Soil Biology & Biochemistry 49 (2012) 132–138

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Perennial plant species from semiarid gypsum soils support higher AMF diversity in roots than the annual *Bromus rubens*

M.M. Alguacil [a,*], E. Torrecillas [a], A. Roldán [a], G. Díaz [b], M.P. Torres [b]

[a] CSIC-Centro de Edafología y Biología Aplicada del Segura, Department of Soil and Water Conservation, P.O. Box 164, Campus de Espinardo, 30100 Murcia, Spain
[b] Departamento de Biología Aplicada, Area de Botánica, Universidad Miguel Hernández, Avda. De la Universidad s/n, 03202-Elche (Alicante), Spain

ARTICLE INFO

Article history:
Received 3 October 2011
Received in revised form
22 February 2012
Accepted 24 February 2012
Available online 7 March 2012

Keywords:
Arbuscular mycorrhizal fungi
Bromus rubens
Diversity
Gypsophilous
Gypsum soils
Herniaria fruticosa
Semiarid ecosystems
Senecio auricula

ABSTRACT

The arbuscular mycorrhizal fungi (AMF) communities composition regulate plant interactions and determine the structure of plant communities. In this study we analysed the diversity of AMF in the roots of two perennial gypsophyte plant species, *Herniaria fruticosa* and *Senecio auricula*, and an annual herbaceous species, *Bromus rubens*, growing in a gypsum soil from a semiarid area. The objective was to determine whether perennial and annual host plants support different AMF communities in their roots. The roots were analysed by nested PCR, cloning, sequencing of the ribosomal DNA small subunit region and phylogenetic analysis. Twenty AMF sequence types, belonging to the *Glomus* group A, *Glomus* group B, *Diversisporaceae*, *Acaulosporaceae*, *Archaeosporaceae* and *Paraglomeraceae*, were identified. Both gypsophyte perennial species, *H. fruticosa* and *S. auricula* had different compositions of the AMF community and higher diversity than *B. rubens*. This annual plant species shared the full composition of its AMF community with both perennial plant species. Seasonal variations in the colonisation of AM fungi could explain the observed differences in AMF community composition, but this is still a working hypothesis that requires the analysis of further data obtained from a higher number of both annual and perennial plant species in order to be fully tested.

1. Introduction

Arid soils developed from gypsum materials support vegetation that is characterised by a high endemicity rate, adaptive convergence and clear discontinuity with the surrounding vegetation. Gypsophylous scrublands are usually very open and, floristically, are characterised mainly by the presence of numerous perennial, gypsophilous plant species (Martínez-Hernández et al., 2011), although annual species are also an important component in the plant communities of these arid areas.

Recent interest in arbuscular mycorrhizal (AM) ecology in arid ecosystems has focused mainly on the diversity (Alguacil et al., 2009, 2011; Li et al., 2010; Martínez-García et al., 2011) and less on the specificity and functionality of AM fungus-plant interactions. There are indications that the diversity and distribution of AM fungi (AMF) in soil depend on the soil properties in addition to the host-plant preferences of the AMF. Several authors suggested that AM fungal communities may be more specific to plant functional groups than to individual plant species (Öpik et al., 2009; Scheublin et al., 2004). Others showed that the host plant is the main determinant of the AM fungal community colonising the roots (Martínez-García et al., 2011; Van der Heijden et al., 1998). Moreover, in an extensive study, Oehl et al. (2010) concluded that the soil type is the major determinant of both the composition and species richness of AM fungal communities in Central European soils.

In arid ecosystems, AMF can exert control over the plant community dynamics and it has been suggested that different shrub species support different AM fungal communities, at least in their associated rhizosphere soil (Martínez-García et al., 2011). Alguacil et al. (2009) reported that, in a gypsophilous plant community, the composition of the AM fungal community was clearly host-plant-dependent and the number of fungal types detected was different in each of the four shrub species studied. Shrub host plants have the potential to establish a network of active AMF ready to colonise later arrivals (such as annual grasses). In this sense, annual plants should have the same community of root AMF as perennial host plants, but annual plants have physiological traits and use of resources different to those of perennials and this may mean that the AMF community composition differs in both abundance and diversity.

