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ORIGINAL PAPER

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Changes in rhizosphere microbial activity mediated by native or allochthonous AM fungi in the reafforestation of a Mediterranean degraded environment

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Abstract This study was carried out in a semiarid degraded 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. and *Retama sphaerocarpa* (L.) Boissier in this area. Associated changes in the soil microbiological properties and aggregate stability related to these AM inocula were also recorded. Eighteen months after planting, *G. claroideum* had increased available P in the rhizosphere of both shrub species. In general, both inoculation treatments increased water-soluble C and water-soluble and total carbohydrates, *G. claroideum* being the most effective inoculum, particularly in *R. sphaerocarpa*. The mixture of native AM fungi was the most effective treatment for increasing the aggregate stability of *R. sphaerocarpa* soil, while that of *O. europaea* was increased only by *G. claroideum*. Increased (dehydrogenase, urease, protease-BAA, acid phosphatase and β-glucosidase) enzyme activities, in particular of dehydrogenase and acid phosphatase, were recorded in the rhizosphere of both mycorrhizal shrub species. The mixture of native AM fungi was the most effective treatment for stimulating the growth of *O. europaea* and *R. sphaerocarpa* (11.6-fold and 3.3-fold, respectively, greater than control plants). The establishment of mycorrhizal shrub species favoured the reactivation of soil microbial activity, which was linked to an increase in aggregate stability.

Keywords Aggregate stability · Enzyme activities · *Glomus claroideum* · *Olea europaea* subsp. *sylvestris* · *Retama sphaerocarpa*

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Introduction

The Mediterranean area of southeast Spain is one of those most threatened by desertification processes due to scarce and irregular rainfall and a long, dry and hot summer. Under these environmental conditions, the loss of plant cover can cause a decrease in soil structure (with an increase in soil erosion), microbial activity, contents of available plant nutrients, and organic matter in soils (Caravaca et al. 2003c).

The establishment of autochthonous plant species is a widely used practice for reclaiming degraded lands in Mediterranean semiarid and arid areas, for restoring the biodiversity of these eroded areas and preventing the processes of erosion and desertification, in accordance with the agricultural policies of the European Union (Requena et al. 2001). In this context, we have used *Olea europaea* L. subsp. *sylvestris* and *Retama sphaerocarpa* (L.) Boissier, which are low-growing shrubs, well-adapted to water stress conditions, and which belong to the natural succession in certain plant communities of semiarid Mediterranean ecosystems in the southeast of Spain (Barea et al. 1992). Moreover, *R. sphaerocarpa* has the additional benefit of being able to fix nitrogen, thus constituting an N input into the ecosystem (Caravaca et al. 2003a).

In reafforestation programmes, inoculation of plants with microsymbionts, such as mycorrhizal fungi, helps plant establishment (Herrera et al. 1993) and can improve the physical, chemical and biological properties of soil (Carrillo-García et al. 1999). There is evidence that mycorrhizae help plants to thrive in arid conditions (Caravaca et al. 2003b) by increasing the supply of nutrients, such as P (Smith and Read 1997), improving soil aggregation in eroded soils (Caravaca et al. 2002) and reducing water stress (Augé 2001).

In these desertification-threatened areas, the native inoculum potential of AM fungi may disappear or, at least, be severely depleted and so it may be necessary to reinforce or replace it by appropriate inoculation (Azcón-Aguilar et al. 2003). 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) and the effectiveness of AM fungi depends on the plant species inoculated (Caravaca et al. 2003b). The use of native mycorrhizal potential may be considered a preferential inoculation strategy to guarantee the successful re-establishment of shrub species in degraded soil (Caravaca et al. 2003b), since native AM fungi are presumably physiologically and genetically adapted to the whole environment of the desertified ecosystems.

There is evidence that mycorrhizas affect the growth, composition and activity of microbial communities by altering root exudation (Wamberg et al. 2003). To date, it is not known whether different strains of AM fungi can produce different effects on the biochemical properties of soil, such as enzyme activities, or which AM fungi, native or allochthonous, are more effective. These facts should be very important when planning reafforestation programmes, since a more effective inoculum to improve the microbial properties of soil may not only be decisive for plant development but also for creating the soil qualities that favour the spontaneous appearance of other autochthonous shrub species.

The objectives of this study were: (1) to determine which type of mycorrhizal inoculation, native or allochthonous AM fungi, is more effective in shrub plant establishment in degraded zones; and (2) to assess the changes in the soil microbiological properties and aggregate stability related to these AM inocula and to ascertain which mycorrhizal treatments may be more effective in improving them.

