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Soil Characteristics Driving Arbuscular Mycorrhizal Fungal Communities in Semiarid Mediterranean Soils

Maria del Mar Alguacil,[a] Maria Pilar Torres,[b] Alicia Montesinos-Navarro,[c] Antonio Roldán[a]

Centro de Edafología y Biología Aplicada del Segura-CSIC, Department of Soil and Water Conservation, Campus de Espinardo, Murcia, Spain[a]; Departamento de Biología Aplicada, Área de Botánica, Universidad Miguel Hernández, Alicante, Spain[b]; Centro de Investigaciones sobre Desertificación-CSIC, Valencia, Spain[c]

ABSTRACT

We investigated communities of arbuscular mycorrhizal fungi (AMF) in the roots and the rhizosphere soil of *Brachypodium retusum* in six different natural soils under field conditions. We explored phylogenetic patterns of AMF composition using indicator species analyses to find AMF associated with a given habitat (root versus rhizosphere) or soil type. We tested whether the AMF characteristics of different habitats or contrasting soils were more closely related than expected by chance. Then we used principal-component analysis and multivariate analysis of variance to test for the relative contribution of each factor in explaining the variation in fungal community composition. Finally, we used redundancy analysis to identify the soil properties that significantly explained the differences in AMF communities across soil types. The results pointed out a tendency of AMF communities in roots to be closely related and different from those in the rhizosphere soil. The indicator species analyses revealed AMF associated with rhizosphere soil and the root habitat. Soil type also determined the distribution of AMF communities in soils, and this effect could not be attributed to a single soil characteristic, as at least three soil properties related to microbial activity, i.e., pH and levels of two micronutrients (Mn and Zn), played significant roles in triggering AMF populations.

IMPORTANCE

Communities of arbuscular mycorrhizal fungi (AMF) are main components of soil biota that can determine the productivity of ecosystems. These fungal assemblages vary across host plants and ecosystems, but the main ecological processes that shape the structures of these communities are still largely unknown. A field study in six different soil types from semiarid areas revealed that AMF communities are significantly influenced by habitat (soil versus roots) and soil type. In addition, three soil properties related to microbiological activity (i.e., pH and manganese and zinc levels) were the main factors triggering the distribution of AMF. These results contribute to a better understanding of the ecological factors that can shape AMF communities, an important soil microbial group that affects multiple ecosystem functions.

Arbuscular mycorrhizal fungi (AMF) represent an important soil microbial group that affects multiple ecosystem functions and processes, including nutrient cycling, plant productivity and competition, and plant diversity. As a consequence, the number of ecological studies concerning AMF has increased considerably in recent years (1–9). Those studies considered AMF communities associated with different host plants in many different ecosystems. However, studies comparing the occurrence of specific AMF species and communities in different soil types are scarce and have focused mainly on cultivated soils and different land uses (10–13). Differences in soil types have been reported to be key factors determining AMF community composition (10), and this is particularly relevant in stressed environments such as serpentine soils (14–16), thermal soils (17), heavy metal soils, and saline soils (18–23).

Traditionally, studies on AMF abundance and distribution have been made by spore extraction from soil and identification based on the morphology and ontogeny of the spores. Thus, identification of spores has also been widely used to characterize AMF communities in soil (10, 24, 25).

The introduction of molecular methods to the study of AMF has revealed a previously unexpected degree of complexity in the ecology of fungi and their relationships with the host plants. PCR-based methods have been used to detect AMF in plant roots and in soil in numerous studies in natural and seminatural ecosystems, including grasslands (26), wetlands (27), agricultural ecosystems (12), urban soils (28), semiarid shrubland (29, 30), and a temperate forest (31). Recently, some investigations have incorporated fungal DNA extraction from soil in addition to root extractions (9, 32–35) as tools to describe the total AMF soil diversity, including actively functioning fungal taxa as well as dormant spores.

In semiarid ecosystems, AMF play key roles in improving the function and adaptation of plant communities to these stressed environments. Despite their importance in semiarid regions, few studies have investigated AMF diversity and community composition, e.g., in plants from gypsum soils (6, 29, 36), in semiarid prairies (30), in degraded areas (37), and in a shrub community (38).

There is clear evidence that AMF community composition and

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Address correspondence to Maria del Mar Alguacil, mmalguacil@cebas.csic.es.

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distribution at different sites or in different habitats are affected mainly by host plant species and environmental factors such as soil type (6, 7, 10, 29, 31, 33, 39–41). If soil type determines the composition and species richness of AMF communities, what are the key soil parameters defining such communities? Is a single parameter or a set of physical, chemical, and/or biological properties involved? In recent studies, Gosling et al. (42) found that different AMF communities in agricultural fields colonized the same host plants, depending on phosphorus concentrations in the soil, and Hazard et al. (40) established that soil pH has a stronger effect than land use itself on AMF communities in agroecosystems and crops. However, those studies considered only a limited set of soil properties.

