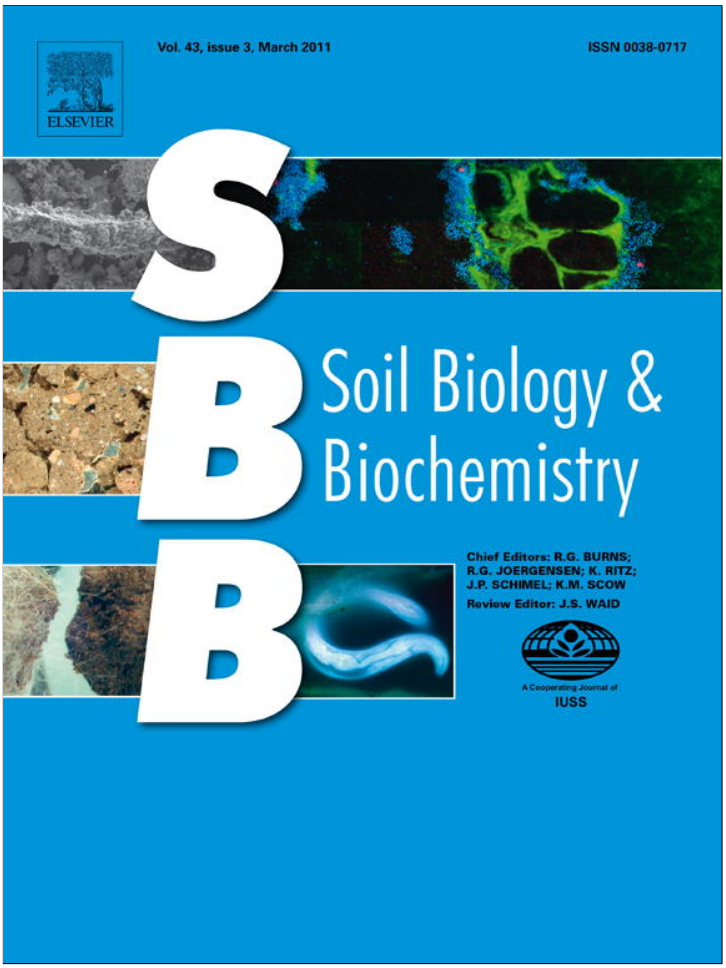

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Shrubs influence arbuscular mycorrhizal fungi communities in a semi-arid environment

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ABSTRACT

Interactions between arbuscular mycorrhizal fungi (AMF) and plants are essential components of ecosystem functioning; however, they remain poorly known in dry ecosystems. We examined the relationship between seven shrub species and their associated AMF community in a semi-arid plant community in southern Spain. Soil characteristics and plant physiological status were measured and related to AMF community composition and genetic diversity by multivariate statistics. We found differences in AMF communities in soils under shrubs and in gaps among them, whereas no differences were detected among AMF communities colonizing roots. Soil nutrients content drove most of the spatial variations in the AMF community and genetic diversity. AMF communities were more heterogeneous in fertile islands with low nitrogen-to-phosphorus ratio and vice versa. AMF genetic diversity increased in soils limited by phosphorus and with high soil organic matter content, while AMF genetic diversity increased in roots growing in soil not limited by phosphorus. Overall, we could not find a clear link between plant performance and the associated AMF community. Our findings show that different shrub species generate islands of fertility which differ in nutrient content and, therefore, support different AMF communities, increasing AMF diversity at the landscape level.

1. Introduction

Understanding interactions below and aboveground is essential to explain the dynamics of plant communities, although most research is focused on aboveground interactions (Callaway and Aschehoug, 2000; Callaway and Walker, 1997; Holmgren et al., 1997; Tilman et al., 1997). In the last decade, however, the importance of diversity and distribution of soil microorganisms for ecosystem functioning has been highlighted, emphasizing the significance of plant–soil interactions in shaping plant communities (Smith and Read, 2008; Van Der Putten, 2003; Wardle et al., 2004); nevertheless, the role of such interactions on plant community dynamics needs more attention (Bardgett et al., 1999; Bartelt-Ryser et al., 2005; Kardol et al., 2007).

In arid ecosystems, where nutrient accessibility by plants is often low because of water scarcity, below and aboveground interactions have been developed as a response to drought. Shrubs and trees often act as nurse plants (Niering et al., 1963), forming resource-rich patches that facilitate growth of other species (Flores and Jurado, 2003; Ludwig et al., 2005; Pugnaire et al., 1996b) and lead to a heterogeneous distribution of soil resources. Growth of arbuscular mycorrhiza fungi (AMF) is enhanced in such patches and mycorrhiza potential (i.e., AMF inoculum) has been shown to increase in the rhizosphere of arid shrub species (Azcón-Aguilar et al., 2003; Moora and Zobel, 2010). Under harsh environmental conditions mycorrhized plants may increase their competitive ability (Johnson et al., 2010). Previous studies in patchy environments have revealed that AMF stimulate growth of shrubs and their responsiveness to drought (Goicoechea et al., 2005; Querejeta et al., 2007) while at the same time shrubs exert a selective pressure on AMF species (Alguacil et al., 2009). There is empirical evidence showing how AMF identity and diversity shape plant communities (Klironomos et al., 2000; van der Heijden et al., 1998a; van der Heijden et al., 1998b). However, more insights on AMF diversity, distribution patterns, and ecological function in arid environments are needed to fully understand plant–AMF interactions, particularly in dry environments due to the relatively low number of field studies compared to temperate regions.

The use of molecular techniques revealed an unexpectedly high diversity of AMF (Helgason et al., 2002; Redecker, 2002) and strong host specificity (Helgason et al., 2007; Johnson et al., 2003; Vandenkoornhuyse et al., 2002) challenging the traditional view of

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a relatively low number of species described (c.152 species in 6 genera, Walker and Trappe, 1993) with a large number of host species (c. at least 80% of vascular plants Trappe, 1987). However, our knowledge is still limited as recent reports have shown that AMF–plant associations may not always be species-specific (Opik et al., 2009; Santos et al., 2006) and factors such as soil properties can determine AMF–plant interactions (Landis et al., 2004; Lekberg et al., 2007).

The incidence and direction of AMF–plant interactions may depend on belowground feedbacks (Casper et al., 2008) which explain how the presence of a given plant species modifies the soil community in a way that eventually alters its own and other species' performance (Bever, 1999, 2003; Klironomos, 2002; Reynolds et al., 2003). When plant performance is improved in association with microorganisms, it leads to increased abundance of both the plant species and its associated microorganisms, displacing others (Bever, 2003; Reynolds et al., 2003). However, the opposite occurs when feedbacks contribute to the persistence of competitor species (Bever, 2003). As a result, belowground feedbacks may lead to changes in species diversity; therefore, knowledge about AMF diversity and distribution both in soil and roots, as well as plant performance is very useful to describe patterns of AMF–plant interactions.

