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Plant type differently promote the arbuscular mycorrhizal fungi biodiversity in the rhizosphere after revegetation of a degraded, semiarid land

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ABSTRACT

It is suggested that the diversity of arbuscular mycorrhizal fungi (AMF) and their association with distinct plants species are crucial in the early stages of revegetation procedures since the AMF roots colonisation plays an important role improving plant establishment and growth. We carried out a study where we analyse the AMF community composition in the roots of *Ephedra fragilis*, *Rhamnus lycioides*, *Pistacia lentiscus* and *Retama sphaerocarpa* fourteen months after revegetation in a Mediterranean semiarid degraded area of southeast Spain in order to verify whether different plant species can variably promote the diversity of AM fungi in their rhizospheres after planted. We analysed a portion of approximately 795 bases pairs of the small-subunit ribosomal DNA by means of nested PCR, cloning, sequencing and phylogenetic analyses. Eight fungal sequence types belonging to *Glomus* group A and B and to the genus *Paraglomus* were identified. The different plant species had different AM fungal community composition. Thus, *R. lycioides* harboured the highest number of four fungal sequence types while from *E. fragilis* only two types could be characterized that were specific for this plant species. *P. lentiscus* and *R. sphaerocarpa* harboured each one three sequence types and two of them were shared. All AMF sequence types were found in the natural soil. These results show that one effective way of restoring degraded lands is to increase the number of plant species used, which would increase the AMF diversity in the soil and thus the below-ground, positive interactions.

1. Introduction

In Mediterranean semiarid areas of southeast Spain, scarce and irregular rainfall and a long, dry period in summer have contributed drastically to the acceleration of the processes of soil degradation. The environmental changes as a consequence of the loss of natural plant communities are often accompanied or preceded by the degeneration of physical and chemical soil properties as well as by the loss or reduction of the below-ground microbial activity, particularly that of arbuscular mycorrhizal fungi (AMF). This produces a reduction in the amount, diversity and activity of mycorrhizal propagules capable of colonising the roots (mycorrhizal potential) in degraded areas (Requena et al., 2001; Azcón-Aguilar et al., 2003). AM fungi are obligate symbiotic fungi which form mutualistic associations with the roots of most land plants. They may help plant to thrive in semiarid conditions (Herrera et al., 1993; Caravaca et al., 2003, 2005) by increasing the supply of nutrients to the plant (Smith and Read, 2008; Kafkas and Ortas, 2009) and improving soil structure in eroded soils (Caravaca et al., 2002). It has been showed that extraradical hyphae contribute to the formation and maintenance of soil aggregates (Bedini et al., 2009; Rillig et al., 2010) through the exudation of glomalin, a glycoprotein that acts as a glue-like agent for soil particles (Wright and Anderson, 2000). Earlier experiments have demonstrated that the AM fungal diversity in soil can affect the diversity and productivity of plants and, therefore, the stability and sustainability of the ecosystem (Van der Heijden et al., 1998, 2006; Vogelsang et al., 2006). Also, it has been shown that the composition and diversity of the plant community influence the structure of the AMF community (Burrows and Pfleger, 2002; Johnson et al., 2003). Thus, mycorrhizal symbiosis seems to be a key ecological factor in the functioning of ecosystems in semiarid Mediterranean regions (Requena et al., 1996).

Several studies carried out in degraded semiarid Mediterranean areas showed that different shrubs representative of these areas differed in their ability to enhance the development of mycorrhizal propagules in the soil (Azcón-Aguilar et al., 2003; Caravaca et al., 2005), but whether these differences in the amount of AM fungal propagules correspond with variations in the AMF diversity is still

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unknown. Several studies developed recently on AMF molecular ecology have shown that AM fungi have host preferences or host specificity and that different plant species are colonised by different AM fungal communities (e.g. Vandenkoornhuyse et al., 2003; Öpik et al., 2006; Alguacil et al., 2009b), although a lack of specificity for some AM fungal species also has been indicated; these AM fungal isolates have been found associated with a wide range of plant species in very different ecosystems (Öpik et al., 2006). Therefore, there is increasing ecological interest in the diversity of AM fungi present in roots of different plants species under field conditions, particularly with respect to revegetation programmes for degraded ecosystem using autochthonous shrubs. On the one hand the most-functional species of AM fungi can serve as a source of inoculum for subsequent use in revegetation and/or recovery programmes for degraded soils. Thus, the selection of plant species able to promote a high AMF biodiversity in their rhizosphere is an important point in the restoration of these difficult sites.