Here we investigated AMF communities in the roots and the rhizosphere soil of *Brachypodium retusum* (Pers.) P. Beauv., a common plant species of broad distribution that grows in different types of soil in semiarid Mediterranean areas. Fungal DNA extracted from both root and soil samples represented the total AMF assemblages, including actively colonizing fungal taxa as well as those present in soil. Natural soils investigated had different chemical, physical, and biological characteristics but virtually the same environmental conditions (mean annual temperatures and precipitation levels). Under these conditions, we hypothesized that physical, chemical, and biological soil characteristics could shape AMF communities. We used indicator species analyses to assess whether certain AMF operational taxonomic units (OTUs) tended to occur in different habitats (root versus rhizosphere) and sites with contrasting physicochemical properties, and then we tested whether OTUs associated with each habitat or site tended to be closely related (i.e., phylogenetic signals). Finally, inasmuch as host plant species and climatic conditions do not vary between sites, the differences in AMF communities in the rhizosphere of *Brachypodium retusum* could be attributable to differences in soil characteristics. Hence, we studied the relationships between physical, chemical, and biological soil characteristics and the compositions of AMF communities found in the roots and in the rhizosphere soil.

MATERIALS AND METHODS

Experimental sites and root and soil sampling. This study was carried out in Campo de Cartagena, Province of Murcia, in southeastern Spain. The area is a coastal plain, where geological complexity yields diverse soil types that differ in edaphic characteristics but are subject to very similar environmental factors and demonstrate the same species compositions in their plant communities. The climate is semiarid, with a pronounced dry season from June to September, an average temperature of 19.1 ± 0.25°C, an average rainfall of 271 ± 4 mm, and annual potential evapotranspiration of 1,000 ± 14 mm (data are averages of records from 6 weather stations located in the zone; for more detailed information, see Table S1 in the supplemental material). The soils surveyed were Lithic Xerorthent (XER), Xeric Torriorthent (TOR), Typic Haplargid (THA), Typic Haplosalid (THS), Lithic Haploxeroll (LHP), and Typic Haploxeroll (THP), according to the Soil Survey Staff (SSS) (43).

In order to reduce the biotic factors affecting the AMF distribution, this study focused in one target plant, namely, *Brachypodium retusum* (Pers.) P. Beauv., a perennial herbaceous species belonging to the family *Poaceae* that is widely distributed in semiarid soils of southeastern Spain and was the most abundant in all of the locations sampled. The plant community was the same in all locations and belonged to the association *Teucrio pseudochamaepitys-Brachypodietum retusi* O. Bolòs. The community was grassland composed mainly of annual and perennial grasses, including *Brachypodium retusum* (Pers.) P. Beauv., *Dactylis glomerata* L., *Lygeum spartum* L., *Stipa tenacissima* L., and *Brachypodium distachyon* (L.) Beauv., as well as small shrub species such as *Rosmarinus officinalis* L., *Asparagus horridus* L., *Thymus hyemalis* Lange, *Rhamnus lycioides* L., and *Anthyllis terniflora* (Lag.) Pau.

All samples were collected in May 2014 (late spring). Three individual plants were sampled in each of 18 sites across soil types (three replication sites per soil type) in different locations (see Table S2 in the supplemental material). Plants, including root systems, were collected and placed in polyethylene bags for transport to the laboratory, where fine roots were separated from rhizosphere soil. Roots were briefly rinsed, quickly dried on paper, and used for molecular analysis. Rhizosphere soil was used partly for characterization of soil properties and partly for molecular analysis.

Soil analysis. Soil pH and electrical conductivity were measured in a 1:5 (wt/vol) aqueous solution. The percentage of stable aggregates was determined according to the method of Lax et al. (44).

Dehydrogenase activity was determined according to the methods of García et al. (45) and Trevors (46). Urease and *N*-α-benzoyl-L-arginine amide (BAA)-hydrolyzing protease activities were determined in 0.1 M phosphate buffer (pH 7); 1 M urea and 0.03 M BAA, respectively, were used as the substrates. Two milliliters of buffer and 0.5 ml of substrate were added to 0.5 g of soil sieved to <2 mm, and the mixture was incubated for 90 min at 30°C (urease) or 39°C (protease). Both activities were determined as the NH_4^+ released in the hydrolysis reaction (47).

Alkaline phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP) (0.115 M) as the substrate. The *p*-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm (48). β-Glucosidase was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNG) (0.05 M) as the substrate. The assay is based on the release and detection of PNP. The amount of PNP was determined at 398 nm (49).

Glomalin-related soil protein (GRSP) was measured in the easily extractable glomalin form, according to the method of Wright and Anderson (50). Total organic C and total N levels were determined by dry combustion using a Leco Tru-Spec CN analyzer (Leco Corp., St. Joseph, MI, USA). Levels of assimilable P extracted with 0.5 M $NaHCO_3$, assimilable K, Ca, Na, and Mg extracted with ammonium acetate, and B, Fe, Mn, Cu, S, and Zn extracted with water were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Iris Intrepid II XDL; Thermo Elemental Co.). Levels of water-soluble carbohydrates and total carbohydrates were determined by the method of Brink et al. (51).