Materials and methods

Study sites

The experimental area was located on the El Picarcho range in the Province of Murcia (southeast Spain: 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 shrubs of *O. europaea* subsp. *sylvestris* and *R. sphaerocarpa*, which were selected as target species. The plant cover is sparse (less than 20% canopy cover) and degraded due to ancient grazing and logging. Nowadays, dwarf shrubs (<1 m high) such as *Rosmarinus officinalis* and *Stipa tenacissima* grass are very common, constituting more than 98% of plant cover. Bare soil surfaces are abundant between the patches of plants. The soil is a Petrocalcic Xerosol (FAO 1998), developed from limestones, with a silt loam texture. Some characteristics of the soil are shown in Table 1.

Plants and mycorrhizal treatments

The plants used, *O. europaea* subsp. *sylvestris* and *R. sphaerocarpa*, are two 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 *Glomus claroideum* Schenck & Smith (EEZ 24) or a mixture of endophytes isolated from Cieza (SE Spain), a semiarid area where the target plants naturally grow: the fungal mixture consisted 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).

Arbuscular mycorrhizal fungal inoculum consisted of a mixture of rhizospheric soil from trap cultures (*Sorghum* sp.) containing spores, hyphae and mycorrhizal root fragments. Once germinated, seedlings were transplanted

Table 1 Some characteristics of the soil used for the revegetation experiment (*PNPp*-nitrophenol, *INTF* iodo-nitrotetrazolium formazan, *MPN* most probable number)

pH (H_2O)	7.6 (0.0)[a]
Electrical conductivity, 1:5 (μS cm^{-1})	144 (3)
Total organic C (g kg^{-1})	20.8 (0.9)
Water-soluble C (μg g^{-1})	134 (6)
Total carbohydrates (μg g^{-1})	1,956 (82)
Water-soluble carbohydrates (μg g^{-1})	1 (0)
Total N (g kg^{-1})	0.7 (0.1)
Available P (μg g^{-1})	20 (4)
Extractable K (μg g^{-1})	177 (47)
Aggregate stability (%)	19.5 (3.0)
Urease activity (μmol NH_3 g^{-1} h^{-1})	0.26 (0.03)
N-α-benzoyl-L-argininamide hydrolysing activity (μmol NH_3 g^{-1} h^{-1})	0.40 (0.05)
Acid phosphatase activity (μmol PNP g^{-1} h^{-1})	0.43 (0.05)
β-glucosidase activity (μmol PNP g^{-1} h^{-1})	0.66 (0.04)
Dehydrogenase activity (μg INTF g^{-1} soil)	101 (11)
Arbuscular mycorrhizal infective propagules (MPN per g^{-1} dry soil)	0.24 (0.01)

[a] Each value is the mean of five soil samples (standard error)

into the growth 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 an 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.

Experimental design and layout

The experiment was conducted as two 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 November 2000, an area of 1,200 m^2 was mechanically prepared with a subsoiler. Three rows (1 m wide, 25 m long, 3 m apart) were established. Seedlings of the two 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.

Sampling procedures

Every 6 months 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 (1 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- to 20-cm depth in the rhizospheres of five individual plants. Each root system was extracted by excavating manually a hole 40 cm wide, 40 cm long and 20 cm deep. To collect the rhizosphere soil the root system with rhizosphere soil adhered was introduced into a plastic bag, shaken and the rhizosphere soil separated from the root system. Every 3 months after planting, five plants (one per block) of each treatment were also harvested.

Soil physical-chemical, chemical and biochemical analyses

Total N was determined by the Kjeldahl method, and the total organic C according to Yeomans and Bremner (1988). Available P, extracted with sodium bicarbonate, was determined by colorimetry, according to Murphy and Riley (1962). Extractable (with ammonium acetate) K was determined by flame photometry.

In a soil (1:5, w/v) aqueous extract, water-soluble C was determined by wet oxidation with $K_2Cr_2O_7$ and measurement of the absorbance at 590 nm (Sims and Haby 1971). Water-soluble carbohydrates and total carbohydrates were determined as reported by Brink et al. (1960).

Dehydrogenase activity was determined according to Benefield et al. (1977). Briefly soil (1 g) at 60% of its field capacity was treated with 0.2 ml 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) for 20 h at 22°C in the dark. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with 10 ml methanol by shaking vigorously for 1 min and filtering through Whatman no. 5 filter paper; INTF was measured spectrophotometrically at 490 nm.

Urease and *N*-(-benzoyl-L-argininamide (BAA) hydrolysing activities were determined in 0.1 M phosphate buffer at pH 7; 1 M urea (Tabatabai and Bremner 1972) and 0.03 M BAA (Ladd and Butler 1972) were used as substrates, respectively. Two millilitre buffer and 0.5 ml substrate were added to 0.5 g sample, which was incubated at 30°C (for urease) or 39°C (for protease) for 90 min. Both activities were determined as the NH_4^+ released in the hydrolysis reaction.

