roots of *S. tenerrimus* growing in soil had lower richness and Shannon diversity indices than *S. tenerrimus* roots growing on *P. dactylifera* (Table 1). These locations were Campus de Espinardo, Palmeral Elche B, and Archena, all of them characterized for being the driest locations in both average rainfall and precipitation during the growth period (see Table S1 in the supplemental material). The highest Shannon diversity index found here corresponded to roots growing in soil in Beniel and Sopalmo (B) with $H' = 1.53$ and $H' = 1.59$, respectively, and both with 6 OTU.

The evenness Pielou index for AMF communities is higher than 0.5 in most locations and exceeds 0.7 in half locations, regardless of the habitat (epiphytic or terrestrial), thus indicating that the relative abundances of the different AMF species composing the AMF community are quite similar or even (i.e., the different AMF species are quite close in numbers [see Table 1]).

Relationship between the diversity of AMF communities and climate variables. The climate variables considered (mean annual temperature, potential evapotranspiration [ETP], mean annual rainfall, rainfall during the last 8 months, and rainfall during the last 3 months) was not significantly correlated with any measure of diversity of the AMF communities found in epiphytic *S. tenerrimus* roots. In contrast, when considering the AMF communities of terrestrial *S. tenerrimus*, both AMF species richness and Shannon index were positively correlated with precipitation in the last 8 and 3 months (but not the mean annual precipitation) (Table 2). When epiphytic and soil AMF communities are pooled together, there are positive correlations between both species richness and Shannon diversity index with rainfall in the last 3 months ($r = 0.363$; $P < 0.05$; $r = 0.369$; $P < 0.05$, respectively) (Fig. 2).