Root and soil DNA extraction and PCR. DNA extractions from 36 samples (1 root sample and 1 soil sample per replicate for each soil type) were carried out. For root samples, 0.1 g fresh root material was placed in a 2-ml screw-cap propylene tube together with two tungsten carbide balls (3 mm) and was ground (for 3 min at 13,000 rpm) using a mixer mill (MM 400; Retsch, Haan, Germany). Total DNA was extracted using a DNeasy plant minikit, following the manufacturer's recommendations (Qiagen). Two extractions per root sample were performed (0.2 g), and the extracted DNA was resuspended in 20 μl of water and stored at −20°C.

For each soil sample, DNA was extracted from 0.5 g of soil using a FastDNA Spin kit for soil, according to the recommendations of the manufacturer (Q-BIOgene, Heidelberg, Germany). The extracted DNA was resuspended in 20 μl of water and stored at −20°C. Several dilutions of extracted DNA (1:10, 1:50, and 1:100) were prepared, and 2 μl was used as the template. Partial small subunit (SSU) rRNA gene fragments were amplified using nested PCR with the universal eukaryotic primers NS1 and NS4 (52). PCR was carried out in a final volume of 25 μl, using PureTaq Ready-To-Go PCR beads (Amersham Pharmacia Biotech), 0.2 μM deoxynucleoside triphosphates (dNTPs), and 0.5 μM each primer; the PCR conditions were as follows: 94°C for 3 min, 30 cycles of 94°C for 30 s, 40°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 10 min.

Two microliters of several dilutions (1:10, 1:20, 1:50, and 1:100) from

the first PCR were used as the template DNA in a second PCR, which was performed using the specific primers AML1 and AML2 (53). PCRs were carried out in a final volume of 25 μl using PureTaq Ready-To-Go PCR beads (Amersham Pharmacia Biotech), 0.2 μM dNTPs, and 0.5 μM each primer; the PCR conditions were as follows: 94°C for 3 min, 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, and a final extension at 72°C for 10 min. Positive and negative controls using PCR-positive products and sterile water, respectively, were included in all amplifications. All PCRs were run on a PerkinElmer 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 units of RedTaq DNA polymerase (Sigma) and reamplification with the AML1 and AML2 primers under the same conditions as described above. Product quality and size were checked in agarose gels as described above. All clones with inserts of the correct size (795 bp) in each library were sequenced. Clones were grown in liquid culture, and the plasmid was extracted using a QIAprep Spin miniprep kit (Qiagen). Sequencing was performed by the Laboratory of Sistemas Genómicos (Valencia, Spain), using the universal primers SP6 and T7.

AMF richness. Sequence editing was performed using the program FinchTV 1.4.0 (Geospiza, Inc., Seattle, WA, USA). Sequence similarities were determined using BLASTn (54), provided by the National Center for Biotechnology Information (NCBI). Phylogenetic analysis was carried out on the sequences obtained in this study and those corresponding to the closest matches from GenBank, as well as sequences from cultured AMF taxa, including representatives of the major taxonomical groups described by Redecker et al. (55). All of the sequences were aligned using the multiple sequence comparison program MAFFT (version 7.0) (http://mafft.cbrc.jp/alignment/software), and the alignment was adjusted manually with BioEdit software (version 7.0.4.1) (56). The program CHIMERA_CHECK 2.7 (Ribosomal Database Project II) (http://rdp.cme.msu.edu) was used to check for chimeric artifacts among the 18S rDNA sequences.

Maximum likelihood (ML) phylogenetic tree inference was performed with MEGA software (version 5.05) (57). 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 Bayesian information criterion (BIC) scores and was deemed to best describe the nucleotide substitution pattern. Initial trees for the heuristic search were obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach. The robustness of all trees obtained was evaluated with 1,000 bootstrap replications. *Endogone pisiformis* Link and *Mortierella polycephala* Coem. were used as the outgroups. Different AMF sequence types or operational taxonomic units (OTUs) were defined as groups of closely related sequences, with a high level of bootstrap support in the phylogenetic analysis (>85%) and/or sequence similarity of ≥97% with every other sequence.

Statistical analysis. The number of clones for each AMF OTU in each soil sample (combining root and rhizosphere soil AMF communities) was used to calculate the rarefaction curves. The rarefaction curves were produced by plotting the number of OTUs observed against the number of sequences obtained, using the freely available Analytic Rarefaction software (version 1.3) (http://www.uga.edu/~strata/software/anRareReadme.html).

We used indicator species analyses to generate a numerical classification of OTUs (58). This method uses a reciprocal averaging ordination to classify the OTUs with respect to apparently important environmental properties (59). It calculates two different probabilities, i.e., (i) the probability that the surveyed site belongs to a given environment, given the fact that the species has been found there (i.e., specificity of the species as an indicator of an environment type), and (ii) the probability of finding the species in sites belonging to a given environment (i.e., fidelity or sensitivity of the species as an indicator of an environment type). Two independent analyses were performed to test whether there were specific OTUs associated with a certain type of habitat (rhizosphere soil versus roots) or soil type (six soil types). The indicator value (IndVal) index (60) was used to measure the associations. Finally, the statistical significance of the relationships was tested using a permutation test with 999 permutations. These analyses were performed using the indcspecies package implemented in R (version 3.2.1) (61).

