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Survival of inocula and native AM fungi species associated with shrubs in a degraded Mediterranean ecosystem

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Abstract

Reconstitution of the potential of soil mycorrhizal inoculum is a key step in revegetation programs for semiarid environments. We tested the effectiveness of inoculation with native arbuscular mycorrhizal (AM) fungi or with an allochthonous AM fungus, *Glomus claroideum*, with respect to the growth of four shrub species, the release of mycorrhizal propagules in soil, within and outside the canopy, and the improvement of soil structural stability. Two years after outplanting, the mixture of native endophytes was more effective than, for *Olea europaea* subsp. *sylvestris*, *Retama sphaerocarpa* and *Rhamnus lycioides*, or equally as effective as, for *Pistacia lentiscus*, the non-native AM fungus *Glomus claroideum*, with respect to increasing shoot biomass and foliar NPK contents. The increases in glomalin concentration and structural stability produced by inoculation treatments in the rhizosphere soil of the all shrub species, except *R. lycioides*, ranged from about 55 to 173% and 13 to 21%, respectively. The mixture of native AM fungi produced the highest levels of mycorrhizal propagules in soil from the center of the canopy of *P. lentiscus* and *R. lycioides*, while plants of *O. europaea* and *R. sphaerocarpa* inoculated with *G. claroideum* had more mycorrhizal propagules than did those inoculated with the mixture of native fungi. The number of mycorrhizal propagules in soil outside the canopy of the four shrub species was 5–35 times higher in inoculation treatments than in soil of the non-inoculated plants.

Keywords: Aggregate stability; Glomalin; Mediterranean shrub species; Mycorrhizal propagules; Revegetation strategies

1. Introduction

The reintroduction of native plant species is a widely used practice for reclaiming degraded lands in semiarid Mediterranean areas. Revegetation programs based on the planting of native shrubs help conserve biodiversity and prevent erosion and desertification of arid and semiarid landscapes (Bochet et al., 1998; Requena et al., 2001). Low potential of mycorrhizal inoculum can slow revegetation because mycorrhizae increase soil fertility, by altering nutrient cycling (Requena et al., 2001). Mycorrhizae may help plants to thrive in arid conditions (Nelson and Safir, 1982), by increasing the supply of nutrients, particularly P, to the plant (Toro et al., 1997), improving soil aggregation in eroded soils (Caravaca et al., 2002), and reducing water stress (Augé, 2001). Recent studies have indicated that arbuscular mycorrhizal (AM) fungi produce glomalin, that stabilizes soil aggregates (Wright and Anderson, 2000). Thus, AM fungi are essential components of ecosystems, for both revegetation of degraded lands and maintenance of soil structure, thereby reducing the risks of erosion and desertification.

Indigenous inoculum levels of AM fungi in degraded semiarid Mediterranean ecosystems are considered as being low (Azcón-Aguilar et al., 2003; Palenzuela et al., 2002; Requena et al., 1996). Thus, successful reafforestation programs use mycorrhizal inoculum. The identification of efficient AM fungi is a prerequisite to inoculation programs, since the level of compatibility between host plants and AM fungi depends upon the species involved (Roldán et al., 1992; Smith and Read, 1997). Requena et al. (2001) showed that a community of mixed native AM fungi could be managed to improve the outplanting performance of

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Anthyllis cytisoides in a degraded Mediterranean ecosystem. These results suggest that revegetation could be regulated by the natural microflora. However, it is unclear whether inoculation with native AM fungi would be equally effective for other shrub species, such as *Olea europaea* L. subsp. *sylvestris*, *Pistacia lentiscus* L., *Retama sphaerocarpa* (L.) Boissier, and *Rhamnus lycioides* L. Furthermore, there are no data on survival of AM fungal propagules introduced and on their dispersal outside the canopy.

The objectives of this study were: (1) to determine if inoculation with native AM fungi or with an allochthonous AM fungus, *Glomus claroideum*, improves the inoculum potential of the soil and promotes dispersal of AM fungal propagules outside the canopy of *O. europaea*, *P. lentiscus*, *R. sphaerocarpa* and *R. lycioides*, and (2) to determine the effect of inoculation with native AM fungi or with *G. claroideum*, on soil structure and on the establishment of these shrub species in a semiarid Mediterranean area.