We addressed these points in an observational study where we determined AMF community distribution and its relationships with the plant community and soil properties in a semi-arid region in SE Spain. We characterized AMF genetic diversity in two compartments, rhizosphere and roots, and related these data with soil nutrient content and plant performance. We assumed that AMF diversity would be equal or higher in soils than in roots due to the different AMF taxa interacting with different plant species. We hypothesized that in this community 1) different islands of fertility generated by shrub species lead to changes in soil AMF communities composition, and 2) root colonization by AMF is species-specific and differs among shrub species.

2. Materials and methods

2.1. Study site and species

The field site is located in the northern foothills of Sierra Alhamilla range (37°01′N, 2°25′W, 350 m elevation) in Almeria, Spain. The plant community is composed of isolated native shrubs which usually create resource islands beneath their canopies, with relative nutrient-rich soils and increased water availability compared to bare ground areas (Pugnaire et al., 2004). Bare ground areas among shrubs are colonized by biological crusts and annual plant species, usually grasses. Soil is an orthic solonchak with calcic regosol inclusions. It had a silt-loamy texture without rock fragments (51.7% silt, 15.1% clay and 9.1% sand). pH is 8.1, carbonate content is 24.0% and potassium is 0.9 μg/g (Perez-Pujalte, 1987). Phosphorus (P), nitrogen (N) and soil organic matter (SOM) were characterized in collected soil samples. The climate is Mediterranean semi-arid with a mean annual precipitation of 242 mm and mean annual temperature of 17.9 °C. Drought is pronounced during the summer season (June to September), when there is almost no rain, and mean maximum temperatures can reach above 40 °C (Pugnaire et al., 2004).

In June 2008 we sampled roots and soils under shrubs and in gaps between them. Target species were *Artemisia barrelieri* (Besser), *Hammada articulata* (Moq.) O. Bolòs & Vigo, *Launaea arborescens* (Batt.) Murb., *Retama sphaerocarpa* (L), *Salsola genistoides* (Juss. ex Poir. in Lam.), *Salsola oppositifolia* (Desf.) and *Thymus hyemalis* (Lange) intermixed in a flat, homogeneous area of ca. 2500 m^2. Shrubs of similar sizes and separated from each other at least 3 m were chosen for sampling. The dominant species is *R. sphaerocarpa* while *T. hyemalis* is the less abundant one, and the other species are equally distributed. All of them are typically found in semi-arid environments of SE Spain (Peinado et al., 1992), and are evergreen species which differ in leaf lifespan and photosynthesis pathway (Table 1), adopting different strategies to cope with drought (Miranda et al., 2010; Padilla et al., 2009).

2.2. Plant physiological status

Plant physiological status ($n = 5$ shrubs per species) was assessed in June 2008. Pre-dawn relative water content (*RWC*) in above plant tissues was determined following Barrs (1968). Maximum photochemical efficiency of photosystem II (*Fv/Fm*) was measured early in the morning with a portable fluorimeter (PEA, Hansatech, Kings Lynn, UK) in leaves dark-adapted for 30 min. Leaf conductance to water vapor (*gs*) and photosynthetic rate (A) were measured on green, mature leaves held perpendicular to the sun beam (1603 ± 25.92 μmol m^{-2} s^{-1}, mean incident PPFD) during early morning hours (07:00–08:30 solar time), i.e., at the time of maximum photosynthetic rates for most of the species in the area (Haase et al., 1999; Munne-Bosch et al., 2003). Measurements were made with a portable infrared gas analyzer (Li-6400; Li-Cor Biosciences, Nebraska USA) under ambient CO_2 concentrations and light conditions. Temperature inside the cuvette never exceeded the external air temperature by more than 2 °C during each measurement, and differences among temperatures along the measurement period never exceeded ±1.5 °C.

2.3. Soil and root sampling

Fine roots from each individual sampled ($n = 3$ per species) and rhizosphere soil ($n = 5$ per species) from the first 15 cm of soil were sampled close to the main trunk of each target plant and from gaps among shrubs ($n = 5$). Sampling tools were cleaned with water and sterilized with 10% NaClO (diluted commercial bleach) and 70% ethanol between samples to avoid cross contamination. Soil and root samples were kept in an ice chest and brought immediately to the lab. Roots were then washed thoroughly with distilled water and a sonicator was used to remove soil particles. Soil samples were sieved through a 2 mm mesh.

Both root and soil samples were divided in two subsamples, one of each was frozen at −80 °C for later DNA extraction and the other dried at 70 °C for 48 h. Dried roots were used for monitoring arbuscular mycorrhizal colonization and dry soil samples for nutrient analyses.

2.4. Soil nutrient analyses

Total N was calculated from colorimetry of NH_3 obtained after Kjeldahl digestions (Bremner, 1965). Available soil phosphate was calculated by colorimetry (Watanabe and Olsen, 1965), and organic

Table 1
Main traits of target species (Miranda et al., 2010; Padilla et al., 2009).

	Family	Rooting depth	Photsynthesis pathway	Flowering time
L. arborescens	Compositae	Shallow	C3, cladodes	Spring–Summer
R. sphaerocarpa	Leguminosae	Deep	C3, cladodes	Spring
S. oppositifolia	Chenopodiaceae	Medium	C4, succulent leaves	Summer–Fall
S. genistoides	Chenopodiaceae	Shallow	C3, cladodes	Summer
A. Barrelieri	Compositae	Medium	C3, leaves	Spring
T. hyemalis	Lamiaceae	Shallow	C3, leaves	Winter–Spring
H. articulata	Chenopodiaceae	Medium	C3, leaves	Summer–Fall

carbon (C) was oxidized by wet digestion and determined by colorimetry (Hesse, 1971). Afterwards, C values were multiplied by 1.72 (Waksman coefficient) to obtained SOM. Soil analyses were performed at the Laboratorio Agroalimentario de Atarfe (Junta de Andalucía, Granada, Spain).

2.5. AMF root length colonization

Dried roots were rehydrated and stained with trypan blue (Phillips and Hayman, 1970). A modified line intersection method (McGonigle et al., 1990) was applied to determine the percentage of roots colonized by AMF. For each sample, presence of hyphae, vesicles, and arbuscules was recorded and a minimum of 50 intersections points were scored in 30–50 cm of roots.

