Plant Soil (2012) 354:97–106
DOI 10.1007/s11104-011-1047-9

REGULAR ARTICLE

Differences in the AMF diversity in soil and roots between two annual and perennial gramineous plants co-occurring in a Mediterranean, semiarid degraded area

Emma Torrecillas · Maria del Mar Alguacil · Antonio Roldán

Received: 1 September 2011 / Accepted: 19 October 2011 / Published online: 18 November 2011

Abstract
Aims In the present study, we analysed the diversity of indigenous arbuscular mycorrhizal fungi (AMF) colonising both the roots and rhizosphere soil of an annual herbaceous species, *Bromus rubens*, and a perennial herbaceous species, *Brachypodium retusum*, co-occurring in the same Mediterranean, semiarid degraded area. The intention was to study whether these two species promoted the diversity of AM fungi in their rhizospheres differently and to ascertain whether the AMF community harboured by an annual plant species differed from that harboured by a perennial species when both grew in the same place.
Methods The AMF large subunit ribosomal RNA genes (LSU) were subjected to nested PCR, cloning, sequencing and phylogenetic analysis.
Results Twenty AMF sequence types belonging to *Glomus* group A, *Glomus* group B and *Diversispora* were identified. The two plant species differed in the AMF community composition in their roots, *B. rubens* showing a higher diversity of AMF than *B. retusum*. However the composition of the AMF communities associated with the two rhizosphere soils was similar.
Conclusions These results suggest that the management of these Mediterranean, semiarid degraded areas should include the promotion of annual herbaceous plant communities in order to maintain the sustainability and productivity of these ecosystems.

Keywords Arbuscular mycorrhizal fungi · Diversity · LSU rDNA · *Bromus rubens* · *Brachypodium retusum* · Semiarid degraded areas

Electronic supplementary material The online version of this article (doi:10.1007/s11104-011-1047-9) contains supplementary material, which is available to authorized users.

E. Torrecillas · M. del Mar Alguacil (✉) · A. Roldán
Department of Soil and Water Conservation,
CSIC-Centro de Edafología y Biología Aplicada del Segura,
P.O. Box 164, Campus de Espinardo,
30100 Murcia, Spain
e-mail: mmalguacil@cebas.csic.es

Introduction

Arbuscular mycorrhizal fungi (AMF) are one of the most common types of symbiosis globally, and the majority of known terrestrial plant species form relationships with them. The AMF are beneficial microorganisms fundamental for the soil fertility of natural and agricultural ecosystems (Smith and Read 2008), since they increase plant growth and nutrient uptake, improve soil structure (Caravaca et al. 2002; Wilson et al. 2009) and improve plant tolerance to root pathogens and drought (Martínez-Medina et al. 2009; Querejeta et al. 2007). All these beneficial effects imply that the plant community structure and productivity in ecosystems are influenced significantly by the AM fungal diversity in the soil (van der Heijden et al. 1998). The impact of AMF on plant community composition and functioning has been the subject of many studies

over recent years and this research has led to the conclusion that the activity of AMF is a key mechanism linking biodiversity and ecosystem functioning (Read 1998). Because of the key role of AMF in plant health and soil performance, they are crucial for the reclamation and sustainability of degraded ecosystems, such as those in semiarid Mediterranean regions (Alguacil et al. 2005).

In the Mediterranean areas of southeast Spain, the climatic conditions, characterised by long, dry and hot summers with scarce and irregular rainfall, make these ecosystems fragile and susceptible to degradation. Under these conditions, besides a loss of vegetation cover and degeneration of physical, chemical and biological soil properties, a reduction in the amount, diversity and activity of AMF is produced (Requena et al. 2001; Azcón-Aguilar et al. 2003). For the adequate management of arid lands, it is of interest to know the diversity of the AMF colonising the natural plant community that develops in these areas, as well as the distribution of the AM fungal communities in relation to the host-plants.

Several studies have shown the effectiveness of native AMF with regard to improving the establishment and growth of autochthonous shrub species used in revegetation programmes for Mediterranean, semiarid degraded soils (Caravaca et al. 2003, 2005; Ferrol et al. 2004). Also, it has been shown that an increase in the number of shrub species used to restore degraded lands increases the AMF diversity in the soil and thus the below-ground, positive interactions (Alguacil et al. 2011). However, little is known of the relationship between AMF and herbaceous plant species growing in semiarid degraded areas. Therefore, determination of the natural diversity of AMF present in the soil and colonising the roots of these plant species is essential for the improved management, sustainability and productivity of these ecosystems.

The aim of the present study was to assess the diversity of the indigenous AM fungal communities in both the rhizosphere and roots of an annual herbaceous species, *Bromus rubens*, and to compare it with that of a perennial herbaceous species, *Brachypodium retusum*, co-occurring in the same Mediterranean, semiarid area.

The objective was to see if these two plant species promoted differently the diversity of AMF in the rhizosphere and to ascertain whether the AMF communities harboured by an annual plant species differed from those harboured by a perennial species when both grew in a degraded, semiarid area.