In the present study, we used the PCR primers, developed recently by Lee et al. (2008) and proposed to be AMF-specific, to analyse the natural AMF communities which participate in the early colonisation of the roots of four shrub species after revegetation. These plants belong to the natural succession in semiarid Mediterranean ecosystems: *Ephedra fragilis* Desf., *Rhamnus lycioides* L., *Pistacia lentiscus* L. and *Retama sphaerocarpa* (L.) Boiss.

The objective of this study was therefore to ascertain whether different plant species promote differently the diversity of AM fungi in their rhizospheres when planted in a semiarid, degraded soil.

2. Materials and methods

2.1. Study site

The experimental area was located in the natural ecological park "*Vicente Blanes*" in Molina de Segura, Province of Murcia (Southeastern Spain) (coordinates 38°12′ N, 1°13′ W, 393 m altitude). The climate is semiarid Mediterranean, with an average annual rainfall lower than 270 mm and the potential evapotranspiration (ETP) reaches approximately 1000 mm. The mean annual temperature is 19.2 °C with absence of frost period. The soil in the experimental area is a Typic Torriorthent (SSS, 2006), very little developed with a low organic matter content and a silty clay texture that facilitates the degradation of soil structure. The vegetation in the zone was dominated by *Piptatherum miliaceum* (L.) Cosson., and some shrubs of *Thymus vulgaris L.* and *Rosmarinus officinalis* L. growing with patchy distribution.

2.2. Experimental design

The experiment was conducted using a randomized factorial design with six replication blocks. The following plant species were selected: *E. fragilis* (Desf.), *R. lycioides* L., *P. lentiscus* L. and *R. sphaerocarpa* (L.) Boiss. They are representative autochthonous shrub species from semiarid scrublands in southeast Spain, well adapted to water stress conditions and, therefore, frequently used in the revegetation of semiarid disturbed lands. Once germinated, seedlings were transplanted into the growth substrate, consisting of peat and cocopeat (1:1, v/v) autoclaved (60 min, 120 °C) in order to avoid any mycorrhizal propagules. They were grown with watering for 8 months under nursery conditions. In early April 2007, an area of 1.200 m^2 was selected to carry out the plantation. 30 seedlings of each plant species (5 per replication block) were planted in individual holes, separated between them by at least 1 m. The experiment was carried out under strictly natural conditions, without any watering or fertilizer treatments.

2.3. Sampling

Fourteen months after plantation, in early June 2008 (late spring), when the highest fungal activity could be expected in Mediterranean semiarid soils (Roldán and Albaladejo, 1993) six plants belonging to each of the four selected species (one per replication block) were sampled (a total of 24 plants). Plants, including root systems, were collected and placed in polyethylene bags for transport to the laboratory, where fine roots were separated from the soil. Roots were then briefly rinsed, quickly dried on paper and used for molecular analysis. Six samples of soil (one per replication block) of 500 g each were also randomly collected at 20–40 cm depths. The soil samples were sieved through 2-mm pores to eliminate large particles and stored in plastic bags at −20 °C until processed.

2.4. Roots and soil DNA extraction and PCR

All PCR experiments were run using DNA preparations consisting of pooled roots of individual plants or soil extracts. DNA extractions from 24 root samples (one root sample from each replication block for each shrub species) and 6 soil samples were carried out.

For each sample, total DNA was extracted from root material (representing approx. 5–8 cm root length) using a DNeasy plant mini Kit following the manufacturer's recommendations (Qiagen). The roots samples were placed into a 2-ml screw-cap propylene tube and the DNA extracts were obtained by disrupting roots with a sterile disposable micro-pestle in liquid nitrogen. The DNA was resuspended in 20 μl of water.

For each of the six soil samples, genomic 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). DNA extracts were stored at −20 °C.

Several dilutions of extracted DNA (1/10, 1/50, 1/100) were prepared and 2 μl were used as template. Partial small-subunit (SSU) ribosomal RNA gene fragments were amplified using nested PCR with the universal eukaryotic primers NS1 and NS4 (White et al., 1990). PCR was carried out in a final volume of 25 μl using the "ready to go" PCR beads (Amershan Pharmacia Biotech), 0.2 μM dNTPs and 0.5 μM of each primer (PCR conditions: 94 °C for 3 min, then 30 cycles at 94 °C for 30 s, 40 °C for 1 min, 72 °C for 1 min, followed by a final extension period at 72 °C for 10 min).

Two μl 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 reaction performed using the specific primers AML1 and AML2 (Lee et al., 2008). PCR reactions were carried out in a final volume of 25 μl using the "ready to go" PCR beads (Amershan Pharmacia Biotech), 0.2 μM dNTPs and 0.5 μM of each primer (PCR conditions: 94 °C for 3 min, then 30 cycles of 1 min denaturation at 94 °C, 1 min primer annealing at 50 °C and 1 min 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 the PCR reactions were run on a Perkin Elmer Cetus DNA Thermal Cycler. Reactions yields were estimated by using a 1.2% agarose gel containing ethidium bromide.