2.6. Composition and genetic diversity of AMF communities

The AMF community under each shrub species was characterized in the rhizosphere and in roots, as well as in bare spaces between shrubs. AMF community composition and genetic diversity was analyzed using terminal restriction fragment length polymorphism (T-RFLP; Liu et al., 1997). DNA was extracted from 200 mg of roots using the DNeasy® Plant Mini Kit (Quiagen, Crawley, UK) and from the soil using the Power Soil DNA Isolation Kit (Mo Bio Labs, CA, USA). DNA amplifications were obtained by nested PCR. In the first PCR we used the primer pair LR1 and FLR2for the amplification of the 5′ end of LSU rDNA sequences in general fungi (Trouvelot et al., 1999; van Tuinen et al., 1998) and in the second we used the AMF specific primers FLR3 and FLR4; (Gollotte et al., 2004; Mummey and Rillig, 2007). The master mix of both reactions consisted of 10 mM of DNTPs, 0.4 mM of each primer, 0.4 μl of BSA (10 mg/ml) (New England BioLabs, MA, USA), 0.1 μl of Taq Polymerasa 5μ/μl (MRC Labs, Cambridge, England) and 2.6 μl of PCR Buffer (MRC Labs, Cambridge, England) plus 1 μl DNA resulting a final volume of 20 μl. Thermal cycling for the PCR reactions started with a denaturing step at 94 °C for 3 min, 35 cycles for 1-min at 93 °C, 1 min at 54 °C for primer pairs LR1 and FLR2 and 56 °C for primers pair FLR3 and FLR4, and 1 min at 72 °C, followed by a final extension step of 72 °C for 10 min.

PCR amplicons from the last PCR reaction were incubated with SAP (shrimp alkaline phospatase) for 1 h at 37 °C and 15 min at 65 °C and digested with restriction enzymes (TaqI; Fermentas Lab., Nunningen, Switzerland) for 6 h at 65 °C. Digestion products were precipitated with ethanol (95%) and sodium acetate 3 M (pH 4.6) and resuspended in deionised water.

TRF was separated by capillary electrophoresis and sizes from each sample were determined using a 3100 automated capillary DNA sequencer with Liz 500 (Applied Biosystems, Foster City, USA) as the size standard and then visualized with Peak Scanner™ Software (Applied Biosystems, Foster City, USA).

Peaks were filtered by setting the minimal cut-off height under 100 fluorescence units to exclude background noise. TRF fragments smaller than 50 base pairs (bp) and bigger than 400 bp were also excluded. TRF occurring in only one sample was considered artefacts of the T-RFLP procedure. Finally, the genetic diversity of the AMF community of each sample was estimated from the TRF counts and the AMF community composition was calculated from the presence/absence TRF matrix constructed from data resulting from T-RFLP.

2.7. Data analysis

Differences in plant physiological status were tested by MANOVA, and soil characteristics, percentage root length infected by AMF, and their genetic diversity by ANOVA. Prior to analyses we checked for the homogeneity of variances using Levene's test. When significant differences were found, differences were analyzed with Tukey's post-hoc tests.

We compared AMF community composition and genetic diversity in roots, rhizosphere, and gaps among shrubs with multivariate methods. A binary code was used for presence (1) and absence (0) of each TRF count. The Sørensen similarity matrix was used to perform a Non-metric Multidimensional Scaling (NMDS) to graphically visualize community composition patterns. The purpose of NMDS is to construct a "map" or configuration of samples which relative distances indicating relative similarity, so that samples with very similar composition are close and vice versa (Bennett et al., 2009). The same matrix was used to perform one-way analysis of similarity (ANOSIM) to test for significant differences in AMF community composition associated with the different shrub species and in gaps among them (Clarke, 1993).

Using principal component analysis (PCA) we related both soil characteristics and plant physiological status to AMF genetic diversity. PCA reduces the number of variables to few axes or components along samples and are spatially distributed. Each component maximized the variance of samples projected onto it and informs about the intensity and direction of the variable. Additionally, we analyzed the correlation among AMF genetic diversity, soil N:P ratio and SOM using Pearson *r* coefficient.

A significance level of 0.05 and marginally significant *P*-level up to 0.08 were considered for General Lineal Models (GLM) and correlations.

General Lineal Models were performed with the Statistica 7.0 software (StatSoft Inc., Tulsa, OK, USA). Multivariate analysis was performed with the PRIMER-E 6.0 software (PRIMER-E, Plymouth, UK; Anderson, 2001).

3. Results

3.1. Soil characteristics

Soil properties under shrubs were overall different among species (Table 2). Soils under *A. barrelieri* had the lowest total N and SOM content and *R. sphaerocarpa* and *H. articulata* the highest. Phosphorus content varied among plant species ($F_{7,30} = 2.76$ $P < 0.05$) and although post-hoc pairwise comparisons were not significant, we observed that soils under *A. barrelieri* and *H. articulata* tended to have the highest P content, whereas the lowest were under *T. hyemalis* and *R. sphaerocarpa* (Table 2).

Table 2
Total nitrogen (N), available phosphorous (P), soil organic matter (SOM), C:N and N:P ratios in soils beneath different shrub species and in bare ground in between (mean ± SE; $n = 5$). * and different letters in a row denote significant differences among species ($P < 0.05$), $^{\Psi}$ denotes $P = 0.06$. Values are means ± 1SE.

	ANOVA	*A. barrelieri*	*H. articulata*	*L. arborescens*	*R. sphaerocarpa*	*S. genistoides*	*S. oppositifolia*	*T. hyemalis*	Gaps
N (%)	$F_{(7,32)} = 4.51$**	0.04 ± 0.00^{b}	0.11 ± 0.02^{a}	0.06 ± 0.01^{ab}	0.09 ± 0.01^{a}	0.07 ± 0.01^{ab}	0.08 ± 0.01^{ab}	0.07 ± 0.01^{ab}	0.06 ± 0.01^{ab}
P (ppm)	$F_{(7,30)} = 2.76$*	5.40 ± 0.67^{a}	5.80 ± 0.80^{a}	3.80 ± 0.49^{ab}	2.50 ± 0.29^{ab}	3.20 ± 0.20^{ab}	3.80 ± 1.62^{ab}	$2.25 \pm 0.25^{b\psi}$	3.00 ± 0.31^{ab}
SOM (%)	$F_{(7,32)} = 3.63$**	0.35 ± 0.08^{c}	1.29 ± 0.34^{a}	0.74 ± 0.11^{abc}	1.09 ± 0.21^{a}	0.8 ± 0.12^{abc}	0.98 ± 0.21^{abc}	0.73 ± 0.07^{abc}	0.52 ± 0.13^{bc}
C:N	$F_{(7,32)} = 3.63$**	3.94 ± 0.61^{b}	5.52 ± 0.40^{ab}	5.56 ± 0.56^{ab}	6.07 ± 0.30^{a}	5.23 ± 0.22^{ab}	6.0 ± 0.45^{a}	5.11 ± 0.20^{ab}	4.15 ± 0.50^{b}
N:P	$F_{(7,30)} = 4.35$***	81.1 ± 10.45^{b}	192.86 ± 25.42^{ab}	182.80 ± 27.82^{ab}	354.13 ± 69.80^{a}	240.83 ± 35.95^{ab}	314.40 ± 66.23^{a}	330.83 ± 54.05^{a}	202.83 ± 26.00^{ab}

