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Establishment of shrub species in a degraded semiarid site after inoculation with native or allochthonous arbuscular mycorrhizal fungi

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Abstract

The re-establishment of native shrub species in the Mediterranean basin serves to restore the characteristic biodiversity and to prevent the processes of erosion and desertification in semiarid areas. A field experiment was carried out in an abandoned semiarid agricultural Mediterranean area to assess the effectiveness of mycorrhizal inoculation, with a mixture of native arbuscular mycorrhizal (AM) fungi or an allochthonous AM fungus (*Glomus claroideum*), on the establishment of *Olea europaea* subsp. *sylvestris* L., *Pistacia lentiscus* L., *Retama sphaerocarpa* (L.) Boissier and *Rhamnus lycioides* L. seedlings in this area. One year after planting, shoot biomass of inoculated *O. europaea* and *P. lentiscus* seedlings was greater, by about 630% and 300%, respectively, than that of non-inoculated plants. Shoot biomass of *G. claroideum*-colonised *R. sphaerocarpa* plants was significantly greater than that of seedlings inoculated with the mixed native AM fungi after 12 months. The increase of *R. lycioides* growth due to inoculation with native AM fungi was significantly greater than that of *G. claroideum*-colonised seedlings during the same growth period. Inoculation with a mix of native AM fungi was the most effective treatment for increasing shoot biomass and N, P and K contents in shoot tissues of *R. lycioides* seedlings. The mixture of native AM fungi was the most effective with respect to colonisation of the roots of *O. europaea* and *R. lycioides*, but the native AM fungi and *G. claroideum* achieved similar levels of colonisation in *P. lentiscus* and *R. sphaerocarpa*. The use of native mycorrhizal potential as a source of AM inoculum may be considered a preferential inoculation strategy to guarantee the successful re-establishment of native shrub species in a semiarid degraded soil.

Keywords: *Glomus claroideum*; *Olea europaea* subsp. *sylvestris*; *Pistacia lentiscus*; *Retama sphaerocarpa*; *Rhamnus lycioides*; Revegetation

1. Introduction

The abandonment of agricultural land has contributed drastically to the acceleration of soil degradation and desertification processes because of the lack or scarcity of plant cover, particularly in the semiarid areas of southeastern Spain. Environmental changes caused by the loss of plant cover include increased soil erosion, decreases in water infiltration and organic matter content and loss of soil structure and micro-organisms (García et al., 1997). Natural revegetation tends to be slow in arid and semiarid

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Mediterranean ecosystems, where the scarcity of water frequently limits or prevents plant establishment and growth. Revegetation programmes based on planting shrubs, rather than tree species, would not only assist in the conservation of biodiversity in these areas, but would also help prevent the erosion and the desertification of arid and semiarid landscapes (Bochet et al., 1998; Requena et al., 2001). It has been proven that shrubs may increase soil–water infiltration rate and water holding capacity by improving the soil structure, through reducing the raindrop impact and adding organic matter from leaf litter. Some shrub species such as *Retama sphaerocarpa* (L.) Boissier have the additional benefit of being able to fix nitrogen, thus constituting an N input into the ecosystem (Requena et al., 2001). In this context, the use of shrub species alone, such as *Olea europaea* L. subsp. *sylvestris*, *Pistacia lentiscus* L., *R. sphaerocarpa* and *Rhamnus lycioides* L., in revegetation programmes in the drier areas has been encouraged recently by the Common Agricultural Policy of the European Union. *O. europaea*, *P. lentiscus*, *R. sphaerocarpa* and *R. lycioides* are low-growing shrubs, well-adapted to water stress conditions, which belong to the natural succession in certain plant communities of semiarid Mediterranean ecosystems in the southeast of Spain (Barea et al., 1992). However, knowledge of revegetation strategies with such shrub species is still very scarce.