It was then determined whether the OTUs identified as being characteristic of different habitats (rhizosphere and roots) or contrasting soils were more closely related than expected by chance, using the method proposed by Maddison and Slatkin (62). This test estimates whether the minimum number of evolutionary steps in a character on a phylogenetic tree is smaller than expected by chance, as determined by comparing the observed minimum number of steps with a null model in which data were reshuffled 1,000 times across the tips of the phylogeny. The character used was the OTU's association or not (i.e., significant or nonsignificant IndVal value) with a given habitat or soil, based on the indicator species analyses. These analyses were performed in R (version 3.2.1), using the function phylo.signal.disc developed by Enrico Rezende.

The relationship between the habitat (roots or rhizosphere soil) and the soil type (explanatory variables) regarding the distribution of AMF sequence types was studied using a combination of principal-component analysis (PCA) and multivariate analysis of variance (MANOVA). We used a constrained ordination process, PCA, to seek the combination of environmental variables that best explained the variations in fungal community composition. To avoid biases mediated by the PCR methods, only the absence or presence (0 or 1) of the different OTUs was considered in the analysis. We performed different principal-component analyses to quantify the amounts of variation explained by different sets of environmental variables. First we calculated the amount of variation in fungal community composition explained by habitat (i.e., soils [$n = 18$ samples] versus roots [18 samples]). These analyses included 3 replicates per soil type for each level. In order to eliminate effects of pseudoreplication, the same analysis was repeated 3 times with one replicate per soil type at a time ($n = 6$ for both soil and root samples), and the results were consistent; therefore, only the results of the first analysis are reported. Then we quantified the variation explained by soil type (6 soil types, with 3 replicates of the combined AMF communities in roots and rhizosphere soil). This analysis was also performed by considering rhizosphere soil and root AMF communities independently.

In order to provide variance partitioning considering both factors (habitat and soil type) at the same time, we performed a principal-component analysis with the community matrix (49 OTUs across 36 samples) and selected a sufficient number of axes to account for 90% of the variance explained. This matrix with the selected axes was used as a proxy of the community composition. Then we performed a multivariate analysis of variance (MANOVA) with this matrix as the dependent variable and habitat (root versus rhizosphere), site, and habitat-site interaction as explanatory variables. Finally, we calculated the proportion of the variance associated with each factor as eta-square. These analyses were performed in R (version 3.2.1), using the heplots package.

Finally, we quantified the amount of fungal community variation in the soil-plant system (root plus rhizosphere soil communities) for each soil type that was explained by specific soil properties. Redundancy analysis (RDA) was then applied. As a forward procedure, Monte Carlo permutation tests were conducted using 999 permutations, and the variables were ranked according to their importance and significance for the distribution of the AMF communities. Only soil variables with significant effects ($P < 0.05$) are shown in the bi-plot diagram. This analysis was conducted with CANOCO for Windows (version 4.5) (63).

Nucleotide sequence accession numbers. A total of 144 representative sequences of OTUs from root and soil samples from different soil types generated in this study have been deposited in GenBank under accession numbers HG380100 to HG380243.

RESULTS

PCR and sequence analysis. All of the root and rhizosphere soil samples extracted were amplified successfully by nested PCR and generated PCR products of the expected band size of approximately 795 bp, which were used for cloning and creation of the clone libraries. We screened 1,152 clones in total from soil and roots (32 clones were analyzed per library); of those, 1,092 clones contained an SSU rDNA fragment and subsequently were sequenced. The BLAST search revealed that 981 sequences had a high degree (≥95%) of similarity to sequences from taxa belonging to the phylum *Glomeromycota*, while the remaining 111 sequences showed BLAST similarity to plants and fungi belonging to *Ascomycotina*.

AMF richness. Forty-nine OTUs (see Table S4 in the supplemental material) could be distinguished on the basis of bootstrap values of more than 85% (see Fig. S2 in the supplemental material). Sequences from the families *Glomeraceae* (29 OTUs), *Paraglomeraceae* (7 OTUs), *Claroideoglomeraceae* (4 OTUs), *Diversisporaceae* (3 OTUs), *Archaeosporaceae* (2 OTUs), *Ambisporaceae* (2 OTUs), *Acaulosporaceae* (1 OTU), and *Gigasporaceae* (1 OTU) were obtained. Ten OTUs clustered with previously identified sequences, i.e., *Glomus macrocarpum* (G3), *Sclerocystis sinuosa* (Sc1), *Rhizophagus clarus* (Rh1), *Rhizophagus intraradices-irregularis-fasciculatus* (Rh2), *Diversispora spurca-aurantia* (D1), *Redeckera fulvum* (Red1), *Acaulospora laevis-lacunosa-spinosa* (Ac1), *Claroideoglomus luteum-claroideum-lamellosum* (Cl1), *Archaeospora schenckii-trappei* (Ar2), and *Ambispora leptoticha* (Amb2). Twenty OTUs clustered with uncultured *Glomeromycota* sequences recorded in the database. The remaining 19 OTUs were *Glomeromycota* not clustering with any sequences in the database.