2.5. 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). Forty putative positive transformants were screened in each resulting SSU rRNA gene library, using 0.7 unit of RedTaq DNA polymerase (Sigma) and a re-amplification with AML1 and AML2 primers with the same conditions described

above. Product quality and size were checked in agarose gels as described above. All clones having inserts of the correct size in each library were sequenced.

Clones were grown in liquid culture and the plasmid extracted using the QIAprep Spin Miniprep Kit (Qiagen). The sequencing was done by Laboratory of Sistemas Genómicos (Valencia, Spain) using the universal primers SP6 and T7. Sequence editing was done using the program Sequencher version 4.1.4 (Gene Codes Corporation). 113 representative sequences of the clones generated in this study have been deposited at the National Centre for Biotechnology Information (NCBI) GenBank (http://www.ncbi.nlm.nih.gov) under the accession numbers FN645952–FN646064.

A search for chimeric sequences was performed using the program CHIMERA_Check 2.7 of the Ribosomal Database Project (http://rdp.cme.msu.edu/html/analyses.html) (Maidak et al., 2001).

2.6. Phylogenetical analysis

Sequence similarities were determined using the Basic Local Alignment Search Tool (BLASTn) sequence similarity search tool (Altschul et al., 1997) provided by GenBank. Phylogenetic analysis was carried out on the sequences obtained in this study and those corresponding to the closest matches from GenBank. Sequences were aligned with other published glomeralean sequences using the program ClustalX (Thompson et al., 1997) and the alignment was adjusted manually in GeneDoc (Nicholas and Nicholas, 1997). Neighbour-joining (NJ) phylogenetic analyses (Saitou and Nei, 1987) was performed with the program PAUP4.08b (Swofford, 2002) and using the default parameters. *Endogone pisiformis* Link and *Mortierella polycephala* Coem, were used as the out-group.

2.7. Statistical analysis

Correspondence analysis (CA) with presence and/or absence data for all AMF sequence types at four host plant species was performed, and the results were summarized in an ordination diagram. CA is a multivariate statistical method that allows comparisons of AM fungal community compositions between all plant species. The effect of host plant species on the number of AMF sequence types found in the root samples was analysed by univariate ANOVA tests. Statistical procedures were carried out with the software package SPSS 17.0 for Windows.

The presence or absence of AMF phylotypes in each root and soil sample was used to construct the sampling effort curves (with 95% confidence intervals) using the software EstimateS 8.00 (Colwell, 2005). The sample order was randomized by 100 replications.

The Shannon–Weaver (H′) index was calculated as an additional measure of diversity, as it combines two components of diversity, i.e., species richness and evenness. It is calculated from the equation $H' = -\sum pi(\ln pi)$, where pi is the proportion of individuals found in the ith species (in a sample, the true value of pi is unknown but is estimated as ni/N, [here and throughout, ni is the number of individuals in the ith species]).

3. Results

3.1. PCR and sequences analysis

Twenty of the 24 root samples extracted and five of the six soil samples generated PCR products of the expected band of approximately 795 bps, which were used for cloning and creating a clone library. From the 25 clone libraries, a total of 1000 clones were screened by PCR (on average, 40 clones were analysed per library); out of these, a total of 267 clones contained an SSU rDNA fragment and subsequently all clones were sequenced. The BLAST search revealed that 176 sequences (65.9%) had a high degree of similarity to sequences from taxa belonging to the phylum *Glomeromycota*, while the 91 remaining sequences (34.1%) were similar to sequences from host plant species (see Table 1 in the Supplemental material for a detailed description of the number of clones of each AMF sequence type that were recovered from each single host plant and soil). Sequences identified as host plant species were detected in 12 clone libraries belonging to the four shrub species studied. In the libraries derived from soil samples, no sequences from plant species were detected.