C:N and N:P ratios in soils differed among shrubs and gaps. Although C:N ratio was always lower than 10, soils under *R. sphaerocarpa* and *S. oppositifolia* had the highest values while soils under *A. barrelieri* and bare ground areas had the lowest (Table 2). N:P ratios ranged 81.1–354.1, the highest values being under *R. sphaerocarpa, S. oppositifolia*, and *T. hyemalis,* and the lowest under *A. barrelieri* (Table 2).

3.2. Plant physiological status

The different shrub species showed different physiological status ($F_{4,24} = 6.31$, $P < 0.001$) regarding *RWC, Fv/Fm, A* and g_s. Summer drought was more stressful for *A. barrelieri*, *H. articulata* and *T. hyemalis*, with RWC values between 0.4 and 0.6, while *L. arborescens*, *R. sphaerocarpa* and both *Salsola* species remained above 0.7 ($F_{6,22} = 19.32$, $P < 0.001$; Fig. 1a). The *Fv/Fm* values showed a trend similar to *RWC*. *L. arborescens* and *R. sphaerocarpa* had values close to the optimum (~0.8; sensu Maxwell and Johnson, 2000) while *T. hyemalis* had the lowest ($F_{6,22} = 11.04$, $P < 0.001$; Fig. 1b).

Early morning gas exchange rates varied among species (Fig. 1c–1d; $F_{6,22} = 12.75$, $P < 0.01$ for *A*, and $F_{6,22} = 2.84$, $P < 0.05$ for g_s). *T. hyemalis* and *A. barrelieri* displayed the lowest *A* values as gas exchange was almost arrested in these species, while *S. oppositifolia*, *H. articulata* and *L. arborescens* showed the highest *A* values. Leaf conductance to water vapor, gs, was marginally higher in *L. arborescens* than in *T. hyemalis*.

3.3. AMF communities

Significant differences among shrubs were found in the percent root length colonized by AMF ($F_{6,10} = 3.60$, $P < 0.05$) but no differences were detected regarding percent of arbuscular and vesicular colonization of roots ($F_{6,10} = 1.83$, $P = 0.19$; $F_{6,10} = 0.50$, $P < 0.80$). The highest AMF colonization was found in *H. articulata*, *R. sphaerocarpa*, *T. hyemalis* and *S. oppositifolia* (ranging 40.21% ± 4.03–31.67% ± 3.10) and the lowest in *L. arborescens* (15.88% ± 4.11).

AMF genetic diversity differed among soil ($F_{7,31} = 4.86$, $P < 0.01$) and root ($F_{6,14} = 2.84$, $P = 0.05$) samples. The highest AMF genetic diversity was found in soils under *R. sphaerocarpa* and in gaps among shrubs, whereas the lowest diversity was found in soils beneath *A. barrelieri* and *H. articulata* (Fig. 2). The highest genetic diversity of AMF in roots was found in *A. barrelieri* and *S. genistoides* while *T. hyemalis*, *H. articulata* and *R. sphaerocarpa* had significantly lower values. Four out of seven shrub species had a higher AMF genetic diversity in their rhizosphere soil than in roots [i.e., *t*-tests of soil vs. roots was significant for *T. hyemalis*, *S. oppositifolia* and *R. sphaerocarpa* ($P < 0.05$), and marginally significant for *L. arborescens* ($P = 0.07$)].

Results of NMDS of soil TRF data separated AMF communities under *A. barrelieri* from the other shrub species and gaps. AMF communities under *T. hyemalis*, *R. sphaerocarpa*, *S. oppositifolia* and *S. genistoides* were closely placed (Fig. 3a), suggesting that these species harbour similar soil AMF communities. In roots, however, the NMDS of TRF data did not group AMF communities according to host species (Fig. 3b). AMF-TRF of roots and soils were clearly differentiated if pooled together in the NMDS analysis (Fig. 3c), suggesting differences between the AMF communities present in the soil and colonizing the roots.

Differences in AMF community composition in the soil rhizosphere of target species and gaps were apparent in the ANOSIM analysis ($R = 0.35$; $P < 0.01$). R statistic indicated that dissimilarity was higher among sample sites (target species and gaps) than within sites. However, we did not find differences in AMF community composition in roots of target species ($R = 0.08$ $P = 0.21$).

Principal components 1, 2 and 3 accounted for 76% of the total sample variance (39%, 23% and 14% for PC1, PC2 and PC3, respectively). The PCA showed that AMF genetic diversity in soils and roots had different trends (Fig. 4a). Soil AMF genetic diversity followed the same pattern as N and SOM but were opposite to P content, while AMF genetic diversity in roots had a trend opposite to N and SOM (Fig. 4a). Plant physiological variables tended to have the same trends as soil AMF genetic diversity, and opposite to root