The successful re-establishment of native plants in degraded soils such as semiarid ecosystems may be limited by the low density of mycorrhizal propagules, which represent a significant factor for soil fertility by governing the cycles of major plant nutrients (Requena et al., 2001). There is evidence that mycorrhizae help plants to thrive in arid conditions (Nelson and Safir, 1982) by increasing the supply of nutrients to the plant (particularly P) (Querejeta et al., 1998), improving soil aggregation in eroded soils (Caravaca et al., 2002) and reducing water stress (Augé, 2001). A rehabilitation approach for these areas, based on revegetation strategies using arbuscular mycorrhizal (AM) inoculations, must begin with the evaluation of the mycorrhizal status of the soil (Requena et al., 1996). If the native inoculum potential of AM fungi in the soil is inadequate to support a revegetation programme, then it may be necessary to reinforce or replace it by appropriate mycorrhizal inoculation technologies. Requena et al. (1996) found low indigenous inoculum levels of AM fungi in a semiarid ecosystem in the southeast of Spain. The selection of efficient AM fungi is a key prerequisite in inoculation programmes, since there are different levels of compatibility between host plants and AM fungi (Roldán et al., 1992; Smith and Read, 1997). Recently, the long-term effectiveness of a managed community of microbial symbionts on the re-establishment of *Anthyllis cytisoides* in a degraded ecosystem has been demonstrated (Requena et al., 2001).

The objectives of this study were: (1) to determine the viability of *O. europaea*, *P. lentiscus*, *R. sphaerocarpa* and *R. lycioides* as target species in soil revegetation programmes for an abandoned semiarid agricultural Mediterranean area, following the mycorrhizal inoculation of the seedlings with an allochthonous AM fungus, *Glomus claroideum*, or with a mixture of native AM fungi, and (2) to select the most efficient treatment of mycorrhizal inoculation for stimulating growth and nutrient uptake of each of the four shrub species in the target ecosystem.

2. Materials and methods

2.1. Study sites

The experimental area was located on the El Picarcho range in the province of Murcia (southeast Spain) (coordinates: 1°10′W and 38°23′N). The climate is semiarid 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%. The climax vegetation was dominated by the shrubs, *O. europaea* subsp. *sylvestris*, *P. lentiscus*, *R. sphaerocarpa* and *R. lycioides*, which were selected as target species. The plant cover is sparse (less than 20% canopy cover) and degraded due to ancient grazing and logging. In this area, dwarf shrubs, (<1 m high) such as *R. officinalis* and *S. tenacissima* grass, are very common, constituting more than 98% of the plant cover. Bare soil surfaces are abundant between the patches of plants. The soil is a Petrocalcic Xerosol (F.A.O, 1988), developed from limestone, with a silt loam texture. Some characteristics of the soil are shown in Table 1.

Table 1
Some characteristics of the soil used for the revegetation experiment

Characteristics	Mean	Standard error
pH (H_2O)	8.54[a]	0.01
Electrical conductivity (1:5) ($\mu S\,cm^{-1}$)	237	4
Total organic carbon ($g\,kg^{-1}$)	22.2	0.7
Water soluble carbon ($\mu g\,g^{-1}$)	134	13
Total nitrogen ($g\,kg^{-1}$)	0.7	0.1
Available P ($\mu g\,g^{-1}$)	3	0
Extractable K ($\mu g\,g^{-1}$)	702	47
AM infective propagules (MPN g^{-1} dry soil)[b]	0.24	0.1

[a] Each value is the mean of five soil samples.
[b] MPN: most probable number.

2.2. Plants and mycorrhizal treatments

The plants used, *O. europaea* subsp. *sylvestris*, *P. lentiscus*, *R. sphaerocarpa* and *R. lycioides*, are four representative shrub species from semiarid scrublands in southeast Spain. They are also well-adapted to water stress conditions and, therefore, frequently used in the revegetation of semiarid disturbed lands.

The mycorrhizal fungi used were either *G. claroideum* Schenck & Smith (EEZ 24) or a mixture of endophytes isolated from Cieza (SE Spain), a semiarid area where the target plants grow naturally, consisting of *Glomus geosporum* (Nicol. & Gerd.) Walker (EEZ 31), *Glomus albidum* Walker & Rhodes (EEZ 39), *Glomus microaggregatum* Koske, Gemma & Olexia (EEZ 40), *Glomus constrictum* Trappe (EEZ 42), *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe (EEZ 43), *Glomus coronatum* Giovannetti (EEZ 44), *Glomus intraradices* Schenck & Smith (EEZ 45) and a *Glomus* sp. (EEZ 46). The acronym EEZ refers to Estación Experimental Zaidín, Granada (Spain).