Materials and methods

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 1,000 mm. The mean annual temperature is 19.2°C with the absence of frost. 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 *Brachypodium retusum*(Pers.) P. Beauv., and some shrubs of *Thymus vulgaris L.* and *Rosmarinus officinalis* L. growing with patchy distribution.

Experimental design

The experiment was conducted using a factorial design with four replication blocks (4×4 m) separated 20 m each other. Two plant species natural growing in this area were selected: a perennial herbaceous species, *B. retusum* (Pers.) P. Beauv. and an annual herbaceous species, *Bromus rubens* L. *B. rubens* germinates in autumn-winter with a flowering period between March and June. The flowering period of *B. retusum* is between April and June. These are similar shaped plant species, both are well adapted to water stress conditions and commonly distributed in Mediterranean semiarid disturbed lands. In the case of *B. rubens*, its occurrence is linked to the rainfall period during winter-spring, when it can be very abundant depending on the amount of accumulated rainfall, but always exceeding 10% of soil vegetation cover. The plant cover for the permanent population of *B. retusum* reaches 20%.

Sampling

All samples were collected in the second half of April 2010. Four completely developed and flowered plants (separated at least 50 cm each other) belonging to each of the two selected species were sampled per replication block (a total of 32 plants). Plants, including root systems and rhizospheric soil at 5–20 cm depth, 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. The soil samples were sieved through 2-mm pores to eliminate large particles and stored in plastic bags at −20°C until processed.

Root DNA extraction and PCR

All PCR experiments were run using DNA preparations consisting of pooled roots of individual plants or soil extracts for each replication block. DNA extractions from 8 root samples (one root sample from each replication block for each plant species) and 8 soil samples were carried out.

For each sample, total DNA was extracted from (0.1 g) root material using a DNeasy plant mini Kit following the manufacturer's recommendations (Qiagen, Hilden, Germany). The roots samples were placed into a 2-ml screw-cap propylene tube together with two tungsten carbide balls (3 mm) and ground (3 min, 13,000 r.p.m.) using a mixer mill (MM 400, Retsch, Haan, Germany). The extracted DNA was resuspended in 20 μl of water.

For each of the eight 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. Two-microlitre samples of genomic DNA were used for the amplification of a partial large-subunit (LSU) rRNA gene region. In order to enhance the efficiency of the amplification and increase the amount of DNA available for cloning, a heminested PCR was carried out using the primer pairs LR1 (van Tuinen et al. 1998) and FLR2 (Trouvelot et al. 1999) for the first amplification step and LR1 and FLR4 (Gollotte et al. 2004) for the second one. PCR reactions were carried out in a final volume of 25 μl using the "ready to go" PCR beads (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom), and 0.5 μM of each primer (PCR conditions: 30 cycles at 93°C for 1 min, 58°C for 1 min, and 72°C for 1 min, followed by a final extension period at 72°C for 5 min).

Two microlitres, from the first PCR, were used as template DNA in a second PCR reaction under the same PCR conditions. 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 GelRed™ (Biotium, Hayward, California).

Cloning and sequencing

The PCR products were purified using a Gel extraction Kit (Qiagen) cloned into pGEM-T Easy (Promega, Madison, Wisconsin, USA) and transformed into *Escherichia coli* (XL2-Blue). Thirty two positive transformants were screened in each resulting LSU rRNA gene library, using 0.8 units of RedTaq DNA polymerase (Sigma-Aldrich, St. Louis, Missouri, USA) and a re-amplification with LR1 and FLR4 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). 83 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 FR871313-FR871395.

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

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) and maximum parsimony (MP) phylogenetic analyses (Saitou and Nei 1987) were performed with the program PAUP4.08b (Swofford 2002) and using the default parameters. *Mortierella polycephala* Coem, was used as the out-group.

Statistical analysis

Multivariate analyses were used in order to investigate the influence of environmental factors (two plant species and their rhizospheres) on the AMF community distribution. Ordination analyses were conducted in CANOCO for Windows, version 4.5 (ter Braak and Smilauer 2004), using the presence/absence data for each root/soil sample. Initial detrended correspondence analysis suggested a unimodal character of the data response to the sample origin (the lengths of gradients were ≥4); therefore, canonical-correspondence analysis (CCA) was used. The statistical significance of the environmental variables was evaluated by manual forward selection using a Monte Carlo permutation test with 499 permutations.

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 $H' = -\sum p_i(\ln p_i)$, where p_i is the proportion of individuals found in the *i*th species (in a sample, the true value of p_i is unknown but is estimated as n_i/N, [here and throughout, n_i is the number of individuals in the *i*th species]).

One-way ANOVAs were used to compare the AMF diversity indices among roots and soil from different plant species. Statistical procedures were carried out with the software package SPSS 19.0 for Windows.