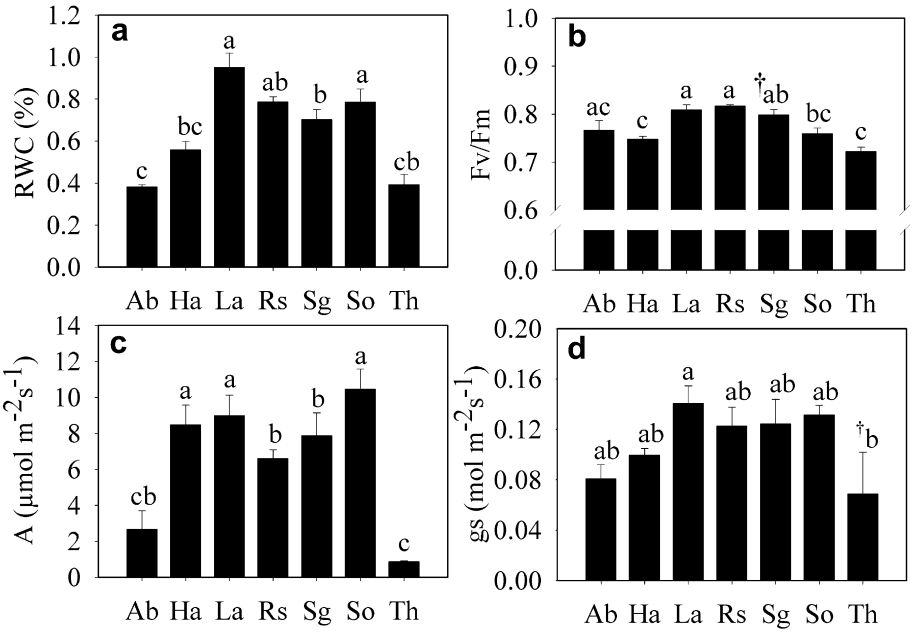

Fig. 1. Relative water content (RWC), maximum photochemical efficiency of photosystem II (Fv/Fm); leaf photosynthetic rate (A) and leaf conductance to water vapor (g_s) of different shrub species (mean ± 1SE; $n = 5$). Within each panel, bars with different letters indicate significant differences among species ($P < 0.05$); † marginal difference ($P = 0.08$). *Artemisia barrelieri* (Ab), *Hammada articulata* (Ha), *Launaea arborescens* (La), *Retama sphaerocarpa* (Rs), *Salsola genistoides* (Sg), *Salsola oppositifolia* (So), *Thymus hyemalis* (Th) and gaps among shrubs (Gp).

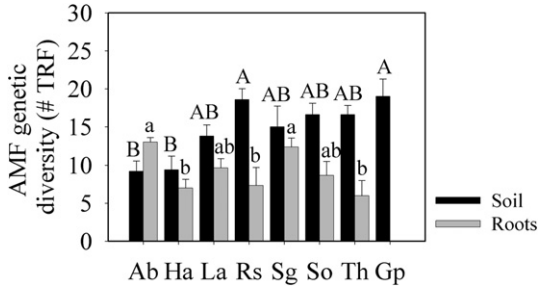

Fig. 2. Genetic diversity of AM communities. Bars are mean number of AMF-TRFs ± 1 SE in soil (solid bars; $n = 5$) and roots (grey bars; $n = 3$) beneath the canopy of seven shrubs species and in bare ground. Different capital case and low case letters indicate significant differences among species for soil and root samples, respectively (Tukey's and Fisher post-hoc test for soils and roots, respectively $P \leq 0.05$). Species abbreviation as in Fig. 1.

AMF genetic diversity (Fig. 4b). These results agree with the positive correlation found between soil AMF genetic diversity and plant RWC ($r = 0.31$; $P = 0.07$), as well as with soil N:P ratio ($r = 0.40$, $P < 0.05$), and SOM content ($r = 0.28$; $P = 0.08$). AMF genetic diversity in roots was inversely correlated to soil N:P ratio ($r = -0.61$; $P < 0.01$).

4. Discussion

Our findings suggest that AMF communities in the rhizosphere of different shrubs species are mainly influenced by soil nutrients content.

As expected, different shrub species generated different soil patches (resources islands) under their canopies and modified soil characteristics in such a way that may have affected the composition of AMF communities in soil. Some of the AMF communities established under shrubs species were similar among each other e.g., *R. sphaerocarpa*, *T. hyemalis*, *S. oppositifolia* and *S. genistoides*. Whereas the composition of AMF communities found under *A. barrelieri* and, to a lesser degree, under *H. articulata* largely differed from the AMF community under other target species and gaps. Since dispersal does not limit fungal distribution at short distances (Dumbrell et al., 2009; Lekberg et al., 2007) the patterns we observed are likely a consequence of plant specific effects on soil nutrient content. It has been shown that soil characteristics such as humidity (Wolfe et al., 2007), structure (Lekberg et al., 2007), fertility (Alguacil et al., 2010; Egerton-Warburton et al., 2007; Landis et al., 2004) and disturbance (Rodriguez-Echeverria and Freitas, 2006) influenced AMF community composition and distribution.

Contrary to our expectations, AMF communities in roots did not differ among shrub species. We chose seven species with contrasting characteristics such as photosynthetic strategy or root system, but all of them were shrubs characterized by forming fertility islands. It is likely that selecting other functional types such as herbs or annual species may have revealed greater differences. In addition, number of samples collected was low and only large differences among species could be detected. These results are, however, in line with recent reports that did not find host specificity (Santos et al., 2006) and suggest that AMF association with plants may occur at higher hierarchical level such as the plant functional type or ecological group (Opik et al., 2009) and not necessarily at the species level (van der Heijden and Horton, 2009).

Soils under our shrubs and in gaps between them had low C:N ratio values (<10) indicating N was mineralized and available for plant use (Osler and Sommerkorn, 2007; Swift et al., 1979). C:N values were higher under *R. sphaerocarpa* than under *A. barrelieri* due to higher litter contribution, which in turn contributed to higher SOM contents under *R. sphaerocarpa*. By contrast, soils showed quite a wide range in N:P values. Although the critical N:P ratio that determines the switch between N or P limitation depends on many different factors (Güsewell and Gessner, 2009), SOM decomposition at high N:P ratios is driven by fungi and limited by P while it is driven by bacteria and limited by N at low N:P values (Güsewell and Gessner, 2009). Our results showed that soils with low P availability for plants (as under *R. sphaerocarpa*, *S. oppositifolia* and *T. hyemalis*) had more homogeneous AMF communities, likely