To investigate the composition of the AM fungal community of perennial and annual plant species in a gypsophilous community, we selected two perennial gypsophyte species, *Herniaria fruticosa*

* Corresponding author. Tel.: +34 968396337; fax: +34 968396213.
E-mail address: mmalguacil@cebas.csic.es (M.M. Alguacil).

0038-0717/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2012.02.024

and *Senecio auricula*, both reported to occur only on gypsum soils, and an annual grass: *Bromus rubens*, a very-common generalistic species. The main objective was to determine the diversity of AMF in three life cycle-diverse plant species and to check whether these plants species share or not the same AM fungi in gypsum soils from an arid ecosystem.

2. Materials and methods

2.1. Study site

The study area was located in "El Rincón" in Lorca, Province of Murcia (Southeastern Spain) (coordinates 37° 40′ N, 1° 41′ W, 753 m altitude). The climate is semiarid, with a potential evapotranspiration (ETP) of 837 mm and an annual average rainfall of 289 mm. The mean annual temperature is 16 °C. The soils are classified as Petrogypsic Gypsiorthid, (SSS, 1999) and Gypsisol (FAO, 1998) with a gypsic and petrogypsic horizon within 100 cm from the surface, developed on gypsum parental rocks.

2.2. Experimental design

The field trial was conducted using a factorial design with three replication blocks (5 × 5 m) separated 10 m each other. Three plant species were selected: two perennial species: a shrub species *Herniaria fruticosa* L., and an herbaceous hemicryptophyte (a plant with perennial buds situated at or just below the soil surface), *S. auricula* Bourg. ex Coss. and a generalistic annual grass, *Bromus rubens* L. Generalistic plant species non adapted to gypsum soils are extremely scarce in the study area. *B. rubens* being the only one which abundance allowed to include it in the block experimental design. All three plant species are well adapted to water stress conditions and commonly distributed in Mediterranean areas from Gypsum soils. In the case of *B. rubens*, its occurrence is linked to the rainfall period during spring.

2.3. Sampling

All samples were collected in the second half of April 2010. Four plants belonging to each of the three selected species were sampled per replication block (a total of 36 plants). Samples of the plant 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.

2.4. DNA extraction and PCR

For each sample, 0.1 g fresh root material was placed into a 2-ml screw-cap propylene tube together with two tungsten carbide balls (3 mm) and ground (3 min, 13 000 r.p.m.) using a mixer mill (MM 400, Retsch, Haan, Germany). Total DNA was extracted using a DNeasy plant mini Kit following the manufacturer's recommendations (Qiagen). The extracted DNA was resuspended in 20 μl of water. Several dilutions of extracted DNA (1/10, 1/50, 1/100) were prepared.

Partial ribosomal small subunit (SSU) DNA 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 (Amersham 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). As a template, 2 μl of extracted DNA was used in all reactions.

Several dilutions (1/10, 1/20, 1/50 and 1/100) 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 (Amersham 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). As a template, 2 μl of extracted DNA was used in all reactions. Positive and negative controls using PCR positive products and sterile water respectively were also included in all amplifications. DNA extracts were stored at −20 °C. 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).

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). Thirty two 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.

They 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). One representative sequence from each plant species and replication belonging to different AMF sequence types have been deposited at the European Molecular Biology Laboratory (EMBL) database under the accession numbers HE576798 to HE576936.

A search for chimeric sequences was performed using the QIIME's ChimeraSlayer wrapper (Caporaso et al., 2010).

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, the corresponding closest matches from GenBank; and sequences representing the most representative eight AM fungal genera. 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-groups.

Different AMF sequence types or phylotypes, were defined as groups of closely related sequences, usually with a high level of bootstrap support in the phylogenetic analyses (higher than 80%) and sequence similarity ≥97%.