Results

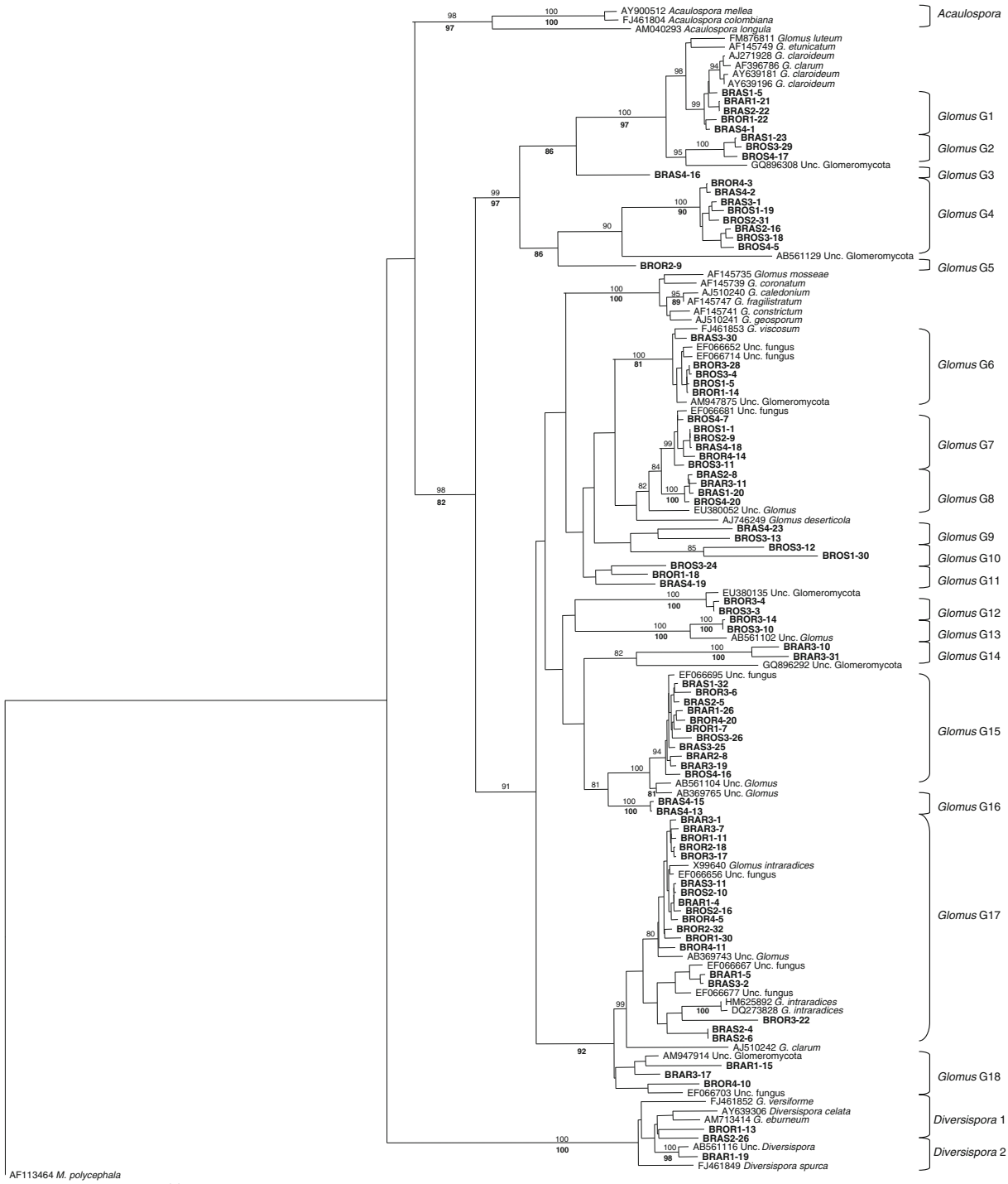
PCR and sequence analysis

All the root and soil samples extracted were amplified successfully by nested PCR and generated PCR products of the expected band, of approximately 720 bp, which were used for cloning and creating a clone library. From the 16 clone libraries, a total of 512 clones were screened by PCR (on average, 32 clones were analysed per library); out of these, a total of 488 clones contained an LSU rDNA fragment and subsequently all clones were sequenced. The BLAST search revealed that 355 sequences (72.7%) had a high degree of similarity to sequences from taxa belonging to the phylum *Glomeromycota*. The rest of the sequences (133) were erroneous sequences.

Phylogenetic analysis of AMF groups

An alignment between 355 glomalean sequences obtained in this study from root and soil samples and 46 sequences downloaded from GenBank was conducted: 83 clones that produced the same sequence were represented just once in the Neighbour-joining (NJ) tree and the 272 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 clades ranged from 98 to 100%. Both the sequence similarities and Neighbour-joining analyses made possible the recognition of 20 sequence groups or phylotypes, on the basis of bootstrap values ≥80%. Of all the AMF sequence types, 13 belonged to *Glomus* group A, five to *Glomus* group B and two to *Diversispora* (Fig. 1). Four AMF sequence types, *G. claroideum* (group *Glomus* G1), *G. viscosum* (group *Glomus* G6), *G. intraradices* (group *Glomus* G17) and *G. eburneum* (group *Diversispora* 1) sequences, clustered with previously identified AMF sequences.