2. Materials and methods

2.1. Study sites

The experimental site was in the El Picarcho range of Murcia (Southeast Spain) (1°10′W and 38°23′N). The climate is semi-arid Mediterranean, with an annual rainfall of 315 mm and a mean annual temperature of 20 °C during the experiment. The topography of the area is mainly flat and slopes do not exceed 6%. Plant cover is sparse (less than 20% canopy cover) and degraded by grazing and logging. In this area, dwarf shrubs less than 1 m high, such as *Rosmarinus officinalis* and *Stipa tenacissima* grass are common, constituting more than 98% of plant cover. A bare soil surface is common between the patches of plants. The soil is a Typic Petrocalcid (Soil Survey Staff, 1999), developed from limestone, with a silt loam texture, containing 3 $\mu g\ g^{-1}$ available P, 0.7 $g\ kg^{-1}$ total N, 22.2 $g\ kg^{-1}$ total organic C, and 702 $\mu g\ g^{-1}$ extractable K. The infectivity of native AM fungi in the bare soil from the experimental site was low, 0.24 (0.12–0.49) infective propagules per gram dry soil.

2.2. Plants and mycorrhizal treatments

Small shrubs such as *Olea europaea* subsp. sylvestris, *Pistacia lentiscus*, *Retama sphaerocarpa*, and *Rhamnus lycioides*, that belong to the climax vegetation of the experimental area, were considered. They are adapted to drought conditions and used frequently in the revegetation of semiarid disturbed lands.

The non-native mycorrhizal fungus was *Glomus claroideum* Schenck and Smith, obtained from the collection of the experimental field station of Zaidín, Granada (EEZ 24).

The mixture of native AM fungi were isolated from the El Picarcho range. We collected soil from the rhizosphere of plants already growing near the experimental site, and used *Sorghum bicolor* L. as trap to enrich the inoculum. AM fungi present in this native inoculum were: *Glomus geosporum* (Nicol. and Gerd.) Walker (EEZ 31), *Glomus albidum* Walker and Rhodes (EEZ 39), *Glomus microaggregatum* Koske, Gemma and Olexia (EEZ 40), *Glomus constrictum* Trappe (EEZ 42), *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe (EEZ 43), *Glomus coronatum* Giovannetti (EEZ 44), *Glomus intraradices* Schenck and Smith (EEZ 45), and a *Glomus* sp. (EEZ 46). Both native and non-native inocula were subjected to a most probable number (MPN) test to determine potential infectivity and to equalize application doses. Both sources of inoculum had a potential infectivity of about 35 infective propagules per gram inoculum.

AM fungal inoculum consisted of a mixture of sorghum rhizospheric soil containing spores, hyphae and mycorrhizal root fragments. Seedlings of test plant species were transplanted into a growth substrate, consisting of peat (*Sphagnum* type) and cocopeat from *Cocos nucifera* L. wastes (1:1, v:v), and AM inoculum (5%, v/v). The same amount of autoclaved inoculum was added to control plants, supplemented with a culture filtrate (0.45 μm mesh) to restore the microbial populations accompanying the mycorrhizal fungi (Jeffries and Barea, 2000). Inoculated and non-inoculated seedlings were grown with watering for 8 months under nursery conditions, without any fertilizer.

2.3. Experimental design and layout

The experiment was conduced as four independent one-factor factorials (one per plant species) with five replicate plots. The factor had three levels: non-inoculated, inoculated with *G. claroideum*, and inoculated with the mixture of native AM fungi. In early January 2000, an area of 5000 m^2 was established. Planting holes 40 cm wide, 40 cm long and 30 cm deep were dug by hand. Seedlings of the four selected shrub species (inoculated and non-inoculated) were planted in individual holes, using a randomized design within each replicate plot at least 1 m apart between holes, with 3 m between plots. Fifteen seedlings per factor level per replicate plot of each shrub species were planted (225 plants per shrub species).