AM fungal inoculum consisted of a mixture of rhizospheric soil from the experimental area; spores, hyphae and mycorrhizal root fragments from cultures of *Sorghum* sp. containing either *G. claroideum* or all eight AM fungal taxa, thus representing the natural diversity in the site. Once germinated, seedlings were transplanted into the growing substrate, consisting of peat and cocopeat (1:1, v/v). The corresponding arbuscular mycorrhizal inoculum was applied at a rate of 5% (v/v). The same amount of autoclaved mixture of the inocula was added to control plants, supplemented with a filtrate (<20 μm) of culture to provide the microbial populations accompanying the mycorrhizal fungi. Inoculated and non-inoculated seedlings were grown for 8 months under nursery conditions without any fertiliser treatment by Paisajes del Sur Ltd. (Granada, Spain).

2.3. Experimental design and layout

The experiment was conducted as four independent one-factor factorials (one per plant species) with five replication blocks. The factor had three levels: non-inoculation, inoculation with *G. claroideum* and inoculation with the mixture of native AM fungi. In early January 2000, an area of 1200 m^2 was mechanically prepared with a subsoiler. Three rows (1 m wide, 25 m long, 3 m apart) were established. Seedlings of the four selected shrub species (inoculated and non-inoculated) were planted in individual holes, at least 1 m apart in a single row and with 3 m between blocks. At least 15 seedlings per factor level per replication block of each shrub species were planted (225 plants per shrub species).

The experiment was carried out under strictly natural conditions, without any watering or fertiliser treatments.

2.4. Sampling and laboratory procedures

One year after planting, five rhizosphere soil samples (defined as soil strongly adhering to roots and collected at 0–4 mm from the root surface) of each treatment were collected (one per block, 15 soil samples, in total, per plant species). Each sample consisted of five bulked subsamples (200 cm^3 soil cores), randomly collected at 0–20 cm depth in the rhizospheres of five individual plants. Every 3 months after planting, five plants (one per block) of each treatment were also harvested, excavating manually a hole 40 cm wide, 40 cm long and 40 cm deep. For mycorrhizal assays, three subsamples from the upper, middle and lower root system were taken. Sampling was based on root colour and morphology to get a mixed age sample and to avoid woody roots. Basal stem diameters and heights of plants were measured with callipers and rulers.

Fresh and dry (105 °C, 5 h) weights of shoots and roots were recorded. 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 Kjeldhal method and plant K was estimated by flame photometry (Schollemberger and Simon, 1954).

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

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous extract. Total soil nitrogen was determined by the Kjeldhal method, and total soil organic C was measured according to Yeomans and Bremner (1989). Available soil P was extracted with sodium bicarbonate and determined colorimetrically according to Murphy and Riley (1962). Extractable (with ammonium acetate) K was determined by flame photometry.

For measurement of the mycorrhizal potential in the soil selected for the revegetation experiment, a dilution technique (Sieverding, 1991) was followed. This method consisted of making serial dilutions of the rhizosphere soil with soil pasteurised by steaming for 1 h on 3 consecutive days. Seedlings of *Sorghum* sp. used as a test plant were placed individually into each of the five replicates of each soil dilution treatment. After 15 days of growth, the number of entry points of AM fungi per root length was calculated after clearing and staining with trypan blue (Phillips and Hayman, 1970). This method allows calculation of the most probable number (MPN) of mycorrhizal propagules able to develop colonisation units on the root of a test plant.

2.5. Statistical analysis

Mycorrhizal inoculation effects on measured variables were tested by a one-way analysis of variance, and comparisons among means were made using the least significant difference (LSD) multiple range test, calculated at $P < 0.05$. Statistical procedures were carried out with the software package Statgraphics for Windows 7.0.