3.2. Phylogenetic analysis of AMF groups

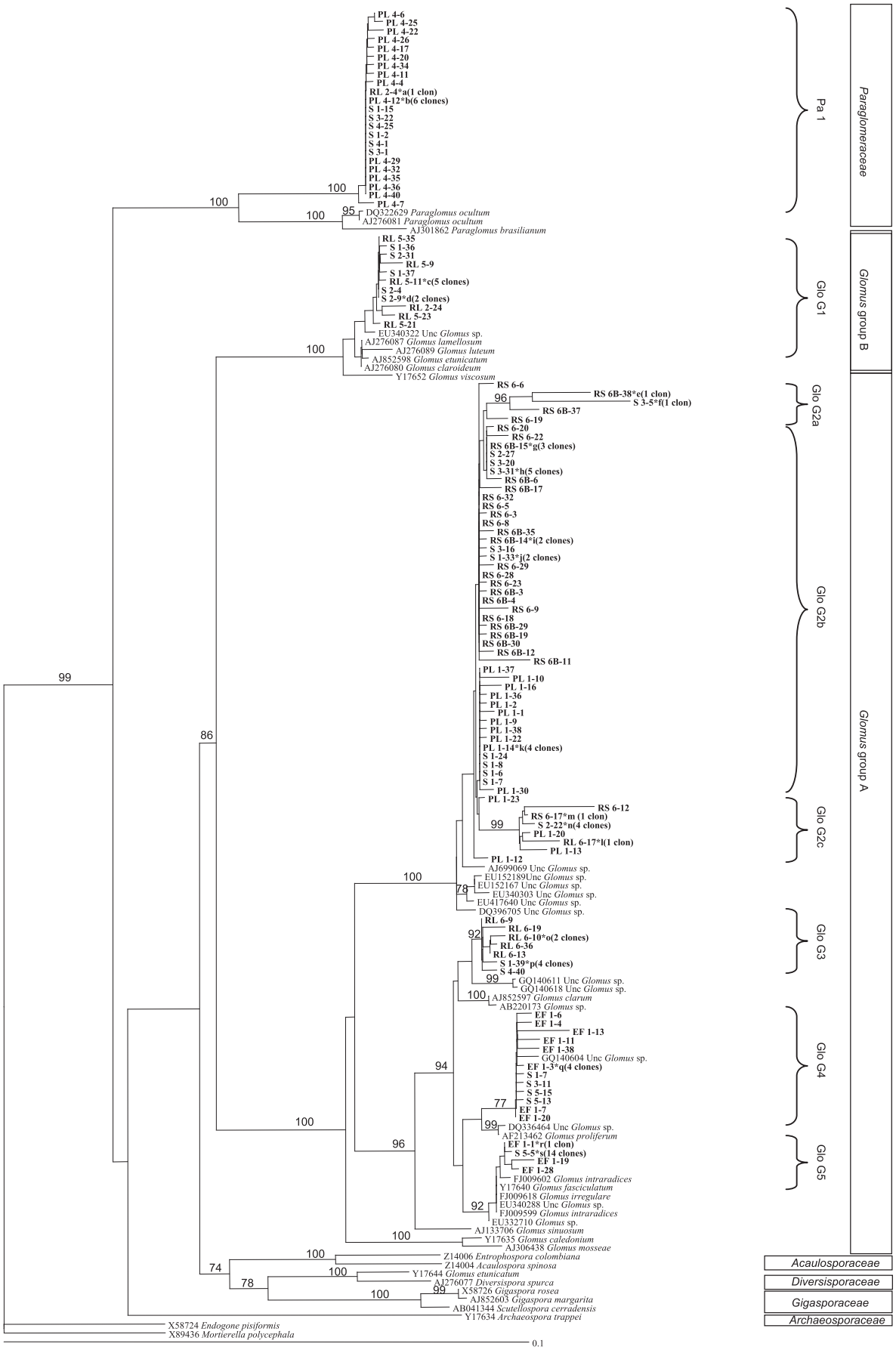
An alignment between 113 different glomalean sequences recovered in this study derived from shrub species root and soil and 41 sequences downloaded from GenBank was conducted. Clones that produced the same sequence were represented just once in the alignment and the 63 remaining identical clones were described in the Supplemental material (see Supplemental material for a detailed description of the clone groups with identical sequences). The pairwise sequence similarities within the grouping varied from 97% to 100%. After Neighbour-joining (NJ) analyses, eight AMF sequence types grouped in the Glomeraceae and Paraglomeraceae families could be distinguished on the basis of bootstrap values $\geq$77% (Fig. 1). Six AMF sequence types belonged to *Glomus* group A, which was the group represented most frequently, one sequence type belonged to *Glomus* group B and one to *Paraglomus*.

Only two AMF sequences types identified in this study showed high similarity to sequences of previously-known glomalean species: Glo G1 clustered together with *Glomus lamellosum* and Glo G5 showed high similarity to the species complex *Glomus irregulare*/*Glomus fasciculatum*. Glo G 2b and Glo G4 showed high similarity to previously-described, root-derived sequences in GeneBank belonging to unknown glomeralean species. Pa 1, although forming a different clade with a high bootstrap value (100%), showed high similarity (95%) with *Paraglomus occultum*. The rest of the sequences (Glo 2a, Glo 2c and Glo G3) received strong support in the phylogenetic analysis but did not seem to be related to any sequences of AM fungi in the database (Fig. 1).