There were other groups that did not seem to be related to any sequences of AMF in the database,



such as *Glomus* G4, *Glomus* G9, *Glomus* G10, *Glomus* G11, *Glomus* G14 and *Glomus* G16.

Although a recent systematic reorganization of the Glomeromycota phylum published by Schüβler and Walker (2010) has been done we have adopted the traditional terminology and only the most representative known sequences of AMF species has been represented in the phylogenetic tree (Fig. 1) for clarity.

◀ **Fig. 1** Neighbour-Joining (NJ) phylogenetic tree showing AM fungal sequences isolated from roots and rhizosphere soil of *Brachypodium retusum* and *Bromus rubens*, and reference sequences from GeneBank. Numbers above the branches indicate the bootstrap values (above 80%, 1000 replicates) of the NJ analysis; numbers below the branches indicate the bootstrap values of the maximum parsimony analysis. Sequences obtained in the present study are shown in bold type. They are labelled with the host plant or rhizosphere soil from which they were obtained (BROR=Roots of *B. rubens*; BROS=Rhizosphere soil of *B. rubens;* BRAR=Roots of *B. retusum*; BRAS=Rhizosphere soil of *B. retusum*) and the clone identity number. (See the supplemental material for a detailed description of the clones identified in this study and included in each group). Group identifiers (for example Glo G1) are AM fungal sequences types found in our study. *Mortierella polycephala* were used as out-group

To determine if the number of clones sequenced was sufficient to represent the AMF diversity in the soil and in the roots, sampling effort curves were constructed (Fig. 2). The results indicate that the number of sequences analysed per sample was sufficient to provide coverage of the AM fungal diversity in the soil and roots from the two plant species studied. In fact, all the curves reached the plateau.

Distribution of AMF sequence types

The Shannon diversity index was calculated in order to compare the degree of richness of AMF in soil and in plant roots. The numbers of AMF sequence types found in soils (13 phylotypes in *B. retusum* soil and 13 in *B. rubens* soil.) were higher than in plant roots (7 phylotypes in *B. retusum* roots and 12 in *B. rubens* roots). Also, the Shannon diversity index for soils corresponded to a higher level of AMF diversity than for plant roots (H_{BROS}=2.21, H_{BRAS}=2.14, H_{BROR}= 1.89 and H_{BRAR}=1.21) (Table 1).

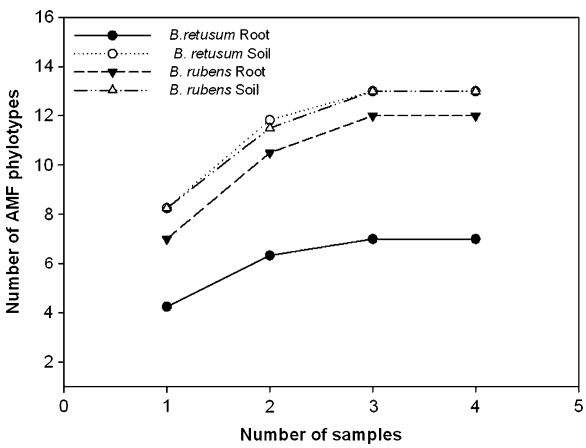


Fig. 2 Sampling effort curves for the AM fungal community in the roots and rhizosphere soil of *B. rubens* and *B. retusum*. The sample order was randomised by 100 replications in EstimateS, version 8.0 (Colwell 2005)

The most abundant phylotypes in this study were *Glomus* G17 (with 32.11% of the clones belonging to this group) and *Glomus* G15 (17.46%), which occurred in the two species of plants in both the roots and the soil, followed by *Glomus* G4 (8.45%), *Glomus* G1 (7.88%), *Glomus* G6 (7%) and *Glomus* G7 (5.9%) (Fig. 3). The abundance of *Glomus* G17 in roots of *B. rubens* was almost twice that in soil samples. The same occurred in the second biggest group, *Glomus* G15, which was five times more abundant in roots of *B. retusum* than in the soil. The groups *Glomus* G2, *Glomus* G3, *Glomus* G9, *Glomus* G10 and *Glomus* G16 were found only in soil samples, while *Glomus* G5, *Glomus* G14, *Glomus* G18 and *Diversispora* 2 occurred exclusively in roots. With regard to differences between the two plant species, four groups (*Diversispora* 2, *Glomus* G3, *Glomus* G14 and *Glomus* G16) were found exclusively in *B. retusum*, while four other groups (*Glomus* G5, *Glomus* G10, *Glomus* G12 and *Glomus* G13) appeared only in *B. rubens*. The CCA biplot diagram (Fig. 4) shows clearly these differences in the composition of the AMF community between the plant species, as the differing distributions of the AMF sequence types. Thus, the symbols representing different plant species are distant to each other, which demonstrate that they hosted distinct AMF. In fact, the influence of the host plant was statistically significant according to the Monte Carlo permutation