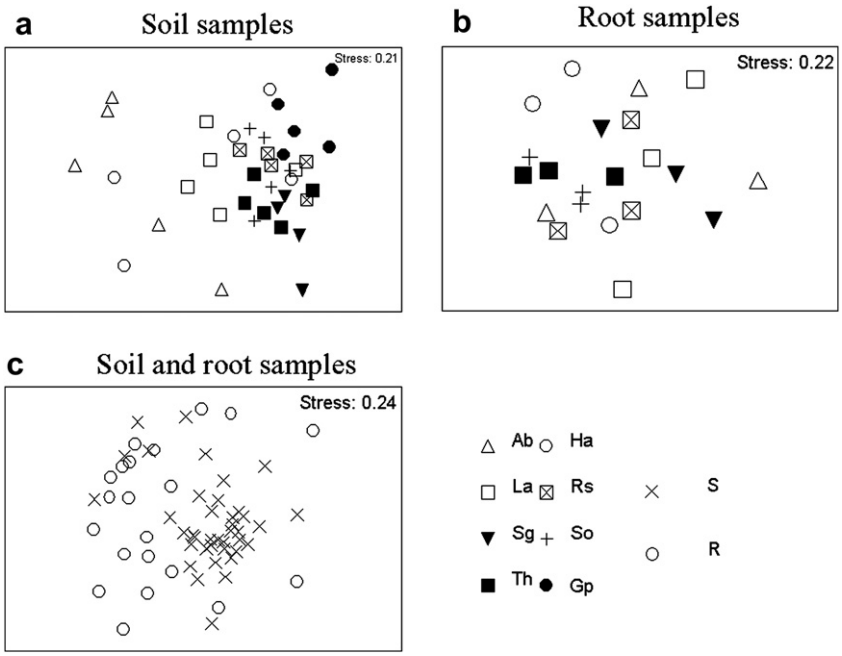

Fig. 3. Non-metric MDS ordination of AM-TRF based on Sørensen similarity matrix (Primer-E). a) patterns of soil AM-TRFs, stress = 0.21; b) patterns of root AM-TRFs, stress = 0.22, and; c) patterns of root and soil AM-TRFs, stress = 0.24. Species abbreviations as in Fig. 1; S for soils and R for roots.

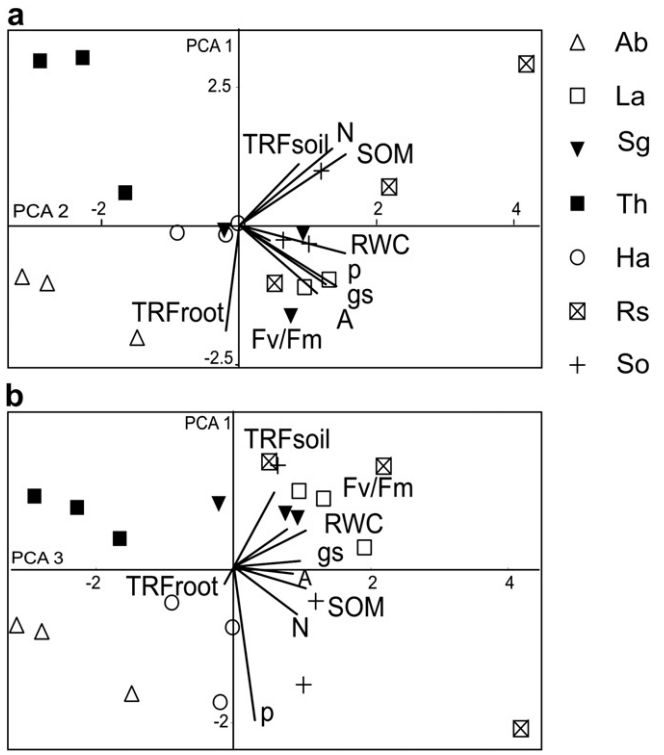

Fig. 4. Results of principal component analysis (PCA) of soil nutrients, physiological variables, and AMF-TRFs. Samples distribution are represented along a) PC1 vs. PC2 and along b) PC1 vs. PC3. Vector orientation indicates direction and vector length indicates rate of increase. Species abbreviations as in Fig. 1.

because AMF ability for P uptake led to competitive exclusion of other soil microorganisms, thus increasing homogeneity as well as diversity of the AMF community. The opposite occurs in soils with low N:P values (as in *A. barrelieri*), that had larger AMF dissimilarities and lower genetic diversity. Here bacteria might exclude fungi and reduce homogeneity and diversity of the AMF community. Overall, we found changes in AMF community composition and diversity among fertile islands and gaps driven by changes in soil nutrient content and their ratios. This results support the idea that resource islands may increase landscape-level diversity by supporting distinct suites of fungi (Bennett et al., 2009).

The fact that AMF diversity was higher in soils than in roots may be explained by the seasonal nature of AMF communities (Clark et al., 2009; Liu et al., 2009) and the influence of neighbouring species (Casper and Castelli, 2007; Mummey et al., 2005). AMF propagules of formerly active symbionts could remain in the soil in addition to propagules of current symbionts. Also, resource islands enhance growth of herbaceous species which might contribute as well to the AMF community found in the understory of shrubs. Both sources (former symbioses and contribution by herbs) may also explain the high AMF diversity found in bare soil, which is surprising since AMF are obligate symbionts. Our data are supported by Johnson et al. (2003), who found higher soil AMF diversity in seedlings growing in bare soil than in soil with *Festuca ovina* monocultures or with a mixture of different species. Therefore, AMF communities in bare areas might come from different points in the landscape leading to higher soil AMF diversity.

In harsh, arid environments community dynamics is rather governed by positive interactions (Callaway and Mahall, 2007; Flores and Jurado, 2003; Pugnaire et al., 1996a; Tirado and Pugnaire, 2003). There are facilitation processes mediated by AMF in arid ecosystems, such as higher availability of AMF inocula in resource islands than in gaps (Azcón-Aguilar et al., 2003; Bashan et al., 2000); greater plant tolerance to drought (Marulanda et al., 2007), and in short, enhanced growth rate of semi-arid shrubs (Al-Karaki et al., 2004; Goicoechea et al., 2004; Marulanda et al., 2007; Querejeta et al., 2007; Sanchez-Blanco et al., 2004). These processes may influence AMF–plant feedbacks. We observed that when AMF genetic diversity in soil was high, RWC tended also to increase; we cannot, however, assume a direct relationship between these variables since most likely soil fertility improved plant water status. Then, we could not detect any link between AMF communities and plant physiological characteristics. Still we noticed that AMF genetic diversity in roots decreased with AMF genetic soil diversity. AMF genetic diversity was higher in roots growing in soils poor in N, supporting results from Santos et al. (2006). The low AMF genetic diversity in *R. sphaerocarpa* roots contrasted with the high variability in soil and its high AMF root length colonization, which could point to feedback dynamics. However, experimental studies would be needed to confirm this point and to explain the strength and direction of feedbacks in patchy environments.

5. Conclusions

Our results showed that soil nutrient contents influenced soil AMF distribution in patchy arid environments. AMF communities under shrubs with soil fertile islands characterized by high N:P values (low P availability) were more homogeneous and diverse than in those shrub fertile islands with low N:P values. In addition, we generally found higher AMF genetic diversity in soil than in roots, likely because of the influence of factors such as neighbouring plants or seasonal dynamics of this semi-arid environment.