2.7. Statistical analysis

We applied General log-linear modellings to check whether the composition of the AM fungal communities differed between the three plant species. Identity of plant species, blocks and their interactions effects on AMF sequence types were tested by a two-

way analysis of variance and comparisons among means were made using the Duncan's test calculated at $P < 0.05$.

Correspondence analysis (CA) with presence and/or absence data for all AMF sequence types at three 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 three plant species. All statistical procedures were carried out with the software package SPSS 19.0 for Windows.

The presence or absence of AMF phylotypes in each root 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 ith 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 ith species]).

3. Results

3.1. AM fungal diversity

Partial SSU rDNA sequences from 36 root samples were amplified successfully by nested PCR. A total of 1152 clones from 36 libraries were screened by PCR (on average, 32 clones were analysed per sample); out of these, 772 contained the SSU rRNA gene fragment and subsequently were sequenced (Table 1). A total of 660 sequences belonging to AMF were obtained, excluding non-AM fungi and cloning vector sequences (112 clones). No chimeric sequences were detected in this study. The 660 AMF sequences were grouped into 20 sequence types based on sequence similarity to those of identified AMF available in GenBank of ≥97% and a level of bootstrap support in the phylogenetic analyses higher than 80% (Fig. 1). Since identical sequences were detected, the clones producing the same sequence for each host plant species were represented once in the alignment for clarity (see the supplementary material for a detailed description of the clone groups with identical sequences).

Of the 20 AMF sequence types obtained, 10 belonged to *Glomus* group A, 3 to *Glomus* group B, 2 to *Glomus* group C or the *Diversisporaceae*, 1 belonged to the *Acaulosporaceae*, 1 to the *Archaeosporaceae* and 3 to the *Paraglomeraceae* (Fig. 1; Table 2). The most-abundant AMF sequence type (represented by 271 clones and found in all three plant species) was Glo G9, which corresponds to the morphologically-defined species *Glomus intraradices*. The second-most-frequent AMF sequence type was Glo G4, which corresponds to *Diversispora aurantia.*

The Aca 1, Glo G3, Glo G11, Glo G12, Glo G13, Arch 1, Para 1 and Para 2 sequence types were supported by very-high bootstrap values in the phylogenetic analysis and did not seem to be related to any sequences of AMF in culture or found previously in the GenBank database; thus, they could be novel AMF sequence types. The rest of the AMF sequence types were related to uncultured AMF species in the database.

The numbers of different AMF sequence types of each plant species were compared by two-way analysis of variance. The block factor did not have a significant effect on the AMF richness ($F = 2.091$; $P = 0.128$); however, plant species identity had a highly-significant influence ($F = 9.969$; $P < 0.001$). The plant harbouring the lowest mean number of AMF sequence types was *B. rubens* (3.87), which differed significantly from *H. fruticosa* (5.15) and *S. auricula* (4.84) according to Duncan's significant difference test.

All three sampling-effort curves showed that the number of root samples analysed was sufficient to detect the majority of AMF sequence types present in the roots, since the curves approach saturation (Fig. 2).

3.2. The AMF community composition in different host plant species

The composition of the AM fungal community colonising the three plant species differed significantly ($\chi^2 = 73.22$, $P < 0.001$), indicating that the community is influenced by the plant factor. In paired comparisons of the plant species, the AMF composition was significantly different for the *H. fruticosa–B. rubens* ($\chi^2 = 47.83$, $P < 0.001$) and *S. auricula–B. rubens* ($\chi^2 = 65.07$, $P < 0.001$) comparisons, but not for *H. fruticosa–S. auricula* ($\chi^2 = 1.38$, $P = 0.241$).