Effects of soil type and habitat (roots or rhizosphere soil) on AMF community composition. In order to determine whether the number of clones sequenced was sufficient to represent the AMF diversity in the roots and in the rhizosphere soil, rarefaction curves were constructed (see Fig. S1A and B in the supplemental material). For the rhizosphere soil samples, the clones sequenced were sufficient to allow the detection of the majority of OTUs. For the root samples, there was a well-defined plateauing of the curves, and it is highly unlikely that the sequencing of more clones would have revealed more OTUs, except in XER soil.

Indicator species analyses were conducted to find specific OTUs associated with root or rhizosphere soil samples and specific OTUs associated with soil types. Seven OTUs were more prone to be found in rhizosphere soil samples; five of them were specific for rhizosphere soil samples (G17, Fu5, P4, Cl4, and Ar2), and the other two both were specific for and showed fidelity for rhizosphere soil samples (Fu1 and Cl1) (Table 1). On the other hand, 4 OTUs were associated with the root habitat; the OTU Rh2 presented more fidelity to the root habitat, and Rh1, G6, and Scu1 showed both specificity and fidelity for roots (Table 1). Although there was a wide range of phylogenetic diversity in both habitats, OTUs significantly associated with roots were more closely related than expected by chance (observed transitions [OTs], 3; null transitions [NTs] [i.e., transitions in the null model], 4; $P = 0.05$), while this was not the case for OTUs associated with rhizosphere soil (OTs, 7; NTs, 7; $P > 0.05$) (Fig. 1).

TABLE 1 Indicator species analyses

OTU and association[a]	Probability[b] A	Probability[b] B	Indicator value index	*P*
OTUs associated with habitat				
Rhizosphere soil				
Fu1	1.000	0.778	0.882	0.001
Cl1	0.708	0.945	0.818	0.003
G17	1.000	0.556	0.745	0.001
Fu5	1.000	0.500	0.707	0.002
P4	1.000	0.389	0.624	0.006
Cl4	1.000	0.333	0.577	0.022
Ar2	1.000	0.278	0.527	0.045
Roots				
Rh1	0.833	0.833	0.833	0.001
G6	0.929	0.722	0.819	0.001
Rh2	0.621	1.000	0.788	0.004
Scu1	0.733	0.611	0.669	0.047
OTUs associated with soil types				
THP				
G18	1.000	1.000	1.000	0.001
G19	1.000	1.000	1.000	0.001
G1	1.000	0.667	0.816	0.003
Fu4	1.000	0.500	0.707	0.017
Amb1	1.000	0.500	0.707	0.008
THA				
G16	1.000	0.500	0.707	0.016
Sc1	1.000	0.500	0.707	0.016
Ac1	1.000	0.500	0.707	0.016
G12	0.429	1.000	0.655	0.009
LHP				
D2	1.000	0.500	0.707	0.018
TOR				
Fu2	1.000	0.500	0.707	0.016
XER				
G11	1.000	0.667	0.816	0.001
G20	0.3529	1.000	0.594	0.038
THS				
G14	1.000	0.500	0.707	0.012

[a] Soil types were as follows: THS, Typic Haplosalid; XER, Lithic Xerorthent; TOR, Xeric Torriorthent; THA, Typic Haplargid; LHP, Lithic Haploxeroll; THP, Typic Haploxeroll.
[b] Probability A, the probability that the surveyed site belongs to a given environment, given the fact that the species has been found; probability B, the probability of finding the species in sites belonging to a given environment.

There were also OTUs associated with specific soil types. Five OTUs tended to occur in Typic Haploxeroll (THP) (G18, G19, G1, Fu4, and Amb1), four in Typic Haplargid (THA) (G16, Sc1, Ac1, and G12), two in Lithic Xerorthent (XER) (G11 and G20), and one each in Lithic Haploxeroll (LHP) (D2), Xeric Torriorthent (TOR) (Fu2), and Typic Haplosalid (THS) (G14) (Table 1). Therefore, only in THP and THA were there enough OTUs to test for a phylogenetic signal in the association with a given soil. In both soils, there was not a phylogenetic signal (THP: OTs, 5; NTs, 5; $P > 0.05$; THA: OTs, 4; NTs, 4; $P > 0.05$) (Fig. 1).