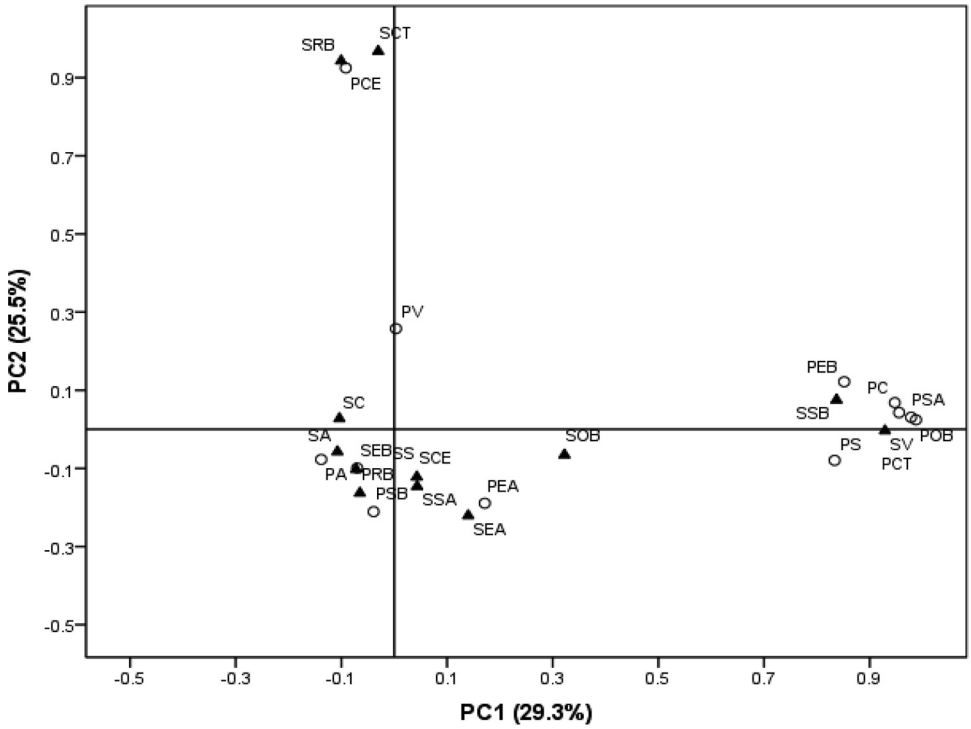

FIG 1 Principal component analysis (PCA) of the AMF community composition found in the *S. tenerrimus* roots growing in soil (with "S" precedes the location codes and represented by full triangles) or in *P. dactylifera* (with "P" preceding the location codes and represented by open circles) in different locations. Location codes: RB, Rincón de Beniscornia; CT, Cabezo de Torres; CE, Campus de Espinardo; B, Beniel; PEA, Palmeral de Elche (A); PEB, Palmeral de Elche (B); S, Sax; C, Carboneras; SA, Sopalmo (A); SB, Sopalmo (B); V, Vera; A, Archena. PCA identified two components that accounted for 54.8% of the total variance, being explained by axis 1 (29.3%) and by axis 2 (25.5%).

DISCUSSION

All sequences analyzed from both epiphytic and terrestrial *S. tenerrimus* plants were grouped in 30 AMF OTU. This is a high level of AMF community richness considering that only a single plant species was studied. In previous studies focusing on much more diverse plant communities in semiarid Mediterranean ecosystems (17–19), we have found values for AMF community richness in individual plant species ranging between 8 and 20. All samples from epiphytic plants showed AMF sequences, thus confirming that *S. tenerrimus* epiphytic plants establish a symbiotic relationship with AMF. Furthermore, microscopic examination of epiphytic roots were performed for some samples and mycorrhizal fungal structures were detected in all cases, with colonization ranging from 10 to 45% (data not shown). There are no previous reports on the mycorrhizal status of epiphytic plant species in semiarid Mediterranean conditions, since the only studies concerning AM symbiosis in epiphytic plants have been conducted in tropical environments. Early studies suggested that arbuscular mycorrhizas generally are absent or rare in tropical epiphytes (6, 20, 21). However, later reports found abundant mycorrhizal

TABLE 1 Richness, Shannon diversity index, and Pielou evenness index in *S. tenerrimus* roots growing in soil or in *P. dactylifera* plants in different locations

	Richness		Shannon diversity index		Pielou evenness index	
Location	Terrestrial	Epiphytic	Terrestrial	Epiphytic	Terrestrial	Epiphytic
Rincón de Beniscornia	2	5	0.15	0.99	0.22	0.62
Cabezo de Torres	4	5	0.91	0.92	0.57	0.66
Campus de Espinardo	5	2	0.83	0.42	0.52	0.61
Beniel	3	6	0.69	1.53	0.63	0.85
Palmeral de Elche (A)	2	3	0.64	1.00	0.92	0.91
Palmeral de Elche (B)	5	3	1.42	0.58	0.88	0.53
Santa Eulalia, Sax	2	5	0.69	1.19	0.99	0.74
Carboneras	5	6	1.10	1.43	0.68	0.80
Sopalmo (A)	3	6	0.74	1.43	0.67	0.80
Sopalmo (B)	4	6	1.06	1.59	0.77	0.89
Vera	2	5	0.35	1.26	0.51	0.78
Archena	4	2	1.32	0.27	0.95	0.39

TABLE 2 Pearson coefficients of correlation and significance levels between the climatic data measured, the richness, and the Shannon diversity and Pielou evenness indexes in *S. tenerrimus* roots growing in soil or in *P. dactylifera* plants[a]

Climatic data[b]	Richness		Shannon diversity index		Pielou evenness index	
	Terrestrial	Epiphytic	Terrestrial	Epiphytic	Terrestrial	Epiphytic
Avg temp	0.247 (0.438)	0.356 (0.256)	–0.073 (0.821)	0.331 (0.294)	–0.558 (0.059)	0.229 (0.473)
ETP	–0.029 (0.928)	–0.136 (0.674)	0.336 (0.285)	–0.147 (0.649)	0.512 (0.089)	–0.321 (0.309)
Avg P	–0.446 (0.146)	0.292 (0.357)	–0.284 (0.371)	0.288 (0.364)	0.225 (0.482)	0.176 (0.585)
Sep-Ap P	–0.366 (0.241)	0.508 (0.092)	–0.203 (0.527)	0.577* (0.049)	0.153 (0.636)	0.487 (0.108)
Feb-Ap P	–0.026 (0.937)	0.725** (0.008)	–0.097 (0.763)	0.792** (0.002)	–0.129 (0.690)	0.646* (0.023)