Ten of the 20 AMF sequence types detected occurred in the roots of all three plant species studied, representing the majority of clones analysed in this study (541 clones) (Table 2). Para 1, Glo G11, Glo G12, Glo G13 and Glo G14 were found exclusively in the two perennial plant species, represented by a number of clones ranging from six to 38. Two sequence types (Para 3 and Aca 1) appeared exclusively in *H. fruticosa* and another two (Para 2 and Glo G5) only in *S. auricula.* No sequence types occurring exclusively in *B. rubens* were detected in this study.

These results were reflected in the Correspondence Analysis diagram (Fig. 3): the perennial gypsophyte plant species are close to each other, indicating that these two species hosted similar AM fungal communities. In contrast, *B. rubens* is distant to both perennial species because of the marked differences in the relative abundances of the Glo G1, Glo G8 and Glo G15 types.

4. Discussion

In this study, we recorded 20 AMF sequence types in total, belonging to five genera (*Acaulospora*, *Glomus*, *Diversispora*, *Archaeospora* and *Paraglomus*). This level of richness is similar to that of a previous study in different gypsum areas (Alguacil et al., 2009). However, the results may not be directly comparable since different primers were used. In the previous study, the AM1-3/NS31 primers developed by Santos-González et al. (2007) were used; these cover a shorter SSU rRNA gene fragment than the AML1/AML2 primers of Lee et al. (2008). In fact, no sequences from

Table 1
Number of clones, AMF sequence types and Shannon- Weaver diversity indices from the three plant species used in the analysis of AMF diversity.

Plant species	Number of libraries analysed by PCR	Total clones examined	Number of clones for sequencing	Number of clones belonging to AMF sequences	Number of AMF sequence types	Shannon-diversity indices
Herniaria fruticosa	12	384	330	290	18	2.12
Senecio auricula	12	384	300	261	16	1.84
Bromus rubens	12	384	142	109	11	1.63
Total	36	1152	772	660	20	–

Fig. 1. Neighbour-Joining (NJ) phylogenetic tree showing AM fungal sequences isolated from roots of *Herniaria fruticosa*, *Senecio auricula* and *Bromus rubens*, and reference sequences from GeneBank. All bootstrap values >80% are shown (1000 replicates). Sequences obtained in the present study are labelled with the host plant from which they were obtained (H = *H. fruticosa* roots; S = *S. auricula* roots; B = *B. rubens* roots) 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* and *Endogone pisiformis* were used as out-groups.

Table 2
Number of clones detected for each arbuscular mycorrhizal fungal (AMF) sequence types in the three different plant species (H, *H.fruticosa*; S, *S. auricula*; B, *B. rubens*).

AMF sequence types	Number of detected clones for each plant species
Para1	H (2), S (5)
Para2	S (2)
Para3	H (2)
Aca1	H (10)
Arch1	H (13), S (7), B (2)
Glo G1	H (3), B (14)
Glo G2	H (9), S (14), B (3)
Glo G3	H (3), S (5), B (4)
Glo G4	H (14), S (74), B (5)
Glo G5	S (4)
Glo G6	H (2), S (10), B (2)
Glo G7	H (57), S (2), B (2)
Glo G8	H (7), S (2), B (13)
Glo G9	H (107), S (106), B (58)
Glo G10	H (13), S (5), B (2)
Glo G11	H (4), S (2)
Glo G12	H (15), S (3)
Glo G13	H (25), S (13)
Glo G14	H (2), S (7)
Glo G15	H (2), B (4)

the present trial showed homology with sequences obtained in the earlier study.