We used principal-component analysis (PCA) to examine the influence of habitat (root or rhizosphere soil) on AMF community variation. Habitat explained 54.3% of the variation in AMF community composition, showing a clear effect of habitat on

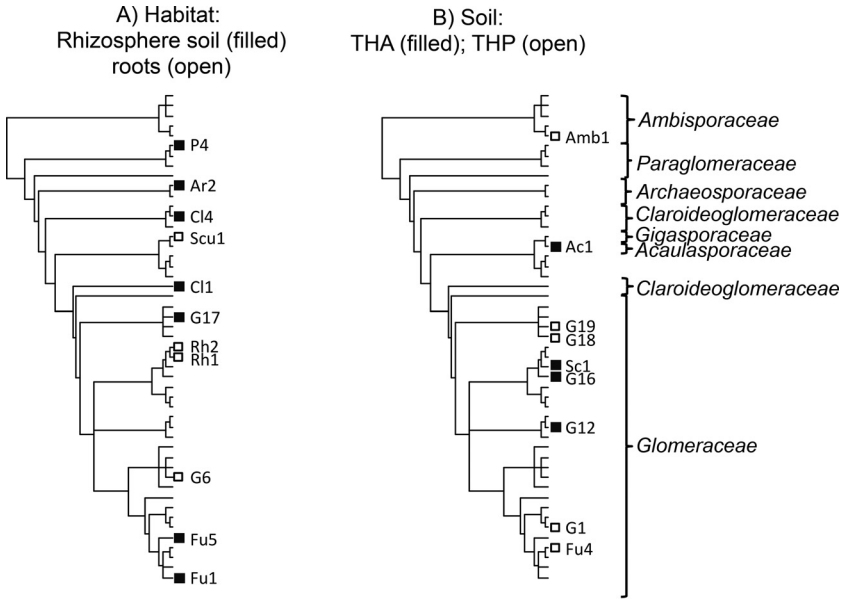

FIG 1 Phylogenetic distribution of the AMF OTUs characteristic of habitat, i.e., rhizosphere soil (■) and roots (□) (A), and the two soils with the most contrasting microbiological activities, i.e., THP (low activity) (□) and THA (high activity) (■) (B). The abbreviations correspond to the codes of the operational taxonomic units. The phylogenetic relationships between the selected OTUs are extracted from Fig. S2 in the supplemental material.

AMF distribution (Fig. 2A). Considering the complete AMF community associated with *Brachypodium retusum* as the sum of the AMF communities of rhizosphere soil and roots, soil type explained 69.7% of the AMF variation (Fig. 2B). When the two factors and their interaction were considered, there was also a significant difference in AMF community composition across habitats ($F = 91.83$, df = 1, partial eta-square = 0.96, $P < 0.001$), sites ($F = 36.19$, df = 5, partial eta-square = 0.69, $P < 0.001$), and habitat-