2.4. Sampling procedures

Plants were harvested after 6 months in June 2000 and after 2 years in December 2001. At each harvest, five plants per treatment, each from a different plot, were taken. Basal stem diameter and plant height were measured and fresh and dry (105 °C, 5 h) weights of shoots and roots obtained. Plant tissues were ground before chemical analysis. The foliar concentrations of nitrogen, phosphorus and potassium were determined after digestion in nitric-perchloric acid (5:3) for 6 h at 210 °C. Plant P was determined colorimetrically according to Murphy and Riley (1962), plant N was determined by the Kjeldahl method, and plant K was

estimated by flame photometry (Schollemberger and Simon, 1954).

After 2 years, soil samples were collected in five replicates with one per plot, i.d., 15 soil samples per plant species. Each sample consisted of five bulked soil subsamples (150 cm^3 soil cores) arbitrarily collected at 0–20 cm depth from five planting holes (considered center canopy soil). The same number of soil samples was simultaneously taken from outside the canopy of the seedlings (at a distance of 40 cm from the planting holes).

2.5. Mycorrhizal determinations

The percentage of root length colonized by AM fungi was calculated by the gridline intersect method (Giovannetti and Mosse, 1980) after staining with trypan blue (Phillips and Hayman, 1970).

Mycorrhizal potential in soil was measured by a dilution technique (Sieverding, 1991) that allows calculation of the MPN of mycorrhizal propagules able to develop colonization units on the root of a test plant. It is based on the probability of whether or not an infection is established in serially diluted media, consisting of sand, vermiculite and soil from experimental area autoclaved. Fourfold dilution series with 5 replications per dilution level were carried out with soil pasteurized by steaming for 1 h on three consecutive days. Seedlings of *S. bicolor* were placed individually into each of five replicates of each soil dilution treatment. After one month of growth, infection was recorded in root fragments cleared and stained with trypan blue (Phillips and Hayman, 1970). The number of infective propagules was calculated according to the formula from Fisher and Yates (1970):

$$d = 10^{\log(x \log a - k)}$$

where x is the mean number of cups with infection, a the factor of dilution, and k a constant from table VIII of Fisher and Yates for fourfold dilutions.

Glomalin was extracted from soil samples with 20 mM sodium citrate (pH 7.0) at a rate of 0.25 g of soil in 2 ml of buffer. Extracts were autoclaved at 121 °C for 30 min (Wright and Anderson, 2000); then centrifuged at $10{,}000 \times g$ for 15 min to remove soil particles. Protein in the supernatant was determined by the Bradford dyebinding assay using bovine serum albumin as the standard.

Soil aggregate stability was determined following the procedure described by Lax et al. (1994) that measures the percentage of soil aggregates between 0.2 and 4 mm that remains stable after being submitted to a simulated rainfall of 150 ml with energy of 270 Jm^{-2}.

2.6. Statistical analysis

Percentage colonization was arcsin-transformed, and the other parameters were log-transformed to compensate for heterogeneity of variance, before analysis of variance. The effect of mycorrhizal inoculation on measured variables was tested by a one-way analysis of variance, and comparisons among means were made with the Least Significant Difference (LSD) test, calculated at $P<0.05$. Statistical procedures were carried out with the software package SPSS 10.0 for Windows (Ferrán Aranaz, 1996).

3. Results and discussion

3.1. Changes in plant growth, nutrient acquisition, and mycorrhizal colonization

At the time of planting, shoot dry weights of the mixed native AM fungi- (0.34 ± 0.02 g dw) or *G. claroideum*-colonized (0.38 ± 0.04 g dw) *O. europaea* plants, the *G. claroideum*-colonized (0.36 ± 0.03 g dw) *P. lentiscus* plants and the *R. lycioides* plants inoculated with the mixture of native AM fungi (0.20 ± 0.02 g dw) were slightly greater than those of the non-inoculated *O. europaea*, *P. lentiscus* and *R. lycioides* plants (0.29 ± 0.01, 0.29 ± 0.02 and 0.14 ± 0.02 g dw, respectively). However, there were no significant differences in growth between non-inoculated (0.52 ± 0.09 g dw) and inoculated (on average, 0.48 ± 0.03 g dw) *R. sphaerocarpa* seedlings prior to transfer to the field.