Our results clearly show that the composition of the AMF community is host-plant-dependent in gypsum soils (Table 1), which agrees with earlier results in different habitats around the world (Helgason et al., 2002; Vandenkoornhuyse et al., 2002, 2003; Öpik et al., 2003; Gollotte et al., 2004; Scheublin et al., 2004; Sýkorovà et al., 2007; Alguacil et al., 2011; Li et al., 2010). It is noteworthy that this effect was due particularly to *B. rubens*, since this plant species had an AMF community whose composition differed significantly from that of both gypsophyte perennial species (*H. fruticosa* and *S. auricula*); also, *B. rubens* showed the lowest Shannon diversity index ($H' = 1.69$). These differences were also shown by the CA analysis (Fig. 3), indicating that, although all the AM fungal types detected in the roots of *B. rubens* were shared by both gypsophyte perennial species, the abundances of Glo G1, Glo G8 and Glo G15 were greater in *B. rubens*. *H. fruticosa* had a higher number of AMF sequence types (18) and a higher Shannon index ($H' = 2.12$) than *S. auricula* (16 AMF types and $H' = 1.84$); however, their AMF community compositions did not differ significantly ($\chi^2 = 1.527$; $P = 0.217$). It is interesting to note that the AMF sequence types Para 1, Glo G11, Glo G12 and Glo G13 did not show homology with any sequences in the EMBL database and were found exclusively in the gypsophyte perennial plants. This suggests host specificity of these endemic plant species for these AMF types.

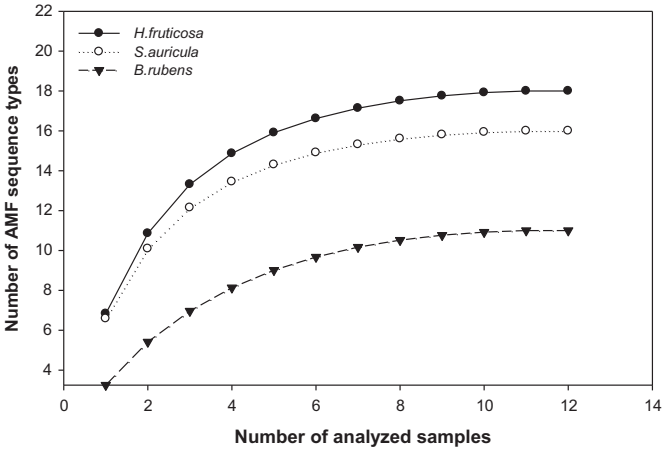

Fig. 2. Sampling effort curves for the AM fungal community in the roots of *Herniaria fruticosa*, *Senecio auricula* and *B. rubens*. The sample order was randomized by 100 replications in EstimateS, version 8.0 (Colwell, 2005).

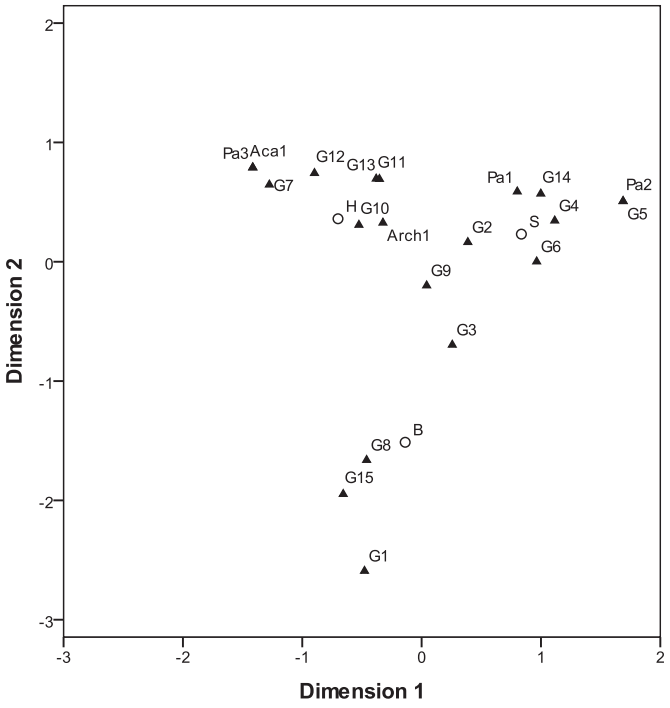

Fig. 3. Correspondence analysis (CA) of the AMF communities found in the roots of *Herniaria fruticosa*, *Senecio auricula* and *Bromus rubens*. The eigenvalues of the first and second axes in the two-dimensional ordination diagrams are as follows: Dimension 1 = 0.50 and Dimension 2 = 0.46. All environmental variables (host plant and AMF types) explained 45% of the total variance. Filled triangles represent the different AMF sequence types and the open circles represent the plant species (H = *H. fruticosa*, S = *S. auricula* and B = *B. rubens*).