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References

Alguacil, M.M., Roldan, A., Torres, M.P., 2009. Complexity of semiarid gypsophilous shrub communities mediates the AMF biodiversity at the plant species level. Microbial Ecology 57, 718–727.
Alguacil, M.D., Lozano, Z., Campoy, M.J., Roldan, A., 2010. Phosphorus fertilisation management modifies the biodiversity of AM fungi in a tropical savanna forage system. Soil Biology and Biochemistry 42, 1114–1122.
Al-Karaki, G., McMichael, B., Zak, J., 2004. Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza 14, 263–269.
Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26, 32–46.
Azcón-Aguilar, C., Palenzuela, J., Roldán, A., Bautista, S., Vallejo, R., Barea, J.M., 2003. Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrublands. Applied Soil Ecology 22, 29–37.
Bardgett, R.D., Lovell, R.D., Hobbs, P.J., Jarvis, S.C., 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. Soil Biology and Biochemistry 31, 1021–1030.
Barrs, H.D., 1968. Determination of water deficits in plant tissues. In: Kozlowski, T.T. (Ed.), Water Deficits and Plant Growth. Academic Press, New York, pp. 235–368.
Bartelt-Ryser, J., Joshi, J., Schmid, B., Brandl, H., Balser, T., 2005. Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. Perspectives in Plant Ecology Evolution and Systematics 7, 27–49.
Bashan, Y., Davis, E.A., Carrillo-Garcia, A., Linderman, R.G., 2000. Assessment of VA mycorrhizal inoculum potential in relation to the establishment of cactus