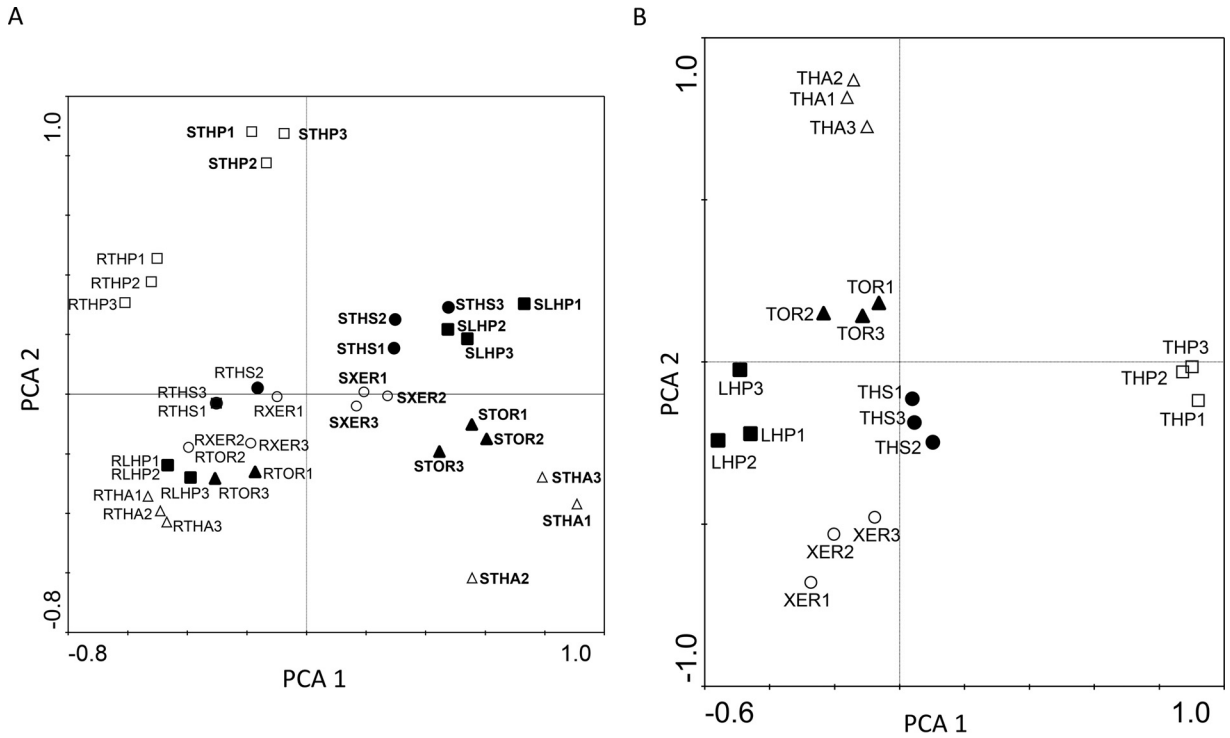

FIG 2 (A) Principal-component analysis (PCA) of the AMF community composition in the roots and in the rhizosphere soil of *Brachypodium retusum* in six different soil types. The amounts of variation explained by the first two PCA axes were as follows: PCA1, 0.20; PCA2, 0.13. The model explained 54.3% of the whole variance. THS, Typic Haplosalid; XER, Lithic Xerorthent; TOR, Xeric Torriorthent; THA, Typic Haplargid; LHP, Lithic Haploxeroll; THP, Typic Haploxeroll; S, rhizosphere soil; R, roots. (B) PCA of the global AMF community composition under *Brachypodium retusum* in six different soil types. The amounts of variation explained by the first two PCA axes were as follows: PCA1, 0.19; PCA2, 0.18. The model explained 69.7% of the whole variance.

site interaction ($F = 23.41$, df = 5, partial eta-square = 0.65, $P < 0.001$), confirming the significant influence of both habitat and soil type on AMF distribution.

Soil characteristics. The evaluation of soil physicochemical and biological parameters showed significant differences among soil types for most of the soil properties considered (see Table S3 in the supplemental material). THS and THA soils presented the highest pH values, although all of the soils had similar pH ranges. Electrical conductivity values were significantly greater for TOR soil. The highest values for aggregate stability were recorded in THA and LHP soils, whereas the lowest values were found in TOR and THS soils.

Regarding the chemical properties of the soils sampled, XER soil showed significantly lower values for total carbon and calcium levels, whereas TOR soil presented the highest values for those properties. The latter soil, TOR, showed the lowest values for most chemical properties, such as nitrogen, organic carbon, available phosphorus, sodium, magnesium, iron, manganese, and zinc levels.

In relation to biological properties (enzyme activities and glomalin-related soil protein levels), the lowest dehydrogenase, urease, protease, and alkaline phosphatase activities and glomalin-related soil protein levels were observed in TOR and THP soils. In contrast, the highest levels were found in THA soil. The highest levels of water-soluble carbohydrates and total carbohydrates were recorded in LHP soil, whereas the lowest levels were found in TOR soil.

Soil properties triggering AMF community structure. The different soils tested differed significantly in most of the properties evaluated, which allowed us to try to establish relationships between the edaphic factors and AMF communities. Multivariate analysis based on constrained ordination, RDA, was used to investigate the influence of soil properties (used as explanatory variables) on the AMF community composition in *B. retusum* rhizospheres (Fig. 3). The first two axes explained 37.1% of the total variance (69.7% for the model). RDA and subsequent forward procedures selected 6 soil properties as significantly triggering AMF community composition, i.e., three related to microbiological activity (namely, urease, dehydrogenase, and total carbohydrate levels; $P < 0.01$), soil pH ($P < 0.01$), and levels of two essential micronutrients (i.e., Mn and Zn; $P < 0.05$ and $P < 0.01$, respectively).

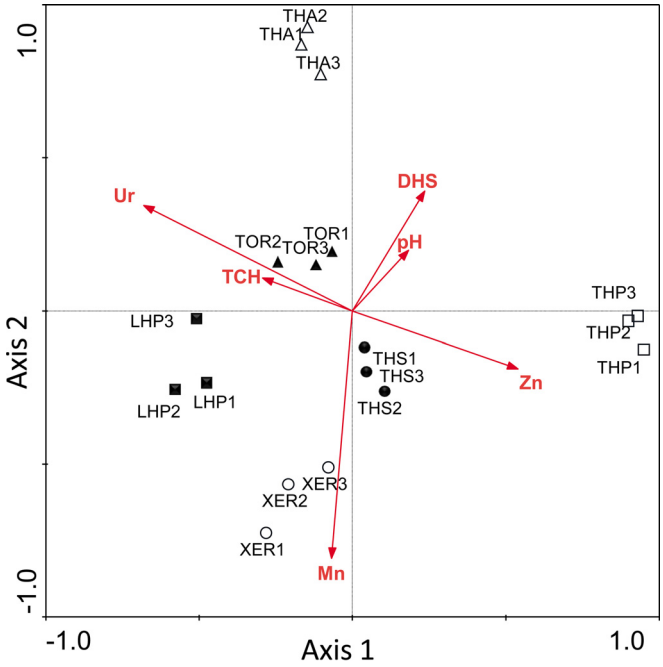

FIG 3 Redundancy analysis (RDA) showing the influence of soil properties on the AMF community composition in *B. retusum* rhizospheres. The first two axes explained 37.1% of the total variance (69.7% for the model). Only soil variables with significant effects in Monte Carlo tests ($P < 0.05$) are shown in the bi-plot diagram. THS, Typic Haplosalid; XER, Lithic Xerorthent; TOR, Xeric Torriorthent; THA, Typic Haplargid; LHP, Lithic Haploxeroll; THP, Typic Haploxeroll; DHS, dehydrogenase; TCH, total carbohydrates; Ur, urease.