Moreover, two AMF sequence types were found exclusively in *H. fruticosa* or *S. auricula*: Para 3 and Aca 1 appeared only in *H. fruticosa* and Para 3 showed very-high similarity (100% identity) with database sequences belonging to uncultured *Paraglomus*, which had been reported previously from *Retama sphaerocarpa* in a semiarid ecosystem (Alguacil et al., 2011), whereas Aca 1 was not related to any AMF sequence type in the database. Para 2 and Glo G5 were *S. auricula*-specific AMF sequence types: Glo G5 showed very-high similarity (98% identity) to *Diversispora* sp. isolated from *Panax japonicus* roots in field crops in Korea (Lee et al., 2008), while Para 2, in contrast, has not been reported previously.

The differences in the AMF community composition between plant species could be explained by different functional plant characteristics, such as life cycle, phenology and physiology (Hawkes et al., 2006). The annual plant species, *B. rubens*, and the two perennial gypsophyte species, *H. fruticosa* and *S. auricula*, differ in the timing and extent of their activity during the year. *B. rubens* only occurs in spring, when the environmental conditions are favourable. The disparity in life forms between these annual and perennial plant species might have affected the establishment of AMF in roots. In fact, it has been shown that the host plant identity and phenology affect the AMF community in host plant roots (Pringle and Bever, 2002; Mummey and Rillig, 2006; Hawkes et al., 2006). In our study, *H. fruticosa* and

S. auricula showed higher AMF diversity and hosted a higher number of AMF sequence types than *B. rubens*. Furthermore, all 12 AMF sequence types hosted by *B. rubens* were found also in the gypsophyte perennial species and no AMF sequence types were hosted exclusively by *B. rubens*. On the other hand these differences between perennial and annual plant species could be due to the fact that perennial plants roots are present in the soil longer giving more opportunities for mycorrhizal colonization over time. Although in other semiarid ecosystems without gypsum soils in the vicinity, annual plant species always yielded higher AMF diversity in their roots (Alguacil et al., 2011; Torrecillas et al., 2011).

Perennial species that form the plant community of gypsum soils establish mycorrhizae with a range of AMF that would be available to differing degrees to annual plants, with certain seasonal variations. Seasonal changes in the composition of the AMF community colonising plant roots have been shown using molecular methods, in previous studies (Husband et al., 2002a,b; Vandenkoornhuyse et al., 2002; Santos et al., 2006; Dumbrell et al., 2011). Moreover, the compositions of AMF communities, estimated by spore counts, have been shown to be seasonal (Gemma and Koske, 1988; Giovannetti, 1985; Sylvia, 1986), as have the frequency and intensity of root colonisation (Mayr and Godoy, 1989; de Oliveira and de Oliveira, 2005) and the relative amounts of AMF hyphae, vesicles and arbuscules within plant roots (Mandyam and Jumpponen, 2008). Therefore, AMF seem to have differing seasonal phenologies (Pringle and Bever, 2002). Dumbrell et al. (2011) showed that the AMF community was significantly-less diverse and abundant during summer than during winter, with an over-dominance of a single AMF taxon; they suggested that during the summer high rates of photosynthesis increase the carbon supply to the AMF. An increase in available carbon resources can reduce both diversity and community evenness, because it favours the more-competitive AMF (Tilman, 1987). In our study, the mechanism reducing the AMF diversity in *B. rubens*, leading to the over-dominance of certain AMF sequence types, may have been similar since this annual plant species germinates only in the most-favourable season for plant photosynthesis in the semiarid Mediterranean areas (spring) and thus may host only the fastest growing AMF species.