Table 2
Effect of inoculation with the allochthonous AM fungus *G. claroideum* and with a mixture of native AM fungi on physical–chemical and nutrient properties of the rhizospheres of *O. europaea* subsp. *sylvestris*, *P. lentiscus*, *R. sphaerocarpa* and *R. lycioides*

	pH (H_2O)	EC ($\mu S\,cm^{-1}$)	TN ($g\,kg^{-1}$)	Available P ($\mu g\,g^{-1}$)	Extractable K ($\mu g\,g^{-1}$)
O. europaea subsp. *sylvestris*					
C	8.31 a	225 a	1.3 a	22 a	360 a
M	8.30 a	228 a	1.3 a	16 a	392 ab
G	8.31 a	250 a	1.2 a	19 a	426 b
P. lentiscus					
C	8.17 a	206 a	1.0 b	22 a	315 a
M	8.14 a	241 a	1.0 b	31 b	339 a
G	8.23 a	210 a	0.8 a	28 ab	305 a
R. sphaerocarpa					
C	8.16 ab	239 a	1.4 a	10 ab	424 a
M	8.18 b	234 a	1.3 a	5 a	411 a
G	8.08 a	232 a	1.2 a	11 b	415 a
R. lycioides					
C	8.35 a	253 a	1.7 a	6 a	311 a
M	8.34 a	246 a	1.9 a	6 a	403 b
G	8.21 a	250 a	2.0 a	7 a	373 b

EC: electrical conductivity; TN: total nitrogen; C: plants non-inoculated; M: plants inoculated with a mixture of native arbuscular mycorrhizal fungi; G: plants inoculated with *G. claroideum*. For plant species, values sharing the same letter are not significantly different ($P < 0.05$) by the LSD test.

3. Results

3.1. Changes in soil physical–chemical and nutrient properties

Inoculation with *G. claroideum* or the mixture of native AM fungi had no significant effect on the physical–chemical properties (pH and electrical conductivity) of the rhizosphere soil of the four shrub species (Table 2). The mycorrhizal inoculation treatments had small, but differing, effects on the nutrient levels in the rhizospheres. The rhizosphere of *G. claroideum*-colonised *P. lentiscus* had lower total N concentration than that of non-inoculated plants and plants inoculated with the mixture of native AM fungi. The concentration of available P was higher in the rhizosphere of *P. lentiscus* seedlings inoculated with the mixture of native AM fungi, with respect to that of non-inoculated plants. For individual shrub species, the highest concentration of extractable K was found in the rhizosphere of *G. claroideum*-colonised