seedlings under mesquite nurse-trees in the Sonoran Desert. Applied Soil Ecology 14, 165–175.
Bennett, L.T., Kasel, S., Tibbits, J., 2009. Woodland trees modulate soil resources and conserve fungal diversity in fragmented landscapes. Soil Biology and Biochemistry 41, 2162–2169.
Bever, J.D., 1999. Dynamics within mutualism and the maintenance of diversity: inference from a model of interguild frequency dependence. Ecology Letters 2, 52–62.
Bever, J.D., 2003. Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytologist 157, 465–473.
Bremner, J., 1965. Methods of Soils Analysis. American Society of Agronomy, Madison, USA.
Callaway, R.M., Aschehoug, E.T., 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. Science 290, 521–523.
Callaway, R.M., Mahall, B.E., 2007. Plant ecology – family roots. Nature 448, 145–147.
Callaway, R.M., Walker, L.R., 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. Ecology 78, 1958–1965.
Casper, B.B., Castelli, J.P., 2007. Evaluating plant–soil feedback together with competition in a serpentine grassland. Ecology Letters 10, 394–400.
Casper, B.B., Bentivenga, S.P., Ji, B.M., Doherty, J.H., Edenborn, H.M., Gustafson, D.J., 2008. Plant–soil feedback: testing the generality with the same grasses in serpentine and prairie soils. Ecology 89, 2154–2164.
Clark, N.M., Rillig, M.C., Nowak, R.S., 2009. Arbuscular mycorrhizal fungal abundance in the Mojave Desert: seasonal dynamics and impacts of elevated CO_2. Journal of Arid Environments 73, 834–843.
Clarke, K.R., 1993. Non parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18, 117–143.
Dumbrell, A.J., Nelson, M., Helgason, T., Dytham, C., Fitter, A.H., 2009. Relative roles of niche and neutral processes in structuring a soil microbial community. ISME Journal 4, 337–345.
Egerton-Warburton, L.M., Johnson, N.C., Allen, E.B., 2007. Mycorrhizal community dynamics following nitrogen fertilization: a cross-site test in five grasslands. Ecological Monographs 77, 527–544.
Flores, J., Jurado, E., 2003. Are nurse–protege interactions more common among plants from arid environments? Journal of Vegetation Science 14, 911–916.
Goicoechea, N., Merino, S., Sanchez-Diaz, M., 2004. Management of phosphorus and nitrogen fertilization to optimize Anthyllis–Glomus–Rhizobium symbiosis for revegetation of desertification semiarid areas. Journal of Plant Nutrition 27, 1395–1413.
Goicoechea, N., Merino, S., Sanchez-Diaz, M., 2005. Arbuscular mycorrhizal fungi can contribute to maintain antioxidant and carbon metabolism in nodules of *Anthyllis cytisoides L.* subjected to drought. Journal of Plant Physiology 162, 27–35.
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 V14, 111–117.
Güsewell, S., Gessner, M.O., 2009. N:P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. Functional Ecology 23, 211–219.
Haase, P., Pugnaire, F.I., Clark, S.C., Incoll, L.D., 1999. Environmental control of canopy dynamics and photosynthetic rate in the evergreen tussock grass *Stipa tenacissima*. Plant Ecology 145, 327–339.
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.
Helgason, T.H.O.R., Merryweather, J.W., Young, J.P.W., Fitter, A.H., 2007. Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. Journal of Ecology 95, 623–630.
Hesse, P., 1971. A Text Book of Soil Chemical Analysis London, GB.
Holmgren, M., Scheffer, M., Huston, M.A., 1997. The interplay of facilitation and competition in plant communities. Ecology 78, 1966–1975.
Johnson, D., Vandenkoornhuyse, P.J., Leake, J.R., Gilbert, L., Booth, R.E., Grime, J.P., Young, J.P.W., Read, D.J., 2003. Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. New Phytologist 161, 503–515.
Johnson, N.C., Wilson, G.W.T., Bowker, M.A., Wilson, J.A., Miller, R.M., 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proceedings of the National Academy of Sciences of the United States of America 107, 2093–2098.
Kardol, P., Cornips, N.J., van Kempen, M.M.L., Bakx-Schotman, J.M.T., van der Putten, W.H., 2007. Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. Ecological Monographs 77, 147–162.
Klironomos, J.N., McCune, J., Hart, M., Neville, J., 2000. The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. Ecology Letters 3, 137–141.
Klironomos, J.N., 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417, 67–70.
Landis, F.C., Gargas, A., Givnish, T.J., 2004. Relationships among arbuscular mycorrhizal fungi, vascular plants and environmental conditions in oak savannas. New Phytologist 164, 493–504.
Lekberg, Y.L.V.A., Koide, R.T., Rohr, J.R., Aldrich-Wolfe, L.A.U.R., Morton, J.B., 2007. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. Journal of Ecology 95, 95–105.
Liu, W.T., Marsh, T.L., Cheng, H., Forney, L.J., 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Applied and Environmental Microbiology 63, 4516–4522.
Liu, Y., He, L., An, L.Z., Helgason, T., Feng, H.Y., 2009. Arbuscular mycorrhizal dynamics in a chronosequence of *Caragana korshinskii* plantations. FEMS Microbiology Ecology 67, 81–92.
Ludwig, J.A., Wilcox, B.P., Breshears, D.D., Tongway, D.J., Imeson, A.C., 2005. Vegetation patches and runoff-erosion as interacting ecohydrological processes in semiarid landscapes. Ecology 86, 288–297.
Marulanda, A., Porcel, R., Barea, J., Azcón, R., 2007. Drought tolerance and antioxidant activities in lavender plants colonized by native drought-tolerant or drought-sensitive *Glomus* species. Microbial Ecology 54, 543–552.
Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence–a practical guide. Journal of Experimental Botany 51, 659–668.
McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, G.A., 1990. A new method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. New Phytologist 115, 495–501.
Miranda, J.D., Padilla, F.M., Martínez-Vilalta, J., Pugnaire, F.I., 2010. Woody species of a semi-arid community are only moderately resistant to cavitation. Functional Plant Biology 37, 828–839.
Moora, M., Zobel, M., 2010. Arbuscular mycorrhizae and plant–plant interactions. Impact of invisible world on visible patterns. In: Pugnaire, F.I. (Ed.), Positive Plant Interactions and Community Dynamics. CRC Press, Boca Raton FL, USA , pp. 79–98.
Mummey, D.L., Rillig, M.C., 2007. Evaluation of LSU rRNA-gene PCR primers for analysis of arbuscular mycorrhizal fungal communities via terminal restriction fragment length polymorphism analysis. Journal of Microbiological Methods 70, 200–204.
Mummey, D.L., Rillig, M., Holben, W.E., 2005. Neighboring plant influences on arbuscular mycorrhizal fungal community composition as assessed by T-RFLP analysis. Plant and Soil 271, 83–90.
Munne-Bosch, S., Jubany-Mari, T., Alegre, L., 2003. Enhanced photo- and antioxidative protection, and hydrogen peroxide accumulation in drought-stressed *Cistus clusii* and *Cistus albidus* plants. Tree Physiology 23, 1–12.
Niering, W.A., Whittaker, R.H., Lowe, C.H., 1963. The Saguaro: a population in relation to environment. Science 142, 15–23.
Opik, 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.
Osler, G.H.R., Sommerkorn, M., 2007. Toward a complete soil C and n cycle: incorporating the soil fauna. Ecology 88, 1611–1621.
Padilla, F.M., Miranda, J.D., Jorquera, M.J., Pugnaire, F.I., 2009. Variability in amount and frequency of water supply affects roots but not growth of arid shrubs. Plant Ecology 204, 261–270.
Peinado, M., Alcaraz, F., Martínez-Parras, J.M., 1992. In: Cramer, J. (Ed.), Vegetation of Southeastern Spain Berlin.
Perez-Pujalte, A., 1987. Mapa de suelos de Tabernas. Escala 1:100000 y memoria. Proyecto LUCDEME ICONA – CSIC – Ministerio de Agricultura, Pesca y Alimentación.
Phillips, J.M., Hayman, D.S., 1970. Improved procedure for clearing roots and staining parasites and vesicular-arbuscular mycorrhizal fungi for rapid assesment of infection. Transation British Mycology Society 55, 158–161.
Pugnaire, F.I., Haase, P., Incoll, L.D., Clarl, S.C., 1996a. Response of the tussok grass *Stipa tenacissima* to watering in a semiarid environment. Functional Ecology 10, 265–274.
Pugnaire, F.I., Haase, P., Puigdef Bregas, J., 1996b. Facilitation between higher plant species in a semiarid environment. Ecology 17, 1420–1426.
Pugnaire, F.I., Armas, C., Valladares, F., 2004. Soil as a mediator in plant-plant interactions in a semi-arid community. Journal of Vegetation Science 15, 85–92.
Querejeta, J.I., Allen, M.F., Alguacil, M.M., Roldan, A., 2007. Plant isotopic composition provides insight into mechanisms underlying growth stimulation by AM fungi in a semiarid environment. Functional Plant Biology 34, 680–691.
Redecker, D., 2002. Molecular identification and phylogeny of arbuscular mycorrhizal fungi. Plant and Soil 244, 67–73.
Reynolds, H.L., Packer, A., Bever, J.D., Clay, K., 2003. Grassroots ecology: plant–microbe–soil interactions as drivers of plant community structure and dynamics. Ecology 84, 2281–2291.
Rodriguez-Echeverria, S., Freitas, H., 2006. Diversity of AMF associated with *Ammophila arenaria* ssp. arundinacea in Portuguese sand dunes. Mycorrhiza 16, 543–552.
Sanchez-Blanco, M.J., Fernandez, T., Morales, M.A., Morte, A., Alarcón, J.J., 2004. Variations in water status, gas exchange, and growth in *Rosmarinus officinalis* plants infected with *Glomus deserticola* under drought conditions. Journal of Plant Physiology 161, 675–682.
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.
Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis. Academic Press., San Diego, CA, USA.
Swift, M.J., Heal, O.W., Anderson, J.M., 1979. Decomposition processes in terrestrial ecosystems Oxford.
Tilman, D., Lehman, C.L., Thomson, K.T., 1997. Plant diversity and ecosystem productivity: theoretical considerations. Proceedings of the National Academy of Sciences of the United States of America 94, 1857–1861.
Tirado, R., Pugnaire, F.I., 2003. Shrub spatial aggregation and consequences for reproductive success. Oecologia 136, 296–301.
Trappe, J.M., 1987. Phylogenetic and Ecologic Aspects of Mycotrophy in the Angiosperms from an Evolutionary Standpoint. CRC Press, Florida, USA.

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 V8, 203–206.

van der Heijden, M.G.A., Horton, T.R., 2009. Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. Journal of Ecology 97, 1139–1150.

van der Heijden, M.G.A., Boller, T., Wiemken, A., Sanders, I.R., 1998a. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79, 2082–2091.

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

Van Der Putten, W.H., 2003. Plant defense belowground and spatiotemporal processes in natural vegetation. Ecology 84, 2269–2280.

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. Molecular Ecology 7, 879–887.

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.

Walker, C., Trappe, J.M., 1993. Names and epithets in the Glomales and Endogonales. Mycological Research 97, 339–344.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.

Watanabe, F.S., Olsen, S.R., 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Science Society of American Journal 29, 677–678.

Wolfe, B.E., Mummey, D.L., Rillig, M., Klironomos, J.N., 2007. Small-scale spatial heterogeneity of arbuscular mycorrhizal fungal abundance and community composition in a wetland plant community. Mycorrhiza 17, 175–183.