Table 1 Shannon diversity indices for AMF communities from roots and rhizosphere soil of *B. rubens* and *B. retusum* (n=4)

	Means ± SE	Total[b]
B. retusum roots	0.79±0.05a	1.21
B. retusum soil	1.76±0.03bc	2.14
B. rubens root	1.36±0.01b	1.89
B. rubens soil	2.09±0.17c	2.21

[a] Values in columns followed by the same letter do not differ significantly (P<0.05) as determined by the Duncan's test

[b] The total Shannon diversity index was calculated by pooling data from four replicate samples

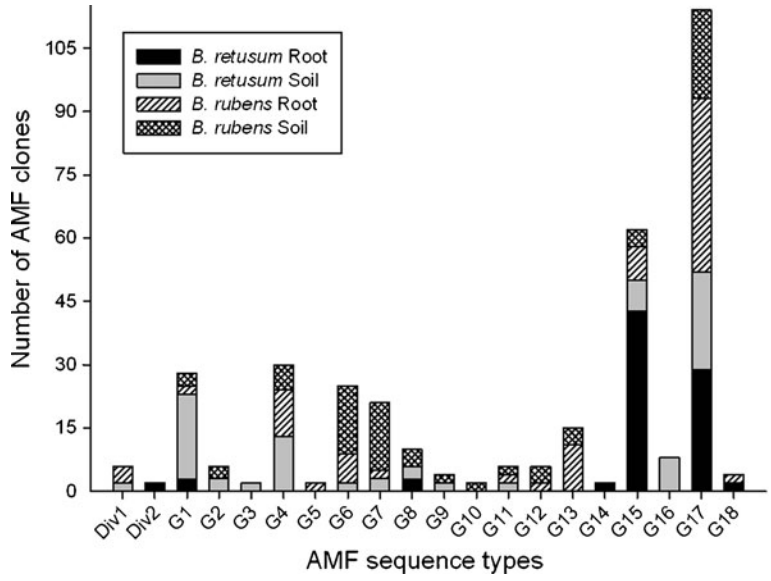


Fig. 3 Bar plot showing the number of clones detected for each AM fungal sequence type in the roots and rhizosphere soil of *B. rubens* and *B. retusum*

test (F=2.21; P=0.022). The diagram also shows the AMF sequence types found exclusively in each plant or soil sample. The variable rizosphere was found to be non-significant (F=1.06; P=0.368).

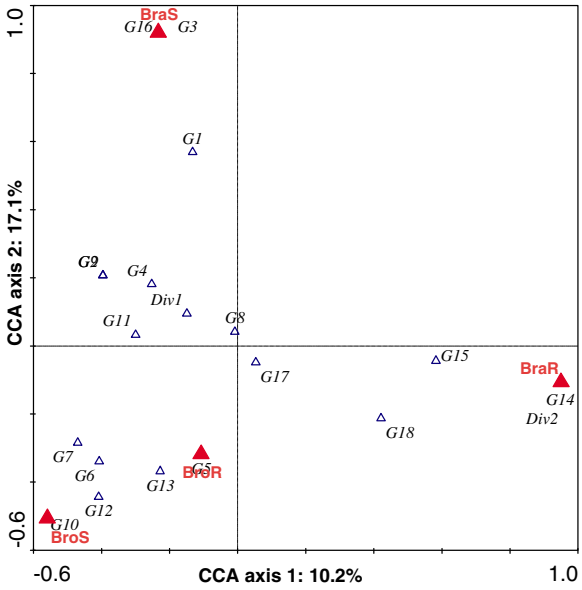


Fig. 4 Canonical Correspondence analysis (CCA) of the AMF communities found in the roots and rhizosphere soil of *B. rubens* and *B. retusum*. (BroR=Roots of *B. rubens*; BroS=Rhizosphere soil of *B. rubens;* BraR=Roots of *B. retusum*; BraS=Rhizosphere soil of *B. retusum*). Open triangles represent the different AMF sequence types and the filled triangles the respective samples. The first axis accounted for 47.8% of the variability explained by all canonical axes. The percentages shown on the first and second axes correspond to the percentages of variance of AMF sequence type data explained by the particular axis

Discussion

This study compared the composition of the community of arbuscular mycorrhizal fungi in roots and soil from an annual herbaceous species with that from a perennial herbaceous species co-occurring in a Mediterranean degraded, semiarid area.