Six months after outplanting, plants of all four species inoculated with *G. claroideum* or with the mixture of native AM fungi had significantly greater height, basal diameter, and shoot dry weight relative to the controls, except for the *G. claroideum*/*R. sphaerocarpa* combination (Table 1). Only in combination with *O. europaea* was *G. claroideum* more effective than the mixture of native AM fungi with respect to increasing the shoot biomass. It is worth noting that the effects of native AM fungi on the growth of shrub species recorded after 6 months relate to a critical growth stage during revegetation, particularly in Mediterranean semiarid areas.

Two years after outplanting, plant survival was about 90% for all treatments and plant species. The mycorrhizal inoculation treatments had various effects on the performance of the four shrub species. There were no significant differences in the growth parameters between *P. lentiscus* seedlings inoculated with the two mycorrhizal inoculation treatments, but, on average, shoot dry weight of inoculated seedlings was about 220% greater than for non-inoculated seedlings at the end of the growth period (Table 2). The mixture of native AM fungi was more effective with respect to increasing shoot dry weight of *O. europaea*, *R. sphaerocarpa*, and *R. lycioides* plants than was *G. claroideum*. This result is consistent with other studies which showed that native AM fungi are important contributors to ecosystem productivity (Requena et al., 2001; Van der Heijden et al., 1998). In this context, the strains of AM fungi recovered from our experimental site can be considered 'ecotypes' (Jeffries and Barea, 2000),

Table 1
Effect of inoculation with the non-native AM fungus *G. claroideum* and with a mixture of native AM fungi on growth parameters and root colonization in *O. europaea* subsp. *sylvestris*, *P. lentiscus*, *R. sphaerocarpa* and *R. lycioides* plants after 6 months in the field

Plant/treatment	Height (cm)	BD (mm)	Shoot (g dw)	Root (g dw)	Colonized root length (%)
Olea europaea					
Control	13.5a[a]	3.2a	0.60a	0.75a	4.0a
Mixture AM fungi	26.3b	4.7b	1.48b	1.34b	57.0b
G. claroideum	31.5c	4.7b	1.91c	1.53b	60.0b
Pistacia lentiscus					
Control	13.4a	2.8a	0.89a	0.37a	13.0a
Mixture AM fungi	15.0b	3.2b	1.30b	0.42ab	68.0b
G. claroideum	17.6c	3.4b	1.46b	0.52b	75.0b
Retama sphaerocarpa					
Control	35.7a	4.1a	2.16a	0.91a	4.0a
Mixture AM fungi	44.2b	5.4b	3.39b	1.51b	70.0b
G. claroideum	40.0ab	4.9b	2.55ab	1.22ab	67.0b
Rhamnus lycioides					
Control	13.3a	1.9a	0.40a	0.35a	9.0a
Mixture AM fungi	23.9c	3.1c	0.82b	0.63b	36.0c
G. claroideum	19.1b	2.6b	0.72b	0.49b	25.0b

[a] For plant species, values sharing the same letter are not significantly different ($P<0.05$) by the LSD test. BD = basal diameter.

which are physiologically and genetically adapted to the environment of the desertification-threatened Mediterranean ecosystems in Southeast Spain. Likewise, Van der Heijden et al. (1998) showed that the below-ground diversity of AM fungi is a major factor contributing to plant species composition, variability, productivity, and biodiversity in artificial microcosms and macrocosms. Our results also indicate that the inoculation treatment with the greatest number of AM fungi taxa, and therefore with the highest AM fungi biodiversity, resulted in the highest productivity and biomass yield.

If the shoot/root ratio reflects the degree of effectiveness of AM (Tobar et al., 1994), then *O. europaea* and *R. lycioides* responded significantly to inoculation with the mixture of native AM fungi (Table 2). Neither of the inoculation treatments had a significant effect on the shoot/root ratio of *P. lentiscus* or *R. sphaerocarpa*. However, plant development, biomass production, and nutrient concentrations and content, as affected by AM colonization, must be considered all together. Thus, total nutrient content can be taken as a representative parameter of mycorrhizal effectiveness, because it takes into account the well-balanced effects on nutrient acquisition and biomass production (Jarrel and Beverley, 1981). The highest effectiveness of the inoculation with the mixture of native AM fungi was confirmed also by the fact that the highest nutrient (NPK) contents were seen in shoot tissues of *O. europaea*, *R. sphaerocarpa* and *R. lycioides* plants