Sampling effort curves verified that the number of root and soil samples analysed was sufficient to detect the majority of PCR-amplifiable sequence types present in the roots of the four shrub species studied and in the soil. These curves showed that there were diversity plateaus after exponential rises for two AMF sequence types for *E. fragilis*, three types for *P. lentiscus* and *R. sphaerocarpa*, four types for *R. lycioides* and eight types for the soil (Fig. 2).

3.3. AMF sequence types distribution

The host plant species had a statistically-significant influence on the AMF communities ($F = 37.8$; $P < 0.001$). *R. lycioides* had the highest AMF richness, hosting four of the eight AM fungal sequence types found in this study (Fig. 3); also, this shrub showed the highest Shannon–Weaver diversity index ($H' = 1.15$). *R. sphaerocarpa* and *P. lentiscus* harboured three AMF sequence types. The AMF communities of *E. fragilis* had the lowest diversity ($H' = 0.56$), with the lowest number of AMF sequence types. The sequence type Glo G2b was the most widespread and accounted for 41% of the AMF sequences detected; it was present only in two shrub species, colonising *R. sphaerocarpa* more frequently than *P. lentiscus*. The next-most-frequent sequence type was Pa 1, which was found almost exclusively in *P. lentiscus*, in a higher proportion than in *R. lycioides*. The Glo G2c fungal sequence type was distributed equally in three shrub species, the exception being *E. fragilis*, while


PL 4-6
PL 4-25
PL 4-22
PL 4-26
PL 4-17
PL 4-20
PL 4-34
PL 4-11
PL 4-4
RL 2-4*a(1 clon)
PL 4-12*b(6 clones)
S 1-15
S 3-22
S 4-25
S 1-2
S 4-1
S 3-1
PL 4-29
PL 4-32
PL 4-35
PL 4-36
PL 4-40
PL 4-7
DQ322629 Paraglomus ocultum
AJ276081 Paraglomus ocultum
AJ301862 Paraglomus brasilianum
RL 5-35
S 1-36
S 2-31
RL 5-9
S 1-37
RL 5-11*c(5 clones)
S 2-4
S 2-9*d(2 clones)
RL 2-24
RL 5-23
RL 5-21
EU340322 Unc Glomus sp.
AJ276087 Glomus lamellosum
AJ276089 Glomus luteum
AJ852598 Glomus etunicatum
AJ276080 Glomus claroideum
Y17652 Glomus viscosum
RS 6-6
RS 6B-38*e(1 clon)
S 3-5*f(1 clon)
RS 6B-37
RS 6-19
RS 6-20
RS 6-22
RS 6B-15*g(3 clones)
S 2-27
S 3-20
S 3-31*h(5 clones)
RS 6B-6
RS 6B-17
RS 6-32
RS 6-5
RS 6-3
RS 6-8
RS 6B-35
RS 6B-14*i(2 clones)
S 3-16
S 1-33*j(2 clones)
RS 6-29
RS 6-28
RS 6-23
RS 6B-3
RS 6B-4
RS 6-9
RS 6-18
RS 6B-29
RS 6B-19
RS 6B-30
RS 6B-12
RS 6B-11
PL 1-37
PL 1-10
PL 1-16
PL 1-36
PL 1-2
PL 1-1
PL 1-9
PL 1-38
PL 1-22
PL 1-14*k(4 clones)
S 1-24
S 1-8
S 1-6
S 1-7
PL 1-30
PL 1-23
RS 6-12
RS 6-17*m (1 clon)
S 2-22*n(4 clones)
PL 1-20
RL 6-17*l(1 clon)
PL 1-13
PL 1-12
AJ699069 Unc Glomus sp.
EU152189Unc Glomus sp.
EU152167 Unc Glomus sp.
EU340303 Unc Glomus sp.
EU417640 Unc Glomus sp.
DQ396705 Unc Glomus sp.
RL 6-9
RL 6-19
RL 6-10*o(2 clones)
RL 6-36
RL 6-13
S 1-39*p(4 clones)
S 4-40
GQ140611 Unc Glomus sp.
GQ140618 Unc Glomus sp.
AJ852597 Glomus clarum
AB220173 Glomus sp.
EF 1-6
EF 1-4
EF 1-13
EF 1-11
EF 1-38
GQ140604 Unc Glomus sp.
EF 1-3*q(4 clones)
S 1-7
S 3-11
S 5-15
S 5-13
EF 1-7
EF 1-20
DQ336464 Unc Glomus sp.
AF213462 Glomus proliferum
EF 1-1*r(1 clon)
S 5-5*s(14 clones)
EF 1-19
EF 1-28
FJ009602 Glomus intraradices
Y17640 Glomus fasciculatum
FJ009618 Glomus irregulare
EU340288 Unc Glomus sp.
FJ009599 Glomus intraradices
EU332710 Glomus sp.
AJ133706 Glomus sinuosum
Y17635 Glomus caledonium
AJ306438 Glomus mosseae
Z14006 Entrophospora colombiana
Z14004 Acaulospora spinosa
Y17644 Glomus etunicatum
AJ276077 Diversispora spurca
X58726 Gigaspora rosea
AJ852603 Gigaspora margarita
AB041344 Scutellospora cerradensis
Y17634 Archaeospora trappei
X58724 Endogone pisiformis
X89436 Mortierella polycephala
0.1
Pa 1
Glo G1
Glo G2a
Glo G2b
Glo G2c
Glo G3
Glo G4
Glo G5
Paraglomeraceae
Glomus group B
Glomus group A
Acaulosporaceae
Diversisporaceae
Gigasporaceae
Archaeosporaceae