As expected, our results clearly show that the two plant species within the same habitat were colonised by different AMF communities. For each plant species, there was a specific community, as shown clearly by the CCA analysis (Fig. 4). This behaviour pattern is consistent with the results of previous studies in different habitats (Vandenkoornhuyse et al. 2002, 2003; Opik et al. 2003; Gollotte et al. 2004; Scheublin et al. 2004; Sýkorová et al. 2007; Alguacil et al. 2009, 2011; Li et al. 2010). Moreover, differing abundances of the shared AM fungal types in the roots of the two plant species were detected, indicating that some AMF types showed a preference for the host-plant species (Vandenkoornhuyse et al. 2002; Gustafson and Casper 2006; Li et al. 2010). These differences in the AMF community composition between the two plant species could be due in part to the differences in plant life cycle, phenology and physiology shown by *B. rubens* and *B. retusum*. The

two species normally differ in the timing and extent of their activity during the year. *B. rubens* germinates in winter and is highly dependent on the periods of rainfall. In a study carried out in California, based on uptake of ^{15}N-labelled ammonium, annual plant roots appeared to be active earlier than standing perennial roots (Hooper and Vitousek 1998). Thus, if roots of *B. rubens* are active earlier in the season than those of *B. retusum*, they may associate with and promote specific groups of AMF. On the other hand, besides differences in life cycle between these two plant species, factors such as plant developmental stage might affect the development of AMF communities in roots. In previous studies (Caravaca et al. 2003, 2005; Alguacil et al. 2011), it was found that AMF-inoculated shrub species showed their most significant increases in growth during the first stages of plant development. In our study, *B. rubens* roots showed higher AMF diversity than *B. retusum* roots. Thus, more AMF species might be helping to improve the growth and development of this annual herbaceous plant. It is also of note that *B. retusum* roots showed the lowest number of AMF sequence types, actually one AMF type (Glo G15) represented 50% of all the AMF sequences obtained in this perennial herbaceous species. Glo G15 appears to be very invasive in *B. retusum* roots and this AMF sequence type might prevent colonisation by other AM fungal species. Moreover, once a hyphal network is established, these fungi could have priority access to perennial plant roots and could thus dominate them (Vierheilig et al. 2000; Vierheilig 2004).

The diversity of AMF associated with *B. rubens* and *B. retusum* rhizosphere soil was similar: 13 AMF sequence types were found in both, of which 10 were common and only three AMF sequence types, represented by a low number of clones, were specific for each rhizosphere soil. The Shannon diversity index also revealed higher AMF diversity in the rhizosphere soil from both plant species than in their roots. In the case of the perennial species, *B. retusum,* its roots showed the lowest AMF diversity index (H'= 1.21) and harboured four AMF sequence types out of 13 AMF sequence types found in the rhizosphere soil. This low overlap in the composition of the AM fungal community between the root and soil fractions of the perennial plant species is in accordance with the findings of different studies (Clapp et al. 1995; Renker et al. 2005; Hempel et al. 2007; Cesaro et al. 2008). Here, we show the efficiency of an annual species (*B. rubens*) with respect to harbouring most of the AMF diversity present in the soil.

Only three AMF sequence types (Glo G1, Glo G15 and Glo G17) out of 20 detected in the whole study were found in both the community of the functionally active AMF within the roots and in the rhizosphere soil of *B. rubens* and *B. retusum*. Of these, Glo G17, that showed very high similarity (99% identity) with the described species *G. intraradices*, was the dominant taxa in our study (32% of sequences). Thus, the cosmopolitan nature of this AM fungal species is confirmed once again (Husband et al. 2002; Öpik et al. 2003; Wirsel 2004; Hijri et al. 2006; Sýkorová et al. 2007; Lekberg et al. 2007; Cesaro et al. 2008).

The AMF sequence type represented by the second highest number of clones was Glo G15, which was related previously with "unknown" sequences detected in roots from *Medicago laciniata* (Pivato et al. 2007). Finally, Glo G1, represented by only 7.9% of the sequences, showed 98% similarity with sequences of *Glomus claroideum* strain BEG14 (Turnau et al. 2001).

Three AMF sequences types (Div2, Glo G5 and Glo G14) were found in roots but not in rizosphere soil. Hempel et al. (2007) also observed this fact in a molecular study of AMF communities in a grassland ecosystem. They suggested that some AMF groups extend short distances in soils relative to its abundance in roots. Also, Hart and Reader (2002) documented major differences in the internal and external mycelium among different AMF species.

In the present study, we obtained 20 AMF sequence types, more than were detected by Gollotte et al. (2004), Cesaro et al. (2008) or Pivato et al. (2007), who identified 10, eight and 12 AMF sequence types, respectively, using our set of primers and molecular methods. Also, in a recent revegetation study of AMF diversity based on SSU rDNA with four shrub species, carried out in the same study area, we detected only eight AMF sequence types 14 months after plant establishment (Alguacil et al. 2011). Thus, the data obtained in the present research suggest that the AMF biodiversity in these Mediterranean, semiarid areas may be considerably higher than previously reported in spite of the primers used in our experiment do not give a complete AMF taxonomic coverage, since FRL4 primer discriminates

above all Diversisporales, Archaeosporales and Paraglomerales orders (Gamper et al. 2009).