Acid phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate. Two millilitre 0.5 M sodium acetate buffer at pH 5.5 (Tabatabai and Bremner 1969) and 0.5 ml substrate were added to 0.5 g soil and incubated at 37°C for 90 min. The reaction was stopped by cooling at 2°C for 15 min. Then, 0.5 ml 0.5 M $CaCl_2$ and 2 ml 0.5 M NaOH were added, and the mixture was centrifuged at 2,287*g* for 5 min. The *p*-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm. Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

β-Glucosidase was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) as substrate. This assay is based on the release and detection of PNP. Two millilitre 0.1 M maleate buffer pH 6.5 and 0.5 ml substrate were added to 0.5 g soil and incubated at 37°C for 90 min. The reaction was stopped by adding 0.1 M Tris-hydroxymethyl aminomethane (THAM) pH 12.0 according to Tabatabai (1982). The amount of PNP was determined at 398 nm (Tabatabai and Bremner 1969).

Physical analysis

The percentage of stable aggregates was determined by the method described by Lax et al. (1994). Sieved (0.2–4 mm) soil (4 g) was placed on a small 0.250-mm sieve and wetted by spray. After 15 min the soil was subjected to an artificial rainfall of 150 ml with energy of 270 Jm^{-2}. The remaining soil on the sieve was placed in a previously weighed capsule (T), dried at 105°C and weighed (P1). Then, the soil was soaked in distilled water and, after 2 h, passed through the same 0.250-mm sieve with the assistance of a small stick to break the remaining aggregates. The residue remaining on the sieve, which was made up of plant debris and sand particles, was dried at 105°C and weighed (P2). The percentage of stable

aggregates with regard to the total aggregates was calculated by (P1− P2)×100/(4− P2+ T).

Percentage of colonised root and growth parameters

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 avoiding woody roots. 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). Fresh and dry (105°C, 5 h) weights of shoots and roots, basal stem diameters and heights of the seedlings were measured at the end of the nursery period and every 3 months after planting.

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) test, calculated at $P < 0.05$. Correlation analysis between all the soil parameters measured was carried out using Pearson's rank correlation coefficients. Statistical procedures were carried out with the software package SPSS 11.0 for Windows.

Results

Changes in soil nutrient properties

At the end of the growth period, the treatment with *G. claroideum* was very effective for increasing the concentration of available P in the rhizosphere soil of *O. europaea* and *R. sphaerocarpa* (by about 62% and 42%, respectively, with respect to control plants). Inoculation with the mixture of native AM fungi had no significant effect on the nutrient levels (total N, available P and extractable K) of the rhizosphere soil of the two shrub species.

Changes in plant growth

Eighteen months after planting, plant survival was about 90% for all treatments and plant species. At the time of planting, shoot and root dry weights of the native AM fungi-colonised or *G. claroideum*-colonised *O. europaea* plants were slightly greater than non-inoculated plants (Fig. 1). At the end of the first year of growth in the field, the *O. europaea* plants inoculated with *G. claroideum* or the mixture of native AM fungi had a greater shoot and root biomass than non-inoculated plants (without significant differences between mycorrhizal treatments). Eighteen months after planting, inoculation with the mixture of native AM fungi or with *G. claroideum* increased the shoot dry weight of *O. europaea* plants about 11.6-fold