Table 2
Effect of inoculation with the non-native AM fungus *G. claroideum* and with a mixture of native AM fungi on growth parameters and root colonization in *O. europaea* subsp. *sylvestris*, *P. lentiscus*, *R. sphaerocarpa* and *R. lycioides* plants after 24 months in the field

Plant/treatment	Height (cm)	BD (mm)	Shoot (g dw)	Shoot/root	Colonized root length (%)
Olea europaea					
Control	11.5a[a]	5.2a	1.42a	1.27a	10.5a
Mixture AM fungi	29.3b	8.5b	16.46c	3.83c	85.0b
G. claroideum	26.5b	7.5ab	6.70b	1.93b	88.2b
Pistacia lentiscus					
Control	17.1a	4.7a	3.40a	3.15a	45.8a
Mixture AM fungi	23.3b	7.5b	10.42b	3.28a	69.8b
G. claroideum	24.1b	7.5b	11.53b	3.29a	71.6b
Retama sphaerocarpa					
Control	31.0a	5.1a	9.46a	2.70a	10.9a
Mixture AM fungi	56.3b	10.2c	29.16c	2.67a	78.8b
G. claroideum	51.0b	7.7b	17.81b	3.11a	73.0b
Rhamnus lycioides					
Control	11.0a	2.5a	0.55a	1.83a	8.2a
Mixture AM fungi	24.3c	5.4c	8.14c	4.23c	68.8b
G. claroideum	17.5b	4.3b	1.76b	2.84b	67.5b

[a] For plant species, values sharing the same letter are not significantly different ($P<0.05$) by the LSD test. BD = basal diameter.

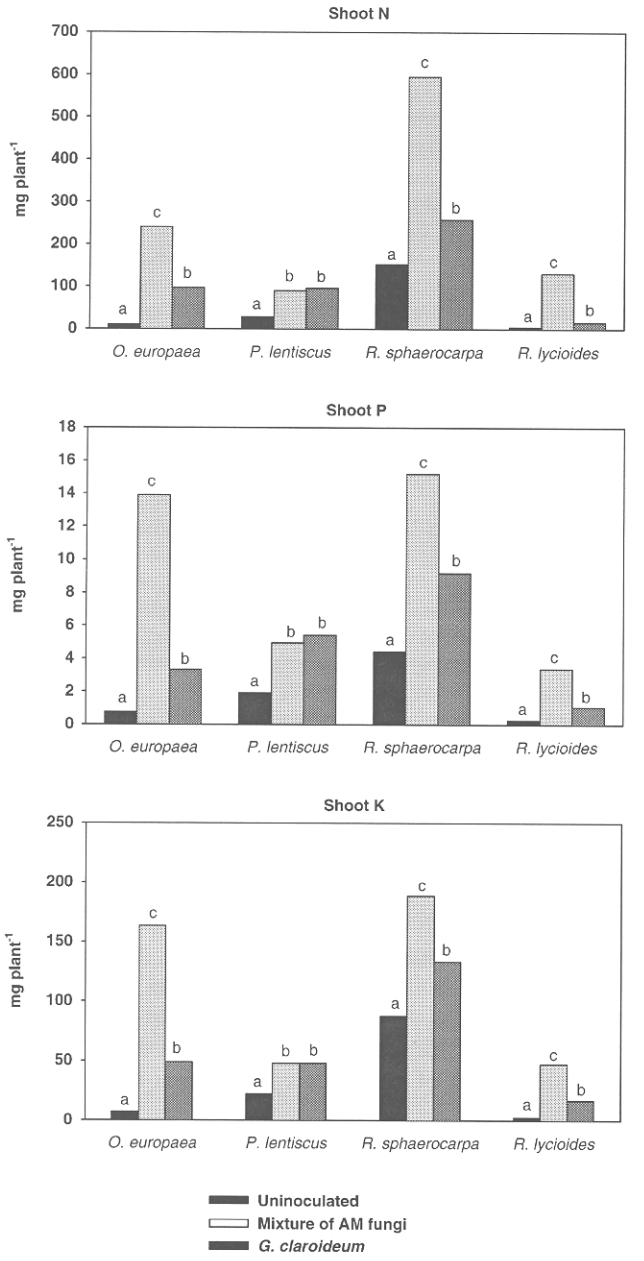

Fig. 1. Effect of inoculation with the non-native AM fungus *G. claroideum* and with a mixture of native AM fungi on total nutrient uptake in *O. europaea* subsp. *sylvestris*, *P. lentiscus*, *R. sphaerocarpa* and *R. lycioides* plants after 24 months in the field. Values with the same letter are not significant different at $P<0.05$, according to the LSD test.