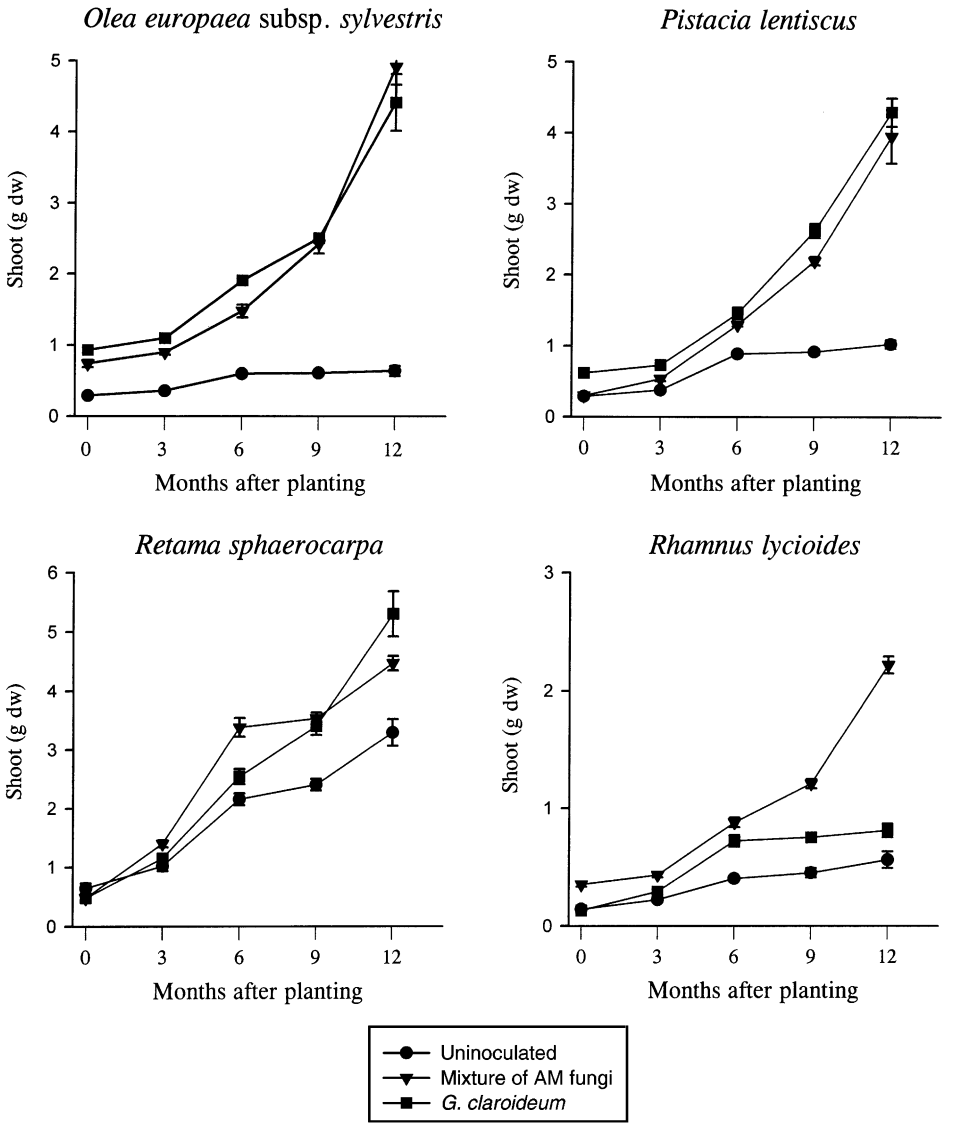

Fig. 1. Shoot dry weight of *O. europaea* subsp. *sylvestris*, *P. lentiscus*, *R. sphaerocarpa* and *R. lycioides* seedlings, subjected to inoculation with the allochthonous AM fungus *G. claroideum* and with a mixture of native AM fungi during a 1-year growth period. Bars represent standard errors.

O. europaea and in the rhizosphere of *R. lycioides* seedlings inoculated with either treatment.

3.2. Changes in plant growth, nutrient acquisition and mycorrhizal colonisation

One year after planting, the percentages of plant survival were about 90% for all treatments and plant species, and there were no significant differences between treatments. At the time of planting, shoot dry weight of the mixture of native AM fungi or *G. claroideum*-colonised *O. europaea*, *G. claroideum*-colonised *P. lentiscus* and *R. lycioides* plants inoculated with the mixture of native AM fungi were slightly greater than non-inoculated plants (Fig. 1). In contrast, there were no significant differences in growth between

non-inoculated and inoculated *R. sphaerocarpa* seedlings previous to planting in the field.

From the time of planting to the end of the initial 6-month growth period, the *O. europaea* plants inoculated with *G. claroideum* had a greater shoot biomass than non-inoculated plants and those inoculated with the native AM fungi (Fig. 1). However, from the autumn growth period onwards (9 months after planting), these differences in growth between the mycorrhizal inoculation treatments disappeared. Shoot biomass of the inoculated plants was significantly greater (about 630%) than that of non-inoculated plants after 12 months.

The effects of inoculation with *G. claroideum* and with the mixture of native AM fungi on *P. lentiscus* seedling growth were generally similar during the