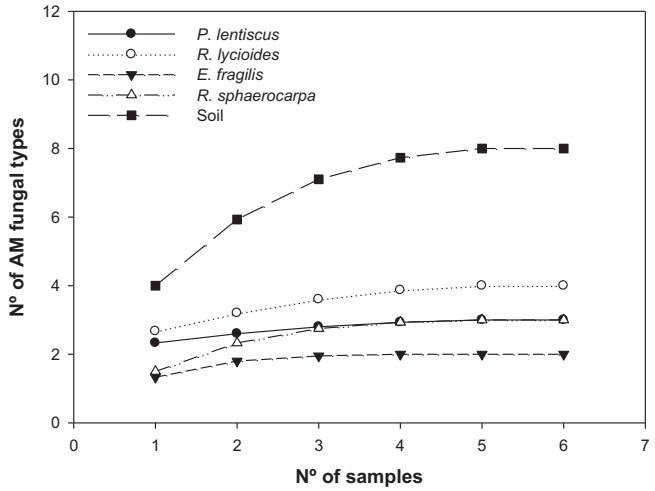


Fig. 2. Sampling effort curves for the AM fungal community in the roots of *R. sphaerocarpa*, *R. lycioides*, *P. lentiscus* and *E. fragilis* and soil. The sample order was randomized by 100 replications in EstimateS, version 8.0 (Colwell, 2005).

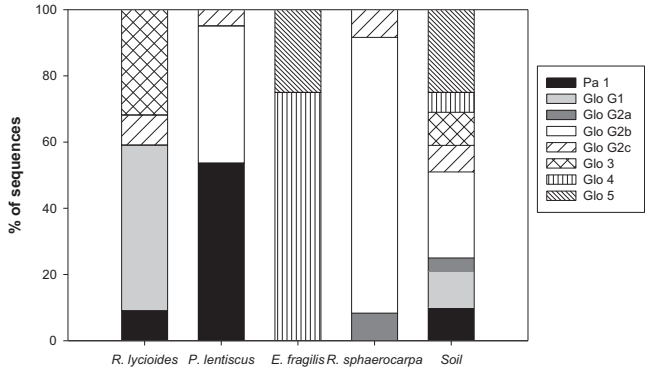


Fig. 3. Bar plot showing the relative abundance of the different AM fungal types observed in *R. sphaerocarpa*, *R. lycioides*, *P. lentiscus* and *E. fragilis* roots and soil.

Glo G1 and Glo G3 represented 15.6% of the AMF sequences and seemed to be specific for *R. lycioides* and Glo G4 and Glo G5 were the only sequence types found exclusively in *E. fragilis* roots. The Glo G2a sequence type appeared in low proportion of sequences (2.6%), exclusively in *R. sphaerocarpa*.

All eight fungal sequence types were also identified from soil samples (Figs. 1 and 3), Glo G5 and Glo G2b being present in the highest proportion. The rest of the sequences had approximately the same quantitative distribution.

The distribution in the CA biplot diagram (Fig. 4) also demonstrates that the different shrub species harboured distinct AMF sequence types, since they appeared distant to each other. The diagram also shows which sequence types were hosted exclusively by each shrub species; thus, Glo G4 and Glo G5 were specific for *E. fragilis*, as were Glo G1 and Glo G3 for *R. lycioides*. The AMF communities in the roots of *P. lentiscus* and *R. sphaerocarpa* did not differ significantly from each other, these plant species appearing nearby in the diagram. A similar trend for *P. lentiscus*, *R. sphaerocarpa* and *R. lycioides* was observed, towards the right of the diagram, due to the sharing of some AMF sequence types among these shrubs.