In conclusion, these data provide evidence in favour of the preferential selection of specific AMF sequence types by a given plant species. The annual herbaceous species, *B. rubens*, showed higher AMF diversity than the perennial herbaceous species, *B. retusum*. Whether this fact can be attributable to the differences in plant phenology is not clear and more studies focusing in more diverse habitats with higher number of both perennial and annual gramineous species could be necessary. Management of these Mediterranean, semiarid degraded areas should also promote annual herbaceous plant communities in order to maintain the sustainability and productivity of these ecosystems.

Acknowledgments MM Alguacil was supported by the Ramón y Cajal programme (Ministerio de Educación y Ciencia, Spain). This research was supported by the Spanish CICYT Project AGL2009.12530-CO2-O1. We are indebted to Ayuntamiento de Molina de Segura (Murcia, Spain) for experimental area facilities.

References

Alguacil MM, Caravaca F, Roldan A (2005) Changes in rhizosphere microbial activity mediated by native or allochthonous AM fungi in the reafforestation of a Mediterranean degraded environment. Biol Fertil Soils 41:59–68

Alguacil MM, Roldán A, Torres MP (2009) Assessing the diversity of AM fungi in arid gypsophilous plant communities. Environ Microbiol 11:2649–2659

Alguacil MM, Torres MP, Torrecillas E, Díaz G, Roldán A (2011) Plant type differently promote the arbuscular mycorrhizal fungi biodiversity in the rhizosphere alter revegetation of a degraded, semiarid land. Soil Biol Biochem 43:167–173

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Azcón-Aguilar C, Palenzuela J, Roldán A, Bautista S, Vallejo R, Barea JM (2003) Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertificationthreatened Mediterranean shrublands. Appl Soil Ecol 22:29–37

Caravaca F, Barea JM, Figueroa D, Roldán A (2002) Assessing the effectiveness of mycorrhizal inoculation and soil compost addition for reafforestation with *Olea europaea* subsp. *sylvestris* through changes in soil biological and physical parameters. Appl Soil Ecol 20:107–118

Caravaca F, Alguacil MM, Figueroa D, Barea JM, Roldán A (2003) Re-establishment of *Retama sphaerocarpa* as a target species for reclamation of soil physical and biological properties in a semi-arid Mediterranean area. Forest Ecol Manag 182:49–58

Caravaca F, Alguacil MM, Barea JM, Roldán A (2005) Survival of inocula and native AM fungi species associated with shrubs in a degraded Mediterranean ecosystem. Soil Biol Biochem 37:227–233

Cesaro P, van Tuinen D, Copetta A, Chatagnier O, Berta G, Gianinazzi S, Lingua G (2008) Preferential colonization of *Solanum tuberosum* L. roots by the fungus *Glomus intraradices* in arable soil of a potato farming area. Appl Environ Microbiol 74:5776–5783

Clapp JP, Young JPW, Merryweather JW, Fitter AH (1995) Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. New Phytol 130:259–265

Colwell R (2005) EstimateS: statistical estimation of species richness and shared species from samples, version 8.0. User's guide. University of Connecticut, Storrs. http://viceroy.eeb.uconn.edu/estimates

Ferrol N, Calvente R, Cano C, Barea JM, Azcón-Aguilar C (2004) Analysing arbuscular mycorrhizal fungal diversity in shrub-associated resource islands from a desertification-threatened semiarid Mediterranean ecosystem. Appl Soil Ecol 25:123–133

Gamper HA, Walker C, Schüβler A (2009) *Diversispora celata* sp. nov: molecular ecology and phylotaxonomy of an inconspicuous arbuscular mycorrhizal fungus. New Phytol 182:495–506

Gollotte A, van Tuinen D, Atkinson D (2004) Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. Mycorrhiza 14:111–117

Gustafson DJ, Casper BB (2006) Differential host plant performance as a function of soil arbuscular mycorrhizal fungal communities: experimentally manipulating cooccurring *Glomus* species. Plant Ecol 183:257–263

Hart MM, Reader JR (2002) Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytol 153:335–344

Hempel S, Renker C, Buscot F (2007) Differences in the species composition of arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland ecosystem. Environ Microbiol 9:1930–1938

Hijri I, Sýkorová Z, Öehl F, Ineichen K, Mader P, Wiemken A, Redecker D (2006) Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. Mol Ecol 15:2277–2289

Hooper DU, Vitousek PM (1998) Effects of plant composition and diversity on nutrient cycling. Ecol Monogr 68:121–149

Husband R, Herre EA, Turner SL, Gallery R, Young JPW (2002) Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. Mol Ecol 11:2669–2678

Lekberg Y, Koide RT, Rohr JR, Aldrich-Wolfe L, Morton JB (2007) Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. J Ecol 95:95–105

Li LF, Li T, Zhang Y, Zhao ZW (2010) Molecular diversity of arbuscular mycorrhizal fungi and their distribution patterns

related to host-plants and habitats in a hot and arid ecosystem, southwest China. FEMS Microbiol Ecol 71:418–427