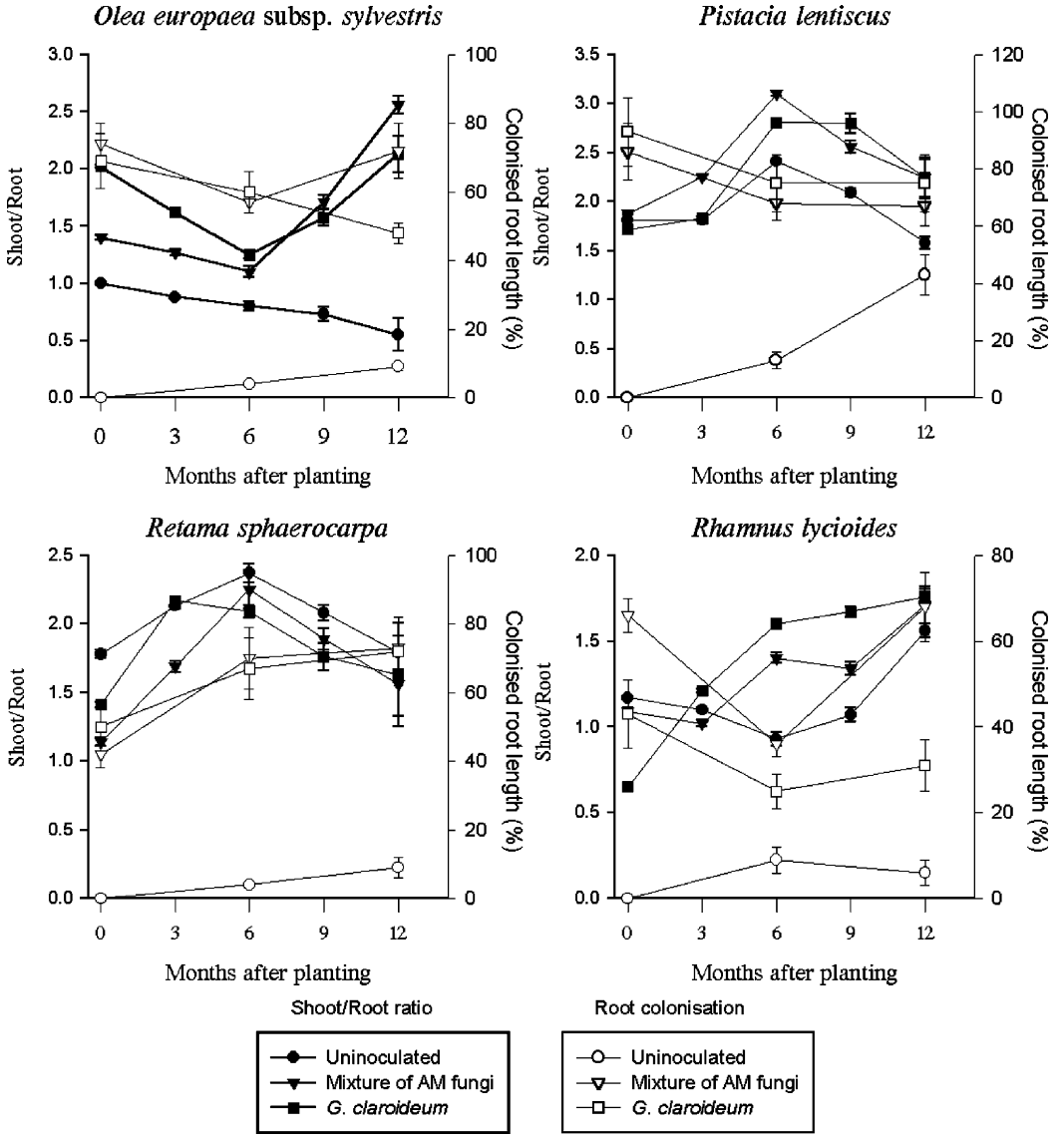

Fig. 2. Root colonisation and shoot/root ratio of *O. europaea* subsp. *sylvestris*, *P. lentiscus*, *R. sphaerocarpa* and *R. lycioides* seedlings subjected to inoculation with the allochthonous AM fungus *G. claroideum* and with a mixture of native AM fungi during a 1-year growth period. Bars represent standard errors.

entire growth period, both producing about 300% more shoot dry matter than the non-inoculated seedlings at the end of this period (Fig. 1).

In the revegetation experiment with *R. sphaerocarpa* seedlings, the mixture of native AM fungi was more effective in increasing shoot dry weight than *G. claroideum* (Fig. 1) during the spring growth period (from 3 months to 6 months after planting). These differences in seedling growth between mycorrhizal inoculation treatments decreased during the summer growth period, so that they had produced similar increases at 9 months. However, shoot dry weight of *R. sphaerocarpa* inoculated with the mixture of native AM fungi was lower than that for *G. claroideum*-colonised seedlings, 12 months after planting.

Inoculation with the mixture of native AM fungi significantly enhanced shoot dry weight of *R. lycioides* seedlings, compared with the non-inoculated seedlings and *G. claroideum*-colonised seedlings, and this difference tended to increase with time (Fig. 1). After 1 year, shoot dry weight of *R. lycioides* seedlings inoculated with the mixed native AM fungi was 293 and 172% greater than that of the non-inoculated seedlings and *G. claroideum*-colonised seedlings, respectively.