4. Discussion

We used *Glomeromycota*-specific PCR AML1-AML2 primers, developed recently by Lee et al. (2008). Until now, diversity studies of AMF communities using these primers in environmental samples, which could be compared with our results, have not been conducted. We found that 34.1% of the sequences analysed were non-AMF in origin; specifically, all belonged to host plant sequences studied. Therefore, our results clearly demonstrate a potential problem with this primer pair for the identification of AMF in roots and soil. However, no amplification of DNA from *Ascomycetes* and/or *Basidiomycetes* fungi was detected, in contrast with the widely-used AM1-NS31 primers (Helgason et al., 2002; Douhan et al., 2005; Santos-González et al., 2007; Alguacil et al., 2008, 2009a,b). Thus, caution must be used when this set of primers is employed to identity AM fungal communities, if DNA sequencing is not applied in all samples and clones. On the other hand, this approach offers better coverage across the order *Glomeromycota*, since these primers amplify sequences from Paraglomeraceae and Archaeosporaceae divergent families – as was observed in our study with the sequences belonging to *Paraglomus* genera. Although there was limited success in obtaining all amplified PCR products and low specificity of the primers used, the sampling effort curves show that the number of sequences analysed per sample was sufficient to cover the overall AM fungal richness in our study.

The diversity of the AMF colonising roots of *R. sphaerocarpa*, *R. lycioides*, *P. lentiscus* and *E. fragilis*, one year after revegetation in a semiarid Mediterranean ecosystem, was found to be rather low ($H' = 0.78$), compared to other ecosystems, such as dry forest ($H' = 2.58$ from one host species; Wubet et al., 2003), tropical forest ($H' = 2.33$ from two host species; Husband et al., 2002), wetland grass ($H' = 2.4$ from one host species; Wirsel, 2004). In general, our findings are in accordance with the fungal richness reported in other semiarid regions (Ferrol et al., 2004; Liu et al., 2009). In the study carried out by Ferrol et al. (2004) in the province of Alicante (eastern Spain), in an ecosystem similar to that of our work, only five AM fungal species were detected, confirming the high degree of degradation of these arid and semiarid ecosystems. Moreover, they found predominance of the *Glomus* and *Paraglomus* genera in accordance with our results. Interestingly, the only AM fungal species recognized morphologically in their study in the rhizosphere soil of *P. lentiscus* was *Paraglomus ocultum*. In our study, we found one of the most-abundant AM fungal sequence types, namely Pa 1 that showed high similarity (95%) with *P. ocultum*, almost exclusively in *P. lentiscus*. This could suggest that *P. ocultum* and *P. lentiscus* preferentially associate in the target ecosystem. It has been reported that small-spore fungi such as *P. ocultum* are better adapted for germination and colonisation of plant roots under environmental stress conditions (Jacobson, 1997), such as those in semiarid ecosystems.

The highest richness of AMF per sample was detected in *R. lycioides* roots. Of four fungal sequence types detected in this species, one of them (Glo G1) belonged to the previously-described

Fig. 1. Neighbour-Joining (NJ) phylogenetic tree showing AM fungal sequences isolated from roots of *R. sphaerocarpa*, *R. lycioides*, *P. lentiscus*, *E. fragilis* and soil and reference sequences from GeneBank. All bootstrap values >75% are shown (100 replicates). Sequences obtained in the present study are shown in bold type. They are labelled with the host plant or soil from which they were obtained (*P. lentiscus* = PL., *R. lycioides* = RL., *R. sphaerocarpa* = RS., and *E. fragilis* = EF., Soil = S.) and the clone identity number. Identical sequences are grouped, followed by the number of clones between brackets having that particular sequence. See the Supplemental material for a detailed description of the clone identifiers included in each group. Group identifiers (for example Glo G1) are AM fungal sequences types found in our study. *Endogone pisiformis* and *Mortierella polycephala* were used as out-groups.