[a] $n = 12$ plants examined. *, significant at $P < 0.05$; **, significant at $P < 0.01$.
[b] ETP, evapotranspiration; P, precipitation; Sep-Ap, September to April; Feb-Ap, February to April.

structures and infection on epiphytic roots (4, 7) showing a significant mycorrhizal presence in epiphytic life forms from tropical forests. The mycorrhizal status of epiphytic vascular plants has only been widely studied and discussed in the family *Orchidaceae* (22–26), but orchids generally form mycorrhizas with *Basidiomycota* (27–29), and no clear evidence exists for arbuscular mycorrhiza colonizing them.

In humid tropical forests, trees show plenty of vascular epiphytes on their branches and trunks, but little is known about vectors for AMF dispersion in these habitats. The occurrence of AMF on epiphytes may be probably attributable to ground-foraging arthropods such as ants, birds that contact soil and can disperse AMF propagules containing spores and/or hyphae (30), or even wasps and rodents. We hypothesize that in Mediterranean semiarid ecosystems the potential biotic dispersers of AMF propagules might be similar to those described by Janos (30) for tropical ecosystems. The evenness Pielou index is very close to 1 in almost all of our study sites, regardless of habitat (epiphytic or terrestrial), thus indicating that the relative abundances of the different AMF OTU composing the AMF community are quite similar or even. This result may indicate that dispersion mechanisms for AMF propagules from soil to epiphytic habitats are generally quite efficient in semiarid areas, since no AMF OTU predominate overwhelmingly over others.

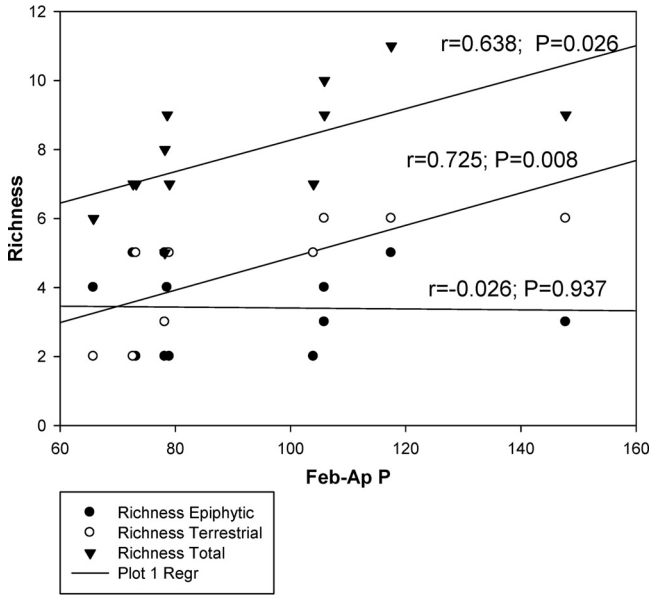

FIG 2 Pearson correlation coefficient relation between OTU richness (terrestrial, epiphytic, and total per site) and precipitation from February to April (Feb-Ap P).