Our conclusions are limited by the fact that, in the experimental work, the main effort was made to establish a sampling block design in order to mitigate the covariation due to spatial variations; consequently, only three plant species were tested. Nevertheless, both gypsophyte perennial species had different AMF communities which differed from that of the annual species. *B. rubens* shared the full composition of its AMF community with both perennial species. However, the explanation of this based on seasonal differences in AMF colonisation is still a working hypothesis that requires the analysis of more data, obtained with a higher number of both annual and perennial plant species, in order to be fully tested.

Acknowledgments

MM Alguacil was supported by the Ramón y Cajal programme (Ministerio de Educación y Ciencia, Spain). This research was supported by the Spanish Ministry of Education and Science-CICYT (Project number REF. CGL2009-12582-CO2-01).

Appendix. Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.soilbio.2012.02.024.

References

Alguacil, M.M., Torres, M.P., 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 Biology and Biochemistry 43, 167–173.

Alguacil, M.M., Roldán, A., Torres, M.P., 2009. Assessing the diversity of AM fungi in arid gypsophilous plant communities. Environmental Microbiology 11, 2649–2659.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25, 3389–3402.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Gonzalez Pena, A., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335–336.

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.

de Oliveira, A.N., de Oliveira, L.A., 2005. Seasonal dynamics of arbuscular mycorrhizal fungi in plants of Theobroma grandiflorum schum and Paullinia cupana mart. of an agroforestry system in Central Amazonia, Amazonas State, Brazil. Brazilian Journal of Microbiology 36, 262–270.

Dumbrell, A.J., Ashton, P.D., Aziz, N., Feng, G., Nelson, M., Dytham, C., Fitter, A.H., Helgason, T., 2011. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytologist 190, 794–804.

FAO, 1998. World Reference Base for Soil Resources. FAO–ISRIC–ISSS, Rome.

Gemma, J.N., Koske, R.E., 1988. Seasonal variation in spore abundance and dormancy of *Gigaspora gigantea* and in mycorrhizal inoculum potential of a dune soil. Mycologia 80, 211–216.

Giovannetti, M., 1985. Seasonal variations of vesicular-arbuscular mycorrhizas and endogonaceous spores in a maritime sand dune. Transactions of the British Mycological Society 84, 679–684.

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.

Hawkes, C.V., Belnap, J., D'Antonio, C., Firestone, M.K., 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant and Soil 281, 369–380.

Helgason, T., Merryweather, J.W., Denison, J., Wilson, P., Young, J.P.W., Fitter, A.H., 2002. Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. Journal of Ecology 90, 371–384.

Husband, R., Herre, E.A., Turner, S.L., Gallery, R., Young, J.P.W., 2002a. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. Molecular Ecology 11, 2669–2678.

Husband, R., Herre, E.A., Young, J.P.W., 2002b. Temporal variation in the arbuscular mycorrhizal communities colonising seedlings in a tropical forest. FEMS Microbiology Ecology 42, 131–136.

Lee, J., Lee, S., Young, J.P.W., 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiology Ecology 65, 339–349.

Li, L.F., Li, T., Zhang, Y., Zhao, Z.W., 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 Microbiology Ecology 71, 418–427.

Mandyam, K., Jumpponen, A., 2008. Seasonal and temporal dynamics of arbuscular mycorrhizal and dark septate endophytic fungi in a tall grass prairie ecosystem are minimally affected by nitrogen enrichment. Mycorrhiza 18, 145–155.

Martínez-García, L.B., Armas, C., Miranda, J.D., Padilla, F.M., Pugnaire, F.I., 2011. Shrubs influence arbuscular mycorrhizal fungi communities in a semi-arid environment. Soil Biology and Biochemistry 43, 682–689.

Martínez-Hernández, F., Pérez-García, F.J., Garrido-Becerra, J.A., Mendoza-Fernández, A.J., Medina-Cazorla, J.M., Martínez-Nieto, M.I., Merlo Calvente, M.E., Mota Poveda, J.F., 2011. The distribution of Iberian gypsophilous flora as a criterion for conservation policy. Biodiversity Conservation 20, 1353–1364.