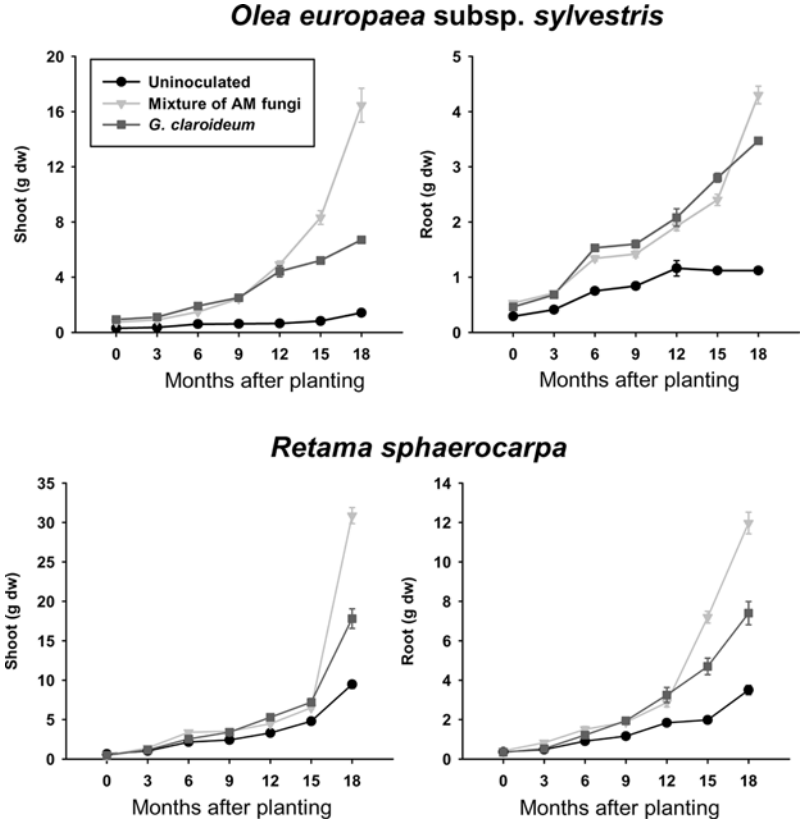

Fig. 1 Shoot and root dry weights of *Olea europaea* subsp. *sylvestris* and *Retama sphaerocarpa* seedlings non-inoculated or inoculated with the allochthonous AM fungus *Glomus claroideum* or with a mixture of native AM fungi, during an 18-month growth period. *Bars* represent standard errors

and 4.7-fold, respectively, in comparison with the control plants (Fig. 1).

At the time of planting, there were no significant differences in growth between non-inoculated and inoculated *R. sphaerocarpa* seedlings. The mixture of native AM fungi was more effective in increasing root and shoot dry weight than *G. claroideum* during the spring growth period (from 3 to 6 months after planting). These differences in seedling growth between mycorrhizal inoculation treatments decreased during the summer growth period, so that the effects of inoculation with *G. claroideum* or with the mixture of native AM fungi on *R. sphaerocarpa* growth were generally similar at 9 months. After 18 months, shoot dry weight of *R. sphaerocarpa* seedlings inoculated with the mixed native AM fungi was 226% and 88% greater than that of the non-inoculated seedlings and the *G. claroideum*-colonised seedlings, respectively. Similar results were obtained for root dry weight of *R. sphaerocarpa* plants (Fig. 1).

Changes in aggregate stability, total organic C, C fractions and root colonisation

Six and 18 months after planting, the inoculation with *G. claroideum* significantly increased the aggregate stability in the rhizosphere soil of *O. europaea*; however, the treatment with the mixture of native AM fungi was more effective in increasing the aggregate stability of the rhizosphere soil of *R. sphaerocarpa* seedlings 18 months after planting (Table 2).

At 18 months after planting, the water-soluble C content of the rhizosphere soil of *O. europaea* was only increased by *G. claroideum* treatment and that of the rhizosphere soil of *R. sphaerocarpa* by both mycorrhizal treatments. At the end of the growth period, both fungal treatments significantly increased the water-soluble carbohydrates content of the rhizosphere soils of *O. europaea* and *R. sphaerocarpa* seedlings; *G. claroideum* was more effective in increasing this C fraction than the mixture of native AM fungi. From 6 to 12 months after planting, both mycorrhizal treatments significantly enhanced the total carbohydrate content of the rhizosphere soil of *O. europaea* seedlings, without significant differences between the two treatments; however, at the end of the growth period, only *G. claroideum* treatment increased, by about 78%, the total carbohydrate content with respect to the control soil. The mixture of native AM fungi or *G. claroideum* inoculum enhanced this C fraction in the rhizosphere soil of *R. sphaerocarpa* seedlings 18 months after planting (Table 2). The content of total organic C of the rhizosphere soil of *O. europaea* seedlings was only increased by *G. claroideum* at the end of the growth period.

Six months after planting and at the end of the growth period, both inoculation treatments produced a similar level of root colonisation in *O. europaea* and *R. sphaerocarpa* seedlings (around 80% at the end of the growth period). The natural colonisation observed in the non-inoculated plants was similar in both shrub species (around 10% at the end of the growth period; Table 2).