One year after planting, inoculation with *G. claroideum* and with the mixture of native AM fungi increased the shoot/root ratio of *O. europaea*, *P. lentiscus* and *R. lycioides* plants about 4.3, 1.4 and 1.1-fold in comparison to the control plants (Fig. 2). However, the shoot/root ratio of inoculated *R. sphaerocarpa* seedlings was significantly lower than that of non-inoculated plants. The mixture of native AM fungi was significantly more effective than *G. claroideum* with respect to the colonisation of the roots of *O. europaea* and *R. lycioides* after 1 year (Fig. 2). In contrast, both inoculation treatments produced a similar level of root colonisation in *P. lentiscus* and *R. sphaerocarpa* (on average, 72% of the root length was colonised in both plant species). The natural colonisation observed in the non-inoculated seedlings was higher in *P. lentiscus* than in the other shrub species.

Inoculation with *G. claroideum* or the mixture of native AM fungi stimulated N, P and K assimilation in shoot tissues of *O. europaea*, *P. lentiscus* and *R. sphaerocarpa* plants (Table 3). There were no significant differences in nutrient contents between seedlings inoculated with different fungal treatments, except that the *O. europaea* seedlings colonised by native AM fungi assimilated more P in shoots than did seedlings colonised by *G. claroideum*. As observed for the growth parameters, the highest N, P and K contents were in the *R. lycioides* plants inoculated with the mixture of native AM fungi. However, inocu-

Table 3
Effect of inoculation with the allochthonous AM fungus *G. claroideum* and with a mixture of native AM fungi on the total nutrient uptake in *O. europaea* subsp. *sylvestris*, *P. lentiscus*, *R. sphaerocarpa* and *R. lycioides* plants

	O. europaea subsp. *sylvestris*			*P. lentiscus*			*R. sphaerocarpa*			*R. lycioides*		
	0 month	6 months	12 months	0 month	6 months	12 months	0 month	6 months	12 months	0 month	6 months	12 months
Nitrogen (mg per plant)												
C	2.20 a	7.22 a	4.80 a	2.57 a	14.06 a	8.35 a	6.38 a	17.76 a	52.69 a	0.47 a	1.55 a	5.05 a
M	7.63 b	19.10 b	68.00 b	2.85 a	24.04 b	33.56 b	6.75 a	35.76 b	91.53 b	5.07 b	12.75 c	33.49 b
G	13.26 c	31.84 c	62.78 b	7.12 b	22.65 b	37.59 b	6.46 a	37.20 b	78.71 ab	1.57 a	7.93 b	7.27 a
Phosphorus (mg per plant)												
C	0.08 a	0.15 a	0.36 a	0.43 a	0.55 a	0.57 a	0.41 a	0.50 a	0.97 a	0.06 a	0.11 a	0.29 a
M	0.57 b	0.82 b	4.00 c	0.63 a	1.23 b	2.44 b	0.53 a	1.66 b	2.42 b	0.26 b	0.39 b	1.38 b
G	0.68 b	1.07 c	2.76 b	2.21 b	1.13 b	2.82 b	0.39 a	1.14 ab	2.75 b	0.07 a	0.30 b	0.44 a
Potassium (mg per plant)												
C	1.70 a	4.68 a	3.52 a	1.76 a	6.01 a	3.91 a	5.10 a	12.78 a	15.59 a	0.52 a	2.42 a	2.09 a
M	6.16 b	10.11 b	34.90 b	1.68 a	10.37 b	27.29 b	6.10 a	25.11 b	26.71 b	3.62 b	5.17 b	6.48 b
G	6.87 b	12.89 b	27.35 b	6.00 b	10.49 b	24.62 b	3.97 a	22.89 b	16.13 b	2.00 a	4.45 b	2.39 a

C: plants non-inoculated; M: plants inoculated with a mixture of native arbuscular mycorrhizal fungi; G: plants inoculated with *G. claroideum*. For plant species, values sharing the same letter are not significantly different ($P < 0.05$) by the LSD test.

lation with *G. claroideum* had no significant effect on N, P and K contents in the shoots of *R. lycioides*.