(Fig. 1). There were no significant differences in nutrient contents between *P. lentiscus* plants inoculated with the different fungal treatments. These results corroborated, under field conditions, the well-known role of AM inoculation in plant nutrition.

3.2. Changes in the AM status and aggregate stability in soil within and outside the canopy of the four shrub species

The number of mycorrhizal propagules that colonizes roots is an important measure of the mycorrhizal inoculum potential of a soil (Brundrett, 1996). As expected (Eom et al., 2000), the four shrubs differed in their ability to enrich the soil with mycorrhizal propagules (Table 3). *O. europaea* plants had the highest capacity for enhancement of the development of AM propagules within the canopy by natural colonization. The mycorrhizal potential of the center canopy soil associated with the non-inoculated plants was significantly less than for the inoculated plants, except for *O. europaea*, and the mycorrhizal potential outside the canopy of non-inoculated plants was even less than in the bare soil [0.24 (0.12–0.49) infective propagules per gram dry soil].

The mycorrhizal inoculation significantly increased the number of 'infective' mycorrhizal propagules in the soil under the plant canopies of the four target shrub species. The mixture of native AM fungi produced the highest mycorrhizal potential in the canopy soil of *P. lentiscus* and *R. lycioides*, while inoculation with *G. claroideum* was more effective for *O. europaea* and *R. sphaerocarpa*. The number of 'infective' mycorrhizal propagules in the soil outside the canopy of the four shrub species also increased following the mycorrhizal inoculation treatments, reaching values 5–35 times higher than in the soil of the non-inoculated plants. The major dispersal of propagules outside the canopy was correlated strongly with the mean MPN of 'infective' mycorrhizal propagules within the canopy ($r^2=0.635$, $P<0.001$), with percentages of root colonization ($r^2=0.695$, $P<0.001$), and with root biomass ($r^2=0.535$, $P<0.001$) of the four shrub species. Mycorrhiza-inoculated *R. sphaerocarpa* was the most effective combination regarding propagule enrichment of the soil outside the canopy and also had the highest root biomass production. With the exception of *R. sphaerocarpa*, the dispersal of AM propagules outside the canopy of the plants did not depend on the assayed mycorrhizal inoculation treatment. This dispersive effect may have been due to the capacity of the extramatrical AM mycelium to release AM propagules. Mycelia extending from mycorrhizal roots usually are the main source of inoculum in semiarid and arid ecosystems, while the importance of soil-borne spores is less recognized (Bashan et al., 2000; Palenzuela et al., 2002). Moreover, there are usually only very low numbers of viable spores in soil from eroded ecosystems (Azcón-Aguilar et al., 2003).

Some shrub species in degraded semiarid ecosystems create 'resource islands' or 'fertility islands', which are points of high biological activity dispersed in a heterogeneous landscape (Reynolds et al., 1990), where the facilitation among plants may be highly fostered (Callaway, 1997). AM propagules in the rhizosphere of mycotrophic shrub species can contribute to the 'nurse' role of these species (Azcón-Aguilar et al., 2003). We have reported recently that, of the relict natural vegetation, currently growing in patches in the target ecosystem, *O. europaea* and *R. sphaerocarpa* had a higher natural mycorrhizal potential in their rhizosphere than *R. lycioides* and *P. lentiscus* (Caravaca et al., 2003). The dispersal of AM propagules to

Table 3
Effect of inoculation with the non-native AM fungus *G. claroideum* and with a mixture of native AM fungi on the development of arbuscular mycorrhizal propagules in soil, glomalin concentration and percentage of stable aggregates within and outside the canopy of *O. europaea* subsp. *sylvestris*, *P. lentiscus*, *R. sphaerocarpa* and *R. lycioides* after 24 months in the field