DISCUSSION

Our results pointed out a tendency of AMF communities in roots to be more similar to each other and different from those in the rhizosphere soil. The indicator species analyses revealed 7 OTUs associated with rhizosphere soil and 4 OTUs with the root habitat. In general, AMF in soil are considered to represent a pool of species from which plants recruit only a fraction at any time (64, 65). Previous morphologically based studies documented differences in AMF present in the roots or rhizosphere of the same plants, probably due to differential sporulation dynamics (66), to seasonal changes in the AMF community (67), or to different life history strategies of AMF (68). Our results coincide with those of Saks et al. (69), showing that root-colonizing AMF represent a phylogenetically clustered subset of AMF available in soil.

In this sense, Varela-Cervero et al. (70) observed that AMF communities detected in root samples from different plant species in a semiarid Mediterranean area were more similar to each other than those in extraradical mycelium and spore fractions, which were more variable. *Glomeraceae* were described previously as abundant in roots but scarce in soil (37, 70, 71). Although our results showed that *Glomeraceae* were also common in rhizosphere soil, three of the four OTUs identified by the indicator species analyses as characteristic of roots are *Glomeraceae* (Fig. 1A), supporting the previous evidence to some extent. This phylogenetic pattern suggests that, among AMF, there are niche preferences and this is probably one important factor regulating AMF community composition (70).

From the findings described above, it seems that a given OTU of an AMF in an individual plant species can be detected in the roots, the rhizosphere soil, or both. In this sense, we considered the AMF communities as the sum of both soil and root populations, to establish their relationships with the edaphic characteristics tested. There is evidence of several biotic factors with strong influences in regulating AMF community composition, with the best studied being the host plant (29, 30, 72–74) and host functional traits (6, 37). Among the abiotic factors that can have relevant roles in driving AMF communities are soil moisture (75), rainfall patterns, and geographical distance (40, 76). There is no doubt that soil type has a role in the AMF distribution (10, 34, 35, 40, 77–80), and our work also demonstrated that soil type is a major factor driving AMF assemblages, after elimination of the host factor and other environmental variables not related to soil characteristics. Although individual soil characteristics have been reported to play important roles in AMF community structure and composition (7, 11, 13, 40, 42, 78, 81–83), very limited data

sets regarding soil characteristics were used in these studies, and the relative incidences of each soil property determined after complete soil characterization have not been reported previously.

Multivariate analysis based on constrained ordination (RDA) identified urease, dehydrogenase, total carbohydrates, pH, Zn, and Mn as the soil properties significantly influencing the AMF community distribution in *Brachypodium retusum* (roots plus rhizosphere). Some of these soil properties have been reported previously to affect the growth and distribution of AMF.

Soil enzyme activities and microbial processes are particularly important because they usually are good indicators of system sustainability (45, 84). Enzyme activities can play an important role, since some authors have reported direct effects of these on AMF colonization development (85). Enzyme activities are involved in nutrient cycling and decomposition of organic matter and respond quickly to any form of change occurring in the system. The importance of urease, dehydrogenase, and total carbohydrates in shaping AMF communities suggests that, in the semiarid soils studied, where biological activity is generally very low (86), the structure of AMF assemblages is partly determined by the soil parameters directly related to microbial activity (13). Our results also showed that the phylogenetic compositions of AMF communities in soils with high (THA) or low (THP) microbiological activity were not phylogenetically clustered. The OTUs of these two contrasting soils tended to belong to different clades in the phylogeny (Fig. 2B), suggesting that there may be some niche segregation between species characteristic of soils with contrasting microbiological activities.

In this survey, pH was found to be a significant factor shaping AMF community composition. Soil acidity is one of the most important drivers (environmental filters) of microbial communities and particularly of AMF communities (7, 87). Recently, Bainard et al. (9) concluded that soil pH is the only environmental variable that appears to be a key factor in the assembly of AMF communities in the Canadian prairie landscape.

According to our results, besides pH, consistent chemical drivers of AMF communities were Zn and Mn soil contents. These micronutrients are important for metabolic processes in plants (88), and their uptake can be positively influenced by AMF (89, 90). Several studies showed strong negative effects of Zn soil contents on AMF abundance and diversity in polluted soils (20–23). The Zn contents in our surveyed soils were far from those found in heavy metal-polluted soils; however, it seems that Zn can be a determinant in shaping the structure of AMF communities also in nonpolluted soils. Little is known about the influence of Mn on the abundance and diversity of AMF, as well as on the composition of their communities. Wei et al. (91) found that AMF root colonization and diversity were negatively correlated with total extractable Mn concentrations in contaminated soils, and they concluded that Mn contamination affected AMF diversity and shaped AMF community structure. As in the case of Zn, our results point to a relevant role of Mn also in noncontaminated soils from semiarid areas.

It can be concluded that both soil type and habitat (root versus rhizosphere) determine the distribution of AMF communities in semiarid Mediterranean soils. In addition, the driving effect of soil type could not be attributed to a single soil characteristic, and the use of extensive soil characterization revealed that up to three soil properties related to microbial activity, i.e., pH and the levels of two micronutrients (Mn and Zn), play significant roles in triggering AMF populations.

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