Mayr, R., Godoy, R., 1989. Seasonal patterns in vesicular–arbuscular mycorrhiza in Melic-BeechForest. Agriculture, Ecosystems and Environment 29, 281–288.

Mummey, D.L., Rillig, M.C., 2006. The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. Plant and Soil 288, 81–90.

Nicholas, K.B., Nicholas, H.B. Jr., 1997. GeneDoc: a tool for editing and annotating multiple sequence alignments. Distributed by the authors.

Oehl, F., Laczko, E., Bogenrieder, A., Stahr, K., Bösch, R., van der Heijden, M., Sieverding, E., 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. Soil Biology and Biochemistry 42, 724–738.

Öpik, M., Metsis, M., Daniell, T.J., Zobel, M., Moora, M., 2009. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. New Phytologist 184, 424–437.

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 Phytologist 160, 581–593.

Pringle, A., Bever, J.D., 2002. Divergent phenologies may facilitate the coexistence of arbuscular mycorrhizal fungi in a North Carolina grassland. American Journal of Botany 89, 1439–1446.

Saitou, N., Nei, M., 1987. The Neighbor-Joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4, 406–425.

Santos, J.C., Finlay, R.D., Tehler, A., 2006. Molecular analysis of arbuscular mycorrhizal fungi colonising a semi-natural grassland along a fertilisation gradient. New Phytologist 172, 159–168.

Santos-González, J.C., Finlay, R.D., Tehler, A., 2007. Seasonal dynamics of arbuscular mycorrhizal fungal communities in roots in a seminatural grassland. Applied and Environmental Microbiology 73, 5613–5623.

Scheublin, T.R., Ridgway, K.P., Young, J.P.W., van der Heijden, M.G.A., 2004. Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. Applied and Environmental Microbiology 70, 6240–6246.

Soil Survey Staff (SSS), 1999. Soil taxonomy. A basic system of soil classification for making and interpreting soil surveys. In: Agriculture Handbook, second ed., vol. 436. U.S.D.A., N.R.C.S, U.S. Gov. Print. Off., 869 pp.

Swofford, D.L., 2002. PAUP: Phylogenetic Analysis Using Parsimony (and other methods), Version 4.08b10 for Machintosh. Sinauer Associates Inc., Sunderland, MA.

Sýkorovà, Z., Wiemken, A., Redecker, D., 2007. Cooccurring *Gentiana verna* and *Gentiana acaulis* and their neighboring plants in two Swiss upper montane meadows harbour distinct arbuscular mycorrhizal fungal communities. Applied and Environmental Microbiology 73, 5426–5434.

Sylvia, D.M., 1986. Spatial and temporal distribution of vesicular-arbuscular mycorrhizal fungi associated with *Uniola paniculata* in Florida foredunes. Mycologia 78, 728–734.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24, 4876–4882.

Torrecillas, E., Alguacil, M.M., Roldán, A., 2011. Differences in the AMF diversity in soil and roots between two annual and perennial gramineous plants co-occurring in a Mediterranean, semiarid degraded area. Plant and Soil. doi:10.1007/s11104-011-1047-9.

Tilman, D., 1987. Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. Ecology Monographs 57, 189–214.

Vandenkoornhuyse, P., Husband, R., Daniell, T.J., Watson, I.J., Duck, J.M., Fitter, A.H., Young, J.P.W., 2002. Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. Molecular Ecology 11, 1555–1564.

Vandenkoornhuyse, P., Ridgway, K.P., Watson, I.J., Fitter, A.H., Young, J.P.W., 2003. Co-existing grass species have distinctive arbuscular mycorrhizal communities. Molecular Ecology 12, 3085–3095.

Van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396, 69–72.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogen-etics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols. A Guide to Methods and Applications. Academic, San Diego, pp. 315–322.