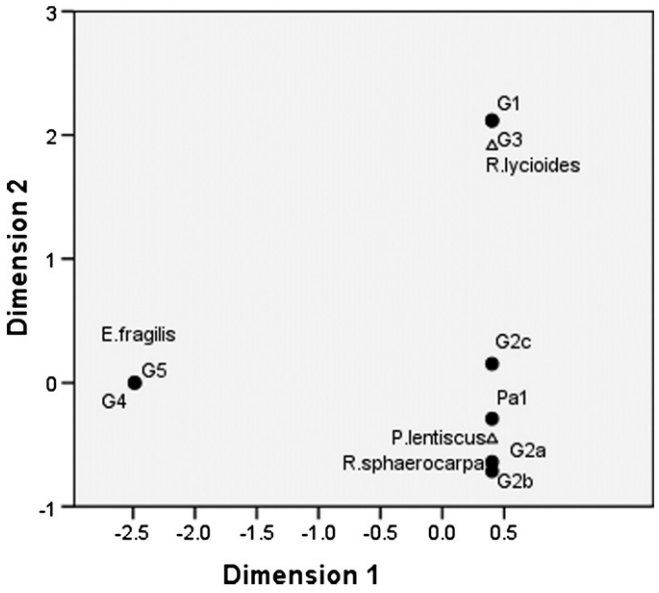


Fig. 4. Correspondence analysis of the AM fungal community composition found in the roots of *R. sphaerocarpa*, *R. lycioides*, *P. lentiscus* and *E. fragilis*. The eigenvalues of the 1st and 2nd axes in the two-dimensional ordination diagrams are as follows: dimension 1 = 0.46 and dimension 2 = 0.38. Host plant species are represented by open triangles and AM fungal types by filled circles.

G. lamellosum group, and was the most-abundant taxon found exclusively in this shrub. The second-most-common sequence type detected was Glo G3, which was found for the first time and was also *R. lycioides*-specific.

The genetic diversities of the AMF associated with *P. lentiscus* and *R. sphaerocarpa* did not differ significantly. In both shrub species, three fungal sequence types were identified, two of which (Glo 2c and Glo 2b) were shared. *E. fragilis* harboured the lowest AM fungal diversity in this study. Glo G4 was the most-frequent fungal sequence type that showed strong evidence of host preference for *E. fragilis*. Only in this shrub did we find sequences grouped in the Glo G5 fungal sequence type, related to a known AMF species, *Glomus intraradices*; recently grouped in the same cluster as *Glomus* sp. DAOM197198 and BEG195 (Stockinger et al., 2009). *G. intraradices* is considered the most-common taxon detected in many host species (Helgason et al., 2007) and ecosystems (Öpik et al., 2006). Scheublin et al. (2004) showed that *G. intraradices* was more frequent in legumes than non-legumes and concluded that this fungal type is a specialist coloniser of plants with high nitrogen (N) concentrations; in contrast we found that *G. intraradices* was present exclusively in *E. fragilis* roots and not in roots of the leguminous *R. sphaerocarpa*.

The importance of plant establishment in the early stages after planting is widely acknowledged and regarded as crucial to the success of restoration. In the early stages, there are greater challenges for the adaptation of plants and mortality rates are increased, especially in semiarid ecosystems. At this time when AMF root colonisation plays an important role, improving plant adaptation and growth. Although AMF diversity increases over the years after planting (Liu et al., 2009), it is essential that different species interact in roots from the first stages.

Our results demonstrate that *R. sphaerocarpa*, *R. lycioides*, *P. lentiscus* and *E. fragilis* had different AM fungal community composition; the randomized block design of this study makes the result particularly compelling as it eliminates the possibility that host and fungus are covarying because both are responding to the same environmental variation. The host preference in arbuscular mycorrhizas has been shown previously in plants belonging to different families (Vandenkoornhuyse et al., 2002; Scheublin et al., 2004; Alguacil et al., 2009b) as well as in plants which are closely-related or belong to the same family (Vandenkoornhuyse et al., 2003; Öpik et al., 2003; Gollotte et al., 2004; Sýkorová et al., 2007). The plants used in our study belong to different families but they are all native, drought-tolerant shrub species, with deep roots enabling them to cope with nutrient stress in the eroded soil (Francis and Thornes, 1990). They belong to the natural succession of the shrubland community of semiarid Mediterranean ecosystems in the southeast of Spain.

Other studies carried out in similar ecosystems with shrub species in other desertified areas of South-eastern Spain (Azcón-Aguilar et al., 2003; Caravaca et al., 2005), showed that different shrubs species growing in natural conditions differed in their ability to enrich the soil with mycorrhizal propagules and that the effectiveness of native AM fungi depended on the host shrub species. Although their results are not comparable with ours, because of their differing methodological approaches, it is clear that plant–fungi symbioses have recognition mechanisms that confer specificity. Plants form mycorrhizal associations with the AM fungi most beneficial for survival and thus plant performance (Requena et al., 2001) and productivity (Caravaca et al., 2005).

Our results showed that all AM fungal sequence types colonising the roots of the different shrubs were detected in the soil, in agreement with those of Hempel et al. (2007) and Cesaro et al. (2008), suggesting that plant specificity is not caused by a lack of other co-occurring AM fungi.

5. Conclusions

This study shows that when using different native shrub species for revegetation purposes, the resulting AMF diversity is strongly dependent on the host plant. Therefore, one effective tool to restore degraded lands is an increase in the number of plant species used, which would increase the AMF diversity in the soil and thus the below-ground, positive interactions.

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Appendix. Supplementary material

Supplementary material associated with this paper can be found, in the online version, at doi:10.1016/j.soilbio.2010.09.029.

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