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ORIGINAL PAPER

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Interaction between AM fungi and a liquid organic amendment with respect to enhancement of the performance of the leguminous shrub *Retama sphaerocarpa*

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Abstract This study examined the interactions between the inoculation with three arbuscular mycorrhizal fungi, namely, *Glomus intraradices*, *Glomus deserticola* and *Glomus mosseae*, and the addition of a liquid organic amendment at different rates (0, 50, 100 or 300 mg C of liquid amendment per kilogram soil) obtained by alkaline extraction of composted dry olive residue with respect to their effects on growth of *Retama sphaerocarpa* seedlings and on some microbiological and physical properties of soil. One year after planting, both mycorrhizal inoculation treatments and the addition of amendment had increased plant growth and dehydrogenase, urease and benzoyl argininamide hydrolysing activities. The inoculation with *G. mosseae* increased plant growth to a greater extent than the addition of the amendment (about 35% greater than plants grown in the amended soil and about 79% greater than control plants) and both treatments produced similar increases in soil aggregate stability (about 31% higher than control soil). The organic amendment produced a very significant decrease in the levels of microbial biomass C and a strong increase in soil dehydrogenase and urease activities, which were proportional to the amendment rate. Only the combined treatment involving the addition of a medium dose of amendment (100 mg C kg^{-1} soil) and the mycorrhizal inoculation with *G. intraradices* or *G. deserticola* produced an additive effect on the plant growth with respect to the treatments applied individually (about 77% greater than plants grown in the amended soil and about 63% greater than inoculated plants).

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Introduction

Legumes are among the most effective species in revegetation programmes because of their ability to form symbiotic associations with both nitrogen-fixing bacteria and arbuscular mycorrhizal (AM) fungi. They have been considered useful for revegetation of dry Mediterranean habitats that have low availability of nitrogen, phosphorus and other nutrients (Moro et al. 1997). A leguminous species common in semi-arid environments of south-east Spain is *Retama sphaerocarpa* (L.) Boissier, which has a deep root system, whose functionality is maintained at depths of more than 25 m (Haase et al. 1996) that allows the plant access to deep water sources; in addition, this shrub is capable of resisting the frequent droughts of arid and semi-arid zones. Also, *R. sphaerocarpa* and its conspicuous understorey vegetation of annual and perennial species constitute "fertility islands" (Moro et al. 1997), which are points of high biological activity scattered in a heterogeneous landscape. Several studies have demonstrated the benefits of AM inoculation of these shrubs in semi-arid soils (Caravaca et al. 2003a). The selection of efficient AM fungi is a major topic in inoculation programmes, especially in disturbed soils where the indigenous inoculum levels of AM fungi are considered as being low, which may limit the successful establishment of plants (Palenzuela et al. 2002; Azcón-Aguilar et al. 2003).

The addition of organic materials can increase fertility and improve the physical and biological properties of degraded soils (Roldán et al. 1996). Among the organic amendments, the agronomic use of dry olive residue (DOR) or "alperujo" has increased steadily in recent years due to its high C and mineral nutrient content (Nogales et al. 1999). However, these residues contain phenolic compounds that may have a negative effect on microbial activity, inhibit the establishment of AM symbioses and decrease the plant

Table 1 Chemical, biochemical, microbiological and physical characteristics of the soil

pH (H_2O)	8.5±0.0[a]
EC (1:5, μs cm^{-1})	225±2
Texture	Loam
Total organic C (g kg^{-1})	10.3±0.3
Total carbohydrates (μg g^{-1})	552±20
Water-soluble C (μg g^{-1})	100±1
Water-soluble carbohydrates (μg g^{-1})	8±0
Total N (g kg^{-1})	0.95±0.02
Available P (μg g^{-1})	7±0
Extractable K (μg g^{-1})	222±4
Microbial biomass C (μg g^{-1})	396±11
Dehydrogenase (μg INTF g^{-1})	51±1
Urease (μmol NH_3 g^{-1} h^{-1})	0.31±0.03
Protease-BAA (μmol NH_3 g^{-1} h^{-1})	0.60±0.04
Phosphatase (μmol PNP g^{-1} h^{-1})	0.28±0.02
β-Glucosidase (μmol PNP g^{-1} h^{-1})	0.46±0.01
Aggregate stability (%)	11.5±0.4

[a]Mean±standard error (*N*=6)

growth (Martín et al. 2002; Linares et al. 2003). Studies have shown that the effect of phenolic compounds contained in DOR on AM root colonization can vary with the type of fungi and the time of inoculation (Martín et al. 2002). DOR composting could minimise its antimicrobial effect, but the composted DOR may contain phytotoxic compounds (Linares et al. 2003). On the other hand, the organic fraction extracted with alkaline solutions from organic materials has been used for improving plant growth and nutrient uptake (Ayuso et al. 1996). This fraction may have a positive effect on plant growth directly and/or through the improvement in soil structure, cation exchange capacity, microbiological activity and for complexing certain soil ions. However, there are no data on the effects of this organic fraction extracted from DOR on colonization of roots by AM fungi or on soil properties in revegetation programmes. Our hypothesis was that the combined actions of this liquid organic amendment and mycorrhiza on the growth of Mediterranean shrub species could be higher than the sum of individual effects.