The AMF community composition was clearly different between epiphytic and terrestrial root samples. In palms, the armpits of cut leaves form microhabitats for facultative epiphytic species such as *S. tenerrimus*. These microhabitats have been considered a nutrient-poor environment often subjected to large periods of low water availability (31). These environmental conditions involve epiphytic adaptations for obtaining and retaining water and nutrients, and these include symbiotic associations with AMF (7, 30). Successful colonization of *S. tenerrimus* roots by AMF in epiphytic habitats is initially influenced by the probability of AMF to be dispersed from the soil and later by ecophysiological factors. That means that the AMF community composition would be expected to be different between both habitats and that richness and diversity would be expected to be higher in soil than in epiphytic roots, as we indeed found in this research. In a recent molecular analysis of mycorrhizal fungi in epiphytic and terrestrial orchids, Martos et al. (22) found differences between epiphytic and terrestrial mycorrhizal communities. These authors suggested that differences in mycorrhizal partnerships may be controlled by environmental, functional, and physiological factors causing terrestrial and epiphytic orchids to associate with mutually exclusive fungal partners. Species-specific dispersal constraints may limit the number of AMF symbionts capable of colonizing the epiphytic habitat. Further, the greater environmental and ecophysiological stress characterizing the epiphytic habitat for both plants and their fungal partners (greater exposure of the substrate to evaporation leading to longer periods of low water availability, limited rooting volume of the substrate, low nutrient availability, etc.) may represent an additional filter selecting for particular AMF. We hypothesize that only drought-tolerant AMF with a low carbon cost to the host plant (due to severe environmental constraints on epiphyte photosynthesis) may be capable of establishing mycorrhizal partnerships with epiphytic plants under these challenging semiarid conditions. Further, the substrate on which epiphytic plants grow in palm tree armpits is highly organic (36% organic carbon content versus an average of 1.5% in adjacent soils), which may further select for particular AMF capable of tolerating such unusual conditions.

Glo G3 was the most frequent and abundant OTU in both terrestrial and epiphytic root samples. In previous research conducted in other Mediterranean semiarid environments, this OTU was also the most abundant in the roots of the analyzed plants (32, 33). Hence, Glo G3 appears to be an AMF of great plasticity that is

well adapted to the environmental conditions prevailing in semiarid areas and which shows the ability to colonize many different plant species and contrasting habitats. Its abundance may favor a better and more effective dispersion and therefore colonization of epiphytic roots.

Six OTU were found exclusively in samples from epiphytes; of these, Glo G2 was the most abundant, although it was only found in two locations. This AMF was described as a new type of *Glomeraceae* by Wubet et al. (34) from the roots of trees in an Afromontane dry forest and was later reported in gypsum ecosystems from semiarid areas (18). Therefore, we cannot ascertain whether Glo G2 is an AMF with a preference for epiphytic life forms. The other OTU found exclusively in epiphytic roots have a low quantitative relevance and their presence could be due to chance. Twelve OTU were found only in samples from *S. tenerrimus* plants growing on soil. In general these are less abundant OTU, so their absence from epiphytic habitats could be due to dispersal constraints derived from their low abundance in soil.

Across sites, the richness and diversity of terrestrial AMF communities was positively correlated with rainfall amount during the most recent growing season. In previous spore-based studies, some authors found a positive correlation between AMF abundance and mean annual rainfall (35, 36). Recently, Hazard et al. (37), in an extensive study of AMF community composition at the landscape scale, suggested that specific environmental variables such as rainfall have a stronger effect than land uses on AMF communities. In contrast, there was no significant correlation between climate variables and AMF richness and diversity for epiphytic AMF communities, which suggests that these communities are less dependent on rainfall conditions during the growing season. In semiarid conditions, rainfall events are very infrequent, and the substrate for epiphytic plants and their associated AMF rapidly loses its available water content to evaporation, so growing periods are shorter than in adjacent terrestrial habitats. These harsh conditions could act as an environmental filter that further constrains the dispersal of AM fungi from adjacent soil, so that only very drought-tolerant AMF are capable of colonizing epiphytic habitats. Furthermore, the most diverse epiphytic AMF communities were found at the driest sites. In this sense, Martos et al. (22) suggested that the stronger water and nutrient limitations found for epiphytic orchids in contrast with the more favorable conditions found in soil-rooted orchids may increase the frequency of mycorrhizal interactions and diversity.

We can conclude that AMF play an important role in cases of facultative epiphytism. Even in semiarid conditions, the epiphytic plants are colonized by rich and diverse AMF communities. These AMF communities are significantly different between plant habitats (terrestrial or epiphytic), and this could be attributed to dispersal constraints and/or the contrasting environmental and ecophysiological conditions prevailing in each habitat. Although the composition of AMF communities associated with *Sonchus tenerrimus* in terrestrial habitats is clearly affected by climatic conditions during the growing season, the composition of AMF communities in epiphytic habitats appears to be largely determined by the availability and dispersion of fungal propagules from adjacent terrestrial habitats.

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