Table 2 Effect of inoculation with the allochthonous AM fungus *Glomus claroideum* and with the mixture of native AM fungi on soil aggregate stability, total organic C and C fractions and root colonisation of *Olea europaea* subsp. *sylvestris* and *Retama sphaerocarpa* seedlings (n =5; *TOC* total organic C, *CH* carbohydrates, *C* control, *M* plants inoculated with the mixture of native AM fungi, *G* plants inoculated with *G. claroideum*). *Values in columns sharing the same letter* do not differ significantly (P <0.05) as determined by the LSD test

Parameters	*O. europaea* subsp. *sylvestris*			*R. sphaerocarpa*		
	6 month	12 month	18 month	6 month	12 month	18 month
Aggregate stability (%)						
C	48.5a	37.8a	59.9	50.2a	40.6a	63.7a
M	50.5ab	40.0a	55.6a	43.5a	41.8a	73.7b
G	55.2b	42.2a	67.4b	50.3a	41.2a	63.4a
Water-soluble C (μg g^{-1})						
C	102a	103a	157a	116a	106a	152a
M	90a	105a	161a	123a	116a	200b
G	104a	115a	206b	116a	106a	233b
Water-soluble CH (μg g^{-1})						
C	5a	12a	11a	13b	11a	9a
M	5a	12a	21b	14b	18b	24b
G	5a	11a	28c	10a	14ab	33c
Total CH (μg g^{-1})						
C	686a	1,100a	2,345a	1,370a	1,450a	1,659a
M	987b	1,900b	2,352a	1,316a	2,000a	2,534b
G	1,219b	2,200b	4,175b	1,603b	2,500a	3,064b
TOC (g kg^{-1})						
C	22.3a	23.1a	25.1a	25.1a	24.2a	24.1a
M	21.3a	23.5a	24.2a	22.5a	24.4a	25.5a
G	23.2a	25.6a	31.8b	23.1a	24.7a	25.9a
Colonised root length (%)						
C	4.0a	9.0a	10.5a	4.0a	9.0a	10.9a
M	57.0b	72.0c	85.0b	70.0b	73.0b	78.8b
G	60.0b	48.0b	88.2b	67.0b	72.0b	73.0b

Changes in soil biochemical properties

Six months after planting, urease, β-glucosidase, dehydrogenase and BAA-hydrolysing activities of the rhizosphere soil of *O. europaea* were not significantly affected by either mycorrhizal inoculation treatments. One year after planting, inoculation with *G. claroideum* or the mixture of native AM fungi stimulated all enzyme activities of the rhizosphere soil. The highest increase in enzyme activities due to both inoculation treatments were observed after 18 months, with the highest increase observed for acid phosphatase activity. At the end of the growth period, acid phosphatase or dehydrogenase activities showed no significant differences between the two mycorrhizal inoculation treatments (Fig. 2).