Plant/treatment	MPN of AM propagules (g^{-1} dry soil)		Glomalin ($\mu g\ g^{-1}$ soil)		Aggregate stability (%)	
	Center canopy soil	Outer canopy soil	Center canopy soil	Outer canopy soil	Center canopy soil	Outer canopy soil
Olea europaea						
Control	1.39(0.68–2.86)[a]	0.12(0.06–0.25)	1567a[b]	2024a	59.9a	55.3a
Mixture AM fungi	1.97(0.96–4.05)	0.87(0.41–1.79)	2437b	1977a	55.6a	51.4a
G. claroideum	4.75(2.92–9.77)	0.85(0.40–1.75)	3470c	2226a	67.4b	64.0b
Pistacia lentiscus						
Control	0.43(0.21–0.88)	0.14(0.07–0.29)	2029a	1570a	46.6a	45.4a
Mixture AM fungi	3.43(1.67–7.05)	0.87(0.41–1.79)	3611b	1833a	55.6b	59.4b
G. claroideum	1.89(0.92–3.89)	0.62(0.29–1.27)	3591b	1518a	57.3b	45.0a
Retama sphaerocarpa						
Control	0.15(0.07–0.31)	0.05(0.02–0.10)	1278a	1518a	63.7a	56.5a
Mixture AM fungi	2.52(1.23–5.18)	1.18(0.55–2.43)	3575b	1681a	73.7b	66.2b
G. claroideum	3.58(1.74–7.36)	1.77(0.83–3.64)	3392b	1823a	63.4a	66.8b
Rhamnus lycioides						
Control	0.09(0.04–0.19)	0.05(0.02–0.10)	2000a	2362a	59.1a	60.0a
Mixture AM fungi	3.35(1.63–6.89)	0.61(0.29–1.25)	2174a	2337a	57.9a	60.2a
G. claroideum	1.90(0.92–3.91)	0.66(0.31–1.36)	3655b	2015a	54.1a	57.8a

MPN, most probable number.
[a] In parenthesis, lower and upper limit of confidence at 95% probability.
[b] For plant species, values sharing the same letter are not significantly different ($P<0.05$) by the LSD test.

soil outside the canopy of the target plant species, following the mycorrhizal inoculation treatments could contribute to the development and functions of the resource islands, for example by enhancing early integration of mycotrophic seedlings into the system. This indicates an important ecosystem function for the mycotrophic target plant species, namely provision of available AM propagules as a source of inoculum for mycotrophic seedlings in directed revegetation processes.

Finally, inoculation with *G. claroideum* or a mixture of native AM fungi significantly increased glomalin concentration and the percentage of stable aggregates in the rhizosphere soil of all shrub species except *R. lycioides* (Table 3). Glomalin is a glycoprotein that increases the hydrophobicity of soil particles and forms soil aggregates (Wright and Anderson, 2000). Roots and associated mycorrhizal hyphae also may form a three-dimensional network that aggregates small soil particles. Thus, increased aggregate stability of the rhizosphere soil of the inoculated shrub species could have resulted from the production of glomalin by AM fungi. It is worth noting that the effect of mycorrhizal inoculation on soil aggregate stability extended to soil outside the canopy of *O. europaea*, *P. lentiscus*, and *R. sphaerocarpa*. Increasing soil structural stability favors the establishment and viability of a stable plant cover, which, in turn, improves the soil quality. So, our results emphasize the importance of the mycorrhizal symbiosis as a determinant of the sustainability and stability of the ecosystem.

In conclusion, our medium-term results demonstrate that mycorrhizal inoculation based on the management of native AM fungi, might be an effective strategy in revegetation of semiarid areas with shrub species. Likewise, the inoculation with *G. claroideum* or a mixture of native endophytes was found to be suitable for promotion of mycorrhizal propagule production and dispersal in the soil around plants, and for improvement of soil structural stability, although the effectiveness of native AM fungi depended on the host shrub species. The effects of the enhanced mycorrhizal propagule availability outside the canopy of the shrubs on the establishment of new plants in the area remain to be determined.

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