Maidak BL, Cole JR, Lilburn TG, Parker CT, Saxman PR, Farris RJ, Garrity GM, Olsen GJ, Schmidt TM, Tiedje JM (2001) The RDP-II (Ribosomal Database Project). Nucleic Acids Res 29:173–174

Martinez-Medina A, Pascual JA, Lloret E, Roldan A (2009) Interactions between arbuscular mycorrhizal fungi and Trichoderma harzianum and their effects on Fusarium wilt in melon plants grown in seedling nurseries. J Sci Food Agr 89:1843–1850

Nicholas KB, Nicholas HB Jr (1997) GeneDoc: a tool for editing and annotating multiple sequence alignments. Distributed by the authors

Öpik M, Moora M, Liira J, Kõljalg U, Zobel M, Sen R (2003) Divergent arbuscular mycorrhizal communities colonize roots of *Pulsatilla* spp. in boreal Scots pine forest and grassland soils. New Phytol 160:581–593

Pivato B, Mazurier S, Lemanceau P, Siblot S, Berta G, Mougel C, van Tuinen D (2007) *Medicago* species affect the community composition of arbuscular mycorrhizal fungi associated with roots. New Phytol 176:197–210

Querejeta JI, Allen MF, Alguacil MM, Roldán A (2007) Plant isotopic composition provides insight into mechanisms underlying growth stimulation by AM fungi in a semiarid environment. Funct Plant Biol 34:683–691

Read DJ (1998) Plants on the web. Nature 369:22–23

Renker C, Blanke V, Buscot F (2005) Diversity of arbuscular mycorrhizal fungi in grassland spontaneously developed on area polluted by a fertilizer plant. Environ Pollut 135:255–266

Requena N, Pérez-Solis E, Azcón-Aguilar C, Jeffries P, Barea JM (2001) Management of indigenous plant–microbe symbioses aids restoration of desertified ecosystems. Appl Environ Microbiol 67:495–498

Saitou N, Nei M (1987) The Neighbor-Joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Scheublin TR, Ridgway KP, Young JPW, van der Heijden MGA (2004) Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. Appl Environ Microbiol 70:6240–6246

Schüβler A, Walker C (2010) The *Glomeromycota*: a species list with new families and new genera. Arthur Schüßler, Christopher Walker, Gloucester. Published in libraries at The Royal Botanic Garden Edinburgh, The Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University

Smith SE, Read DJ (2008) Mycorrhizal symbiosis (Third Edition). Academic, New York, 787 pp

Soil Survey Staff (SSS) (2006) Keys to soil taxonomy, 10th edn. USDA. Natural Resources Conservation Service, Washington, DC

Swofford DL (2002) PAUP: phylogenetic analysis using parsimony (and other methods), Version 4.08b10 for Machintosh. Sinauer Associates Inc, Sunderland

Sýkorová Z, Ineichen K, Wiemken A, Redecker D (2007) The cultivation bias: different communities of arbuscular mycorrhizal fungi detected in roots from the field, from bait plant transplanted to the field, and from a greenhouse trap experiment. Mycorrhiza 18:1–14

Ter Braak CFJ, Smilauer P (2004) CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Biometris, Wageningen, The Netherlands

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24:4876–4882

Trouvelot S, van Tuinen D, Hijri M, Gianinazzi-Pearson V (1999) Visualization of ribosomal DNA loci in spore interphasic nuclei of glomalean fungi by fluorescence in situ hybridization. Mycorrhiza 8:203–206

Turnau K, Ryszka P, Gianinazzi-Pearson V, Van Tuinen D (2001) Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland. Mycorrhiza 10:169–174

Van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72

van Tuinen D, Jacquot E, Zhao B, Gollotte A, Gianinazzi-Pearson V (1998) Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. Mol Ecol 7:879–887

Vandenkoornhuyse P, Husband R, Daniell TJ, Watson IJ, Duck JM, Fitter AH, Young JPW (2002) Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. Mol Ecol 11:1555–1564

Vandenkoornhuyse P, Ridgway KP, Watson IJ, Fitter AH, Young JPW (2003) Co-existing grass species have distinctive arbuscular mycorrhizal communities. Mol Ecol 12:3085–3095

Vierheilig H (2004) Further root colonization by arbuscular mycorrhizal fungi in already mycorrhizal plants is suppressed after a critical level of root colonization. J Plant Physiol 161:339–341

Vierheilig H, Garcia-Garrido JM, Wyss U, Piché Y (2000) Systemic suppression of mycorrhizal colonization of barley roots already colonized by AM fungi. Soil Biol Biochem 32:589–595

Wilson GWT, Rice CW, Rillig MC, Springer A, Hartnett DC (2009) Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecol Lett 12:452–461

Wirsel SGR (2004) Homogenous stands of a wetland grass harbour diverse consortia of arbuscular mycorrhizal fungi. FEMS Microbiol Ecol 48:129–138