4. Discussion

The inoculation of seedlings, with an allochthonous AM fungus or a mixture of native AM fungi, stimulated significantly the production of shoot biomass by the four target shrub species, *O. europaea*, *P. lentiscus*, *R. sphaerocarpa* and *R. lycioides*, selected for revegetation of a semiarid Mediterranean area. These results confirm the requirement of mycorrhizal symbiosis for the successful establishment and growth of plants in a degraded area, where the mycorrhizal inoculum potential is low. However, the different shrub species showed different levels of response to inoculation with AM fungi. Assuming that the root/shoot ratio reflects the degree of AM effectiveness (Tobar et al., 1994), *O. europaea* was the plant with the greatest response to AM, reaching the most significant changes in shoot/root ratio followed by *P. lentiscus* and *R. lycioides*. Decreased shoot/root ratio in inoculated *R. sphaerocarpa* plants with respect to non-inoculated plants indicates low mycorrhizal activity in relation to plant biomass production. However, plant development, biomass production and nutrient concentration and content, as affected by AM colonisation, must all be considered together. Thus, total nutrient content can be taken as a representative parameter of mycorrhizal effectiveness because it takes into account the well-balanced effect of nutrient acquisition/biomass production. In all the four shrub species, mycorrhizal inoculation appeared effective in improving nutrient content, particularly in inoculated *O. europaea* plants.

The mycorrhizal inoculation treatments showed different levels of effectiveness in improving the performance of the four shrub species. In most of the species, the mixture of native AM fungi was equally as (in *O. europaea* and *P. lentiscus*) or even more (in *R. lycioides*) effective than the allochthonous AM fungus *G. claroideum* regarding increases in plant growth. Inoculation with the mixture of native AM fungi also markedly increased shoot biomass of *R. sphaerocarpa* and this increase was significantly above that of *G. claroideum*-colonised seedlings in the first stages of growth (6 months after planting), which are the most critical for revegetation, particularly in Mediterranean semiarid areas. These results correlate with other studies which show that native AM fungi are important contributors to plant biodiversity and ecosystem productivity (Van der Heijden et al., 1998; Requena et al., 2001). From three years after planting, Requena et al. (2001) observed significant improvement in the growth of *Anthyllis* plants inoculated with a mixture of native AM fungi. However, in our study the effect of native AM fungi on growth of shrub species was recorded in the short-term. Increased growth associated with AM infection in nutrient deficient soils has been attributed to enhanced nutrient uptake, especially N and P (Smith and Read, 1997; Toro et al., 1998). In all four shrub species, inoculated plants had higher N and P contents than non-inoculated plants. Arbuscular mycorrhiza help plants to compensate for deficiencies of immobile nutrients such as phosphate, by exploring a greater volume of soil than roots alone, and by providing a greater surface area for phosphate uptake (Jakobsen et al., 1992). In addition to increasing the surface area of infected roots, AM fungi take up phosphate much more rapidly than non-mycorrhizal roots and transfer it rapidly to the host plant (Joner et al., 2000).

The extent of mycorrhizal infection is of importance when studying the influence of AM fungi on the host plant. High infection may not be a prerequisite for growth responses in all plants inoculated with AM fungi. Thus, Requena et al. (1996) observed that native fungi were ineffective at promoting growth of *Anthyllis cytisoides* despite colonising a relatively large percentage of the roots. In our revegetation experiment, the effect of AM inoculation on shoot biomass was positively related to the colonisation level of the AM fungi tested in the roots of all shrub species except *R. sphaerocarpa*. However, the effect of mycorrhizal inoculation on plant growth not only depended on the extent of mycorrhizal infection but also on the shrub species infected. At the end of the growth period, the infection level of the mixture of native AM fungi was similar in the roots of all four shrub species, but the increase in shoot biomass was higher in *O. europaea* seedlings than in the other species.

AM fungi can enhance the growth of native shrub species in the short term, which in turn creates a more favourable environment for the development of ecosystem processes. Likewise, the inoculation of shrubs with AM fungi could reduce the substantial

amounts of N and P fertilisers required for optimal plant growth in degraded soils, with a considerable benefit for the environment (Barea et al., 1997). Finally, the use of native mycorrhizal potential as a source of AM inoculum may be considered a preferential inoculation strategy to guarantee the re-establishment success of native shrub species in a semiarid degraded soil.

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