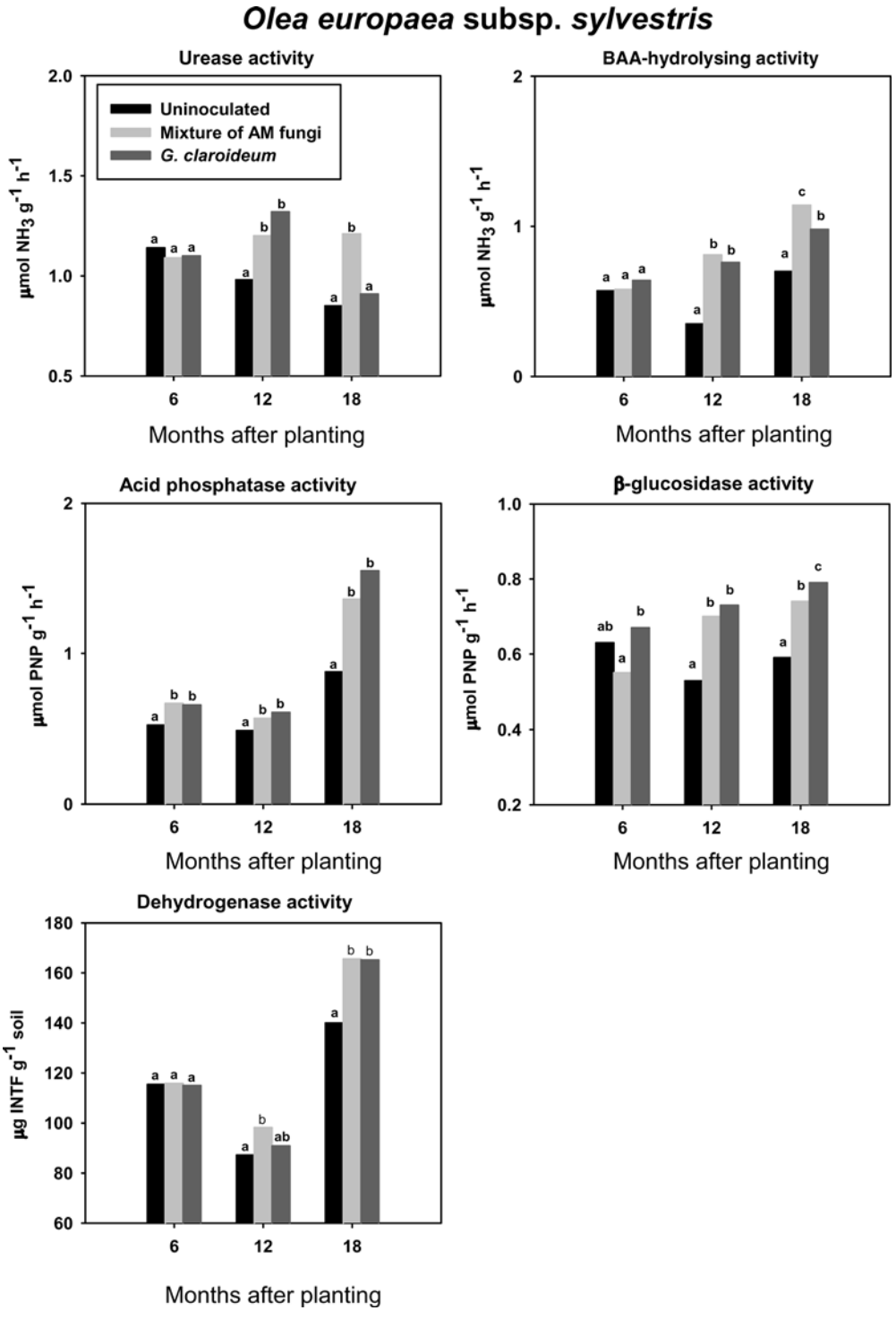

Fig. 2 Changes in the biochemical properties of the rhizosphere of *Olea europaea* subsp. *sylvestris*, in response to inoculation with the allochthonous AM fungus *G. claroideum* or with a mixture of native AM fungi, during an 18-month growth period. For each enzyme activity and sampling, *values sharing the same letter* are not significantly different (P <0.05), according to the LSD test

Six months after planting, inoculation with *G. claroideum* increased significantly the urease activity of the rhizosphere soil of *R. sphaerocarpa*. Urease activity was stimulated by inoculation with *G. claroideum* or the mixture of native species, 1 year after planting and at the end of the growth period, without significant differences between the fungal treatments. One year after planting, the mixture of native AM fungi was more effective in increasing acid phosphatase and dehydrogenase activities of the rhizosphere soil of *R. sphaerocarpa* than the *G. claroideum* treatment. Eighteen months after planting, the two mycorrhizal treatments increased similarly all enzyme activities of the rhizosphere soil of *R. sphaerocarpa* seedlings, except that inoculation with *G. claroideum* was more effective in increasing the acid phosphatase activity (about 276% greater with respect to the control soil; Fig. 3).