The objectives of this study were (1) to assess the interactions between the inoculation with three AM fungi and the addition of a liquid organic amendment obtained by alkaline extraction of composted DOR, with respect to their effects on the growth of *R. sphaerocarpa* seedlings in a degraded semi-arid Mediterranean soil, and (2) to establish the optimum levels of amendment addition, thereby avoiding possible negative effects on plant growth, AM colonization and soil properties considered to be indicators of soil quality such as labile C fractions (microbial biomass C and water-soluble C), enzyme activities (dehydrogenase, urease, protease-BAA, and β-glucosidase) and soil aggregate stability.

Materials and methods

Materials

The soil was a Typic Haplocalcid (Soil Survey Staff 1999) with a texture loam developed from Quaternary sediments (Table 1). It was collected from Los Cuadros in the province of Murcia, south-east Spain (coordinates 1°05'W and 38°10'N). The climate is semi-arid Mediterranean with an average annual rainfall of 300 mm and a mean annual temperature of 19.2°C; the potential evapotranspiration reaches 1,000 mm $year^{-1}$.

The amendment used was the organic fraction extracted with KOH from a composted DOR (Alburquerque et al. 2005) collected from an olive mill in Granada, Spain. The extract was obtained by mechanically shaking the composted DOR with 0.1 M KOH (1:20, w/v) for 24 h (12 h at 25°C and 12 h at 70°). The suspension was centrifuged at 14,644×*g* for 20 min. After centrifugation the particulate matter was eliminated of supernatant. The analytical characteristics of the amendment are shown in Table 2.

The plant used for the reafforestation experiment was *R. sphaerocarpa*, a low-growing shrub reaching a height of 1.3–2.5 m and widely distributed in the Mediterranean area. It is also well adapted to drought and, therefore, frequently used in the reafforestation of semi-arid disturbed lands.

Mycorrhizal inoculation of seedlings

The mycorrhizal fungi used in the experiment, *Glomus intraradices* Schenck & Smith, *Glomus deserticola* (Trappe, Bloss & Menge) and *Glomus mosseae* (Nicol & Gerd.) Gerd. & Trappe, were obtained from the collection of the experimental field station of Zaidín, Granada.

Table 2 Chemical characteristics of the KOH-soluble extract of dry olive residue

Ash (%)	30.4
pH	9.83
EC (mS cm^{-1})	9.78
Organic matter (%)	69.6
Extractable C (g l^{-1})	8.6
Fulvic acid C (g l^{-1})	2.1
Humic acid C (g l^{-1})	6.5
Water-soluble carbohydrates (g l^{-1})	1.03
Phenols (g l^{-1})	1.58
Total N (mg l^{-1})	320
Na (mg l^{-1})	186
K (mg l^{-1})	1,776
P (mg l^{-1})	62
Ca (mg l^{-1})	136
Mg (mg l^{-1})	28.9
Fe (mg l^{-1})	3.4
Cu (mg l^{-1})	0.2
Mn (mg l^{-1})	0.3
Zn (mg l^{-1})	1.2

The AM fungal inoculum consisted of a mixture of rhizosphere soil from trap cultures (*Sorghum* sp.) containing spores, hyphae and mycorrhizal root fragments. Once germinated, seedlings were transplanted into the growth substrate consisting of peat and cocopeat (1:1, v/v). The corresponding AM 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 (Whatman No. 1 paper) of the pot culture of all three AM fungi to provide the microbial populations accompanying the mycorrhizal fungi. Inoculated and non-inoculated seedlings were grown for 8 months under nursery conditions without any chemical fertiliser treatment.

Experimental design and layout

The experiment was a mesocosm assay, conducted as a completely randomized factorial design with two factors. The first factor had four levels: without and with the addition of low, medium or high doses of the liquid amendment to the soil. The second factor had four levels: non-inoculation and inoculation of *R. sphaerocarpa* plants with one of three AM fungi (*G. intraradices*, *G. deserticola* or *G. mosseae*) in the nursery. Four replicates per treatment were carried out, making a total of 64 pots.

Two kilograms of substrate, consisting of soil and vermiculite at a ratio of 3:1 (v/v), were placed in 3.6-l pots. In March 2003, *R. sphaerocarpa* seedlings (inoculated and non-inoculated) were transplanted to the pots (one per pot). Two weeks after planting, the amendment was applied to the pots at a rate of 0, 5.8, 11.7 or 35.1 ml kg^{-1} soil/vermiculite mixture, which corresponds to the addition of 0, 50, 100 or 300 mg C liquid amendment kg^{-1} soil. The experiment was carried out in the nursery of the University of Murcia (Murcia, Spain) without the addition of chemical fertiliser. The plants were well watered and kept outdoors under ambient irradiance, temperature and air humidity. One year after planting, the plants were harvested and rhizosphere soil samples of the pots were taken. Rhizosphere soil samples, air-dried to 20% moisture content and sieved to less than 2 mm, were divided into two subsamples. One subsample was stored at 2°C for microbiological analysis, and another subsample was allowed to dry at room temperature for physical–chemical analysis.

Plant analyses

Basal stem diameters and heights of plants were measured with callipers and rulers. Fresh and dry (105°C, 5 h) masses of shoots and roots were