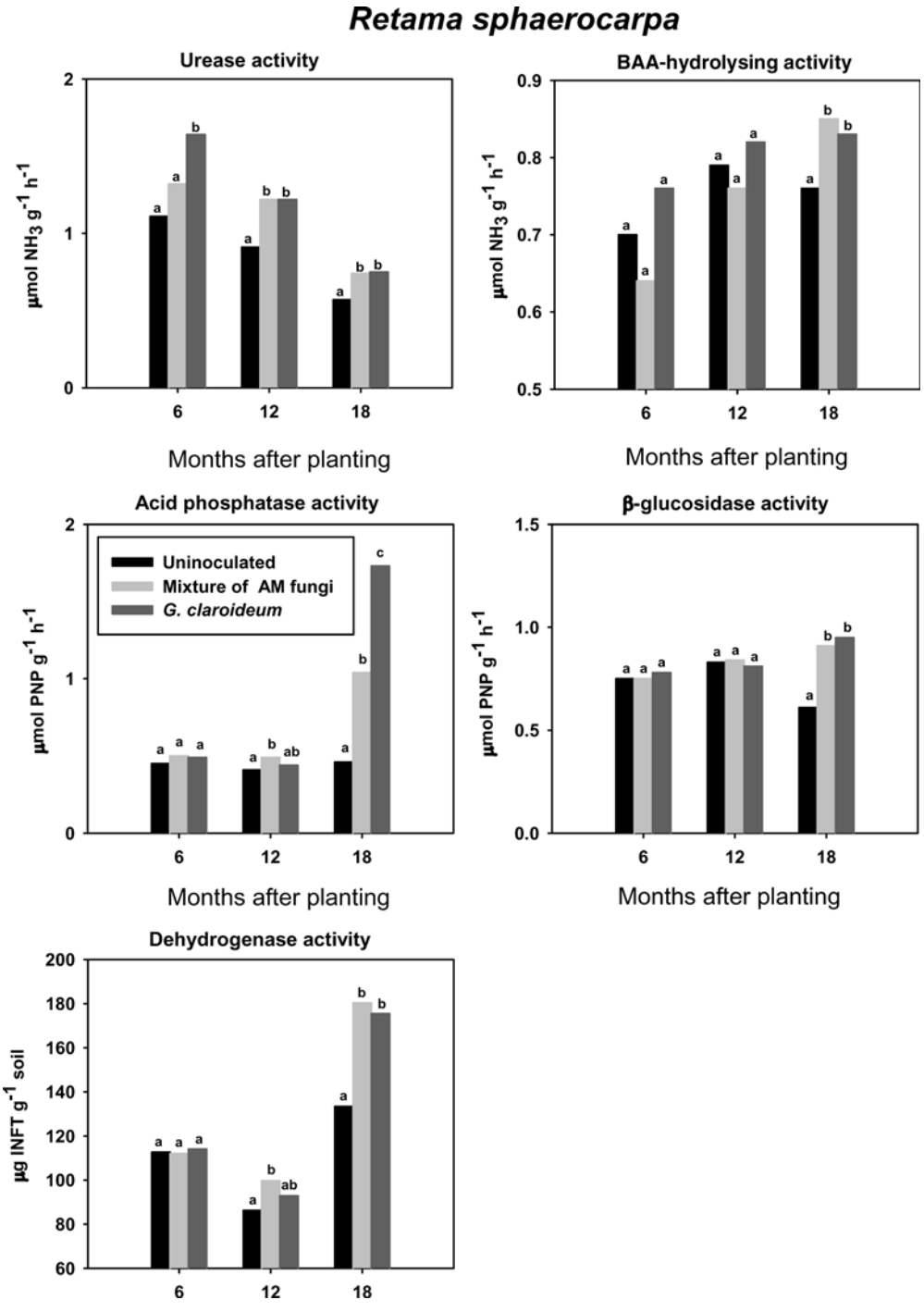

Fig. 3 Changes in the biochemical properties of the rhizosphere of *Retama sphaerocarpa*, in response to inoculation with the allochthonous AM fungus *Glomus claroideum* or with a mixture of native AM fungi, during an 18-month growth period. For each enzyme activity and sampling, *values sharing the same letter* are not significantly different (P <0.05), according to the LSD test

Discussion

This study confirms the key role of mycorrhizae in sustaining the plant cover in soils that are deficient in nutrients (particularly in N and P), as well as showing the necessity of including mycorrhizal inoculation to guarantee plant performance in revegetation programmes for degraded areas where the mycorrhizal inoculum potential is low. This fact is supported by the low effectivity of natural colonisation for increasing the growth of non-inoculated plants. Moreover, the growth of both shrub species (*O. europaea* and *R. sphaerocarpa*) was correlated significantly (P <0.01) with the level of colonisation by AM fungi in their roots. The mycorrhizal inoculation treatments showed different levels of effectiveness in improving the performance of the two shrub species. The mixture of native AM fungi was more effective in increasing shoot dry weight of *O. europaea* and *R. sphaerocarpa* plants than was *G. claroideum*, despite the fact that the percentage of root colonisation was similar with the two inoculation treatments. Our results also indicated that the inoculation treatment with the greater

number of AM fungal taxa, and, therefore, with the higher AM fungal biodiversity, resulted in higher productivity and biomass yield.

Arbuscular mycorrhizal fungi can interact with other rhizosphere microorganisms (Jeffries et al. 2003) and can affect rhizodeposition and thus the quantity and quality of organic C delivered to the soil via fungal hyphae (Marschner et al. 1997). Indeed, the concentrations of water-soluble C, total and water-soluble carbohydrates were higher in the rhizosphere soil of both shrub species inoculated with AM fungi. It is worth noting that the percentage of colonised root was significantly correlated to these labile C fractions in the rhizosphere soil of *O. europaea* and *R. sphaerocarpa*. The higher release of carbohydrates into the rhizosphere of mycorrhizal plants probably affected the composition, activity and size of the rhizosphere soil microflora (Wamberg et al. 2003). In both shrub species, the increase of microbial activity did not depend on the assayed mycorrhizal inoculation treatment. Mycorrhiza-inoculated *R. sphaerocarpa* was the most effective at increasing dehydrogenase activity (by about 34% with respect to the control). Dehydrogenase activity responded to the treatments in a similar manner to the water-soluble C fractions, i.e. increasing with the mycorrhizal inoculation treatments. A positive correlation between the soluble C fractions and microbial activity exists in soil (Ghani et al. 2003). Water-soluble C, as a component of the labile C pool, may also be sensitive to perturbation and stress in soil-plant ecosystems (Doran and Parkins 1994) and, therefore, it could be used as a sensitive indicator of soil quality. Increased microbiological activity was also revealed by the variations in urease, acid phosphatase, β-glucosidase and BAA-hydrolysing activities. The measurement of these hydrolase activities can provide an early indication of changes in soil fertility, since they are related to the mineralisation of such important nutrient elements as N, P and C (Ceccanti et al. 1994). Enzyme activities also are sufficiently sensitive to indicate perturbations caused by microbial inoculation (Naseby and Lynch 1997). The increases observed in urease, β-glucosidase and BAA-hydrolysing activities may be related mainly to increase of the rhizosphere microbial population as a consequence of the inoculation treatments. To our knowledge, there is no evidence regarding the secretion of urease, β-glucosidase and proteases enzymes by AM fungi. In contrast, increased acid phosphate activity in the rhizosphere of mycorrhizal plants may be due to a direct fungal secretion or an induced secretion by the plant roots, as pointed out by Joner et al. (2000). Phosphatases are enzymes with a relatively broad specificity, capable of hydrolysing various organic phosphate esters, and are involved in the P cycle. The highest increase in phosphatase activity was recorded in the rhizosphere soil of mycorrhizal *R. sphaerocarpa*, colonised by *G. claroideum*. The fact that the highest concentrations of available P occurred in the rhizosphere of both shrub species inoculated with *G. claroideum* may be due to the hydrolysis of organic P compounds catalysed by extracellular fungal phosphatase activities. However, the quantitative contribution of extracellular enzymes to the P nutrition of AM plants is considered to be insignificant (Joner et al. 2000).

Soil structure and other soil properties affect soil quality and fertility, which favour the establishment and viability of a stable plant cover. Indeed, *R. sphaerocarpa* yield parameters and soil aggregate stability were significantly (P <0.001) correlated. The present study confirms the influence of mycorrhizal inoculation treatments on soil aggregate stability. The mixture of native AM fungi was more effective in increasing the aggregate stability of the rhizosphere soil of *R. sphaerocarpa*, while that of *O. europaea* was increased only by *G. claroideum*. The mechanisms involved in aggregate stabilisation are based on the enmeshment of soil particles by hyphae and roots, and the exudation of polysaccharides (Bearden and Petersen 2000). The water-soluble C fraction is also regarded as one of the key labile components of organic matter responsible for soil aggregation (Puget et al. 1999). The levels of both soil total carbohydrates and water-soluble C were significantly correlated to the percentage stable aggregate in rhizosphere soil of *O. europaea* (P <0.001). The increased levels of stable aggregates resulting from mycorrhizal inoculation treatments can also be attributed to the proliferation of fungal hyphae in the rhizosphere soil (Roldán et al. 1994; Jeffries and Barea 2000). According to Roldán et al. (1994), the binding effect of roots and hyphae is long-lived, while that of polysaccharides is transient because they are decomposed rapidly by microbes. On the other hand, the fact that the highest microbial activity was in the rhizosphere soil of both mycorrhizal shrub species might be due to high levels of stable aggregates, which protect the organic fraction (on which extracellular enzymes and soil microorganisms are immobilised) from microbial degradation (Nannipieri 1994).

Facilitative interactions between plants have been identified as one of the main processes affecting the composition of vegetation cover in arid and semiarid environments (Maestre et al. 2003). It has been reported that *R. sphaerocarpa* plants facilitate the introduction of annual and perennial species through self-promoting changes in microclimate and soil fertility, thus acting as "nurse plants" (Moro et al. 1997). We have recently shown that within the relict natural vegetation currently growing in patches in the target ecosystem, the rhizosphere soil of *R. sphaerocarpa* showed a higher microbial activity than that of the rhizosphere soil of *O. europaea* (Caravaca et al. 2003c). The improvement in soil quality due to the increase in microbial activity and aggregate stability in the rhizosphere of *O. europaea* and *R. sphaerocarpa* plants inoculated with AM fungi can contribute to the nurse role of these species, thus benefiting plant-plant interactions. In summary, AM fungi can enhance the growth of native shrub species both directly, through improved nutrient assimilation or water supply, and indirectly, by favouring the development of rhizosphere microorganisms and their activities, which lead to an improvement of aggregate stability and an acceleration of nutrient cycles.

In conclusion, mycorrhizal inoculation with the mixture of native AM fungi was the more effective treatment for stimulating the growth of the shrub species. The establishment of mycorrhizal shrub species favoured the reactivation of soil microbial activity, which was linked to an increase in aggregate stability. Finally, the improved soil physical and microbiological quality could facilitate the establishment and development of new plants in the surrounding area, which would aid the revegetation of semiarid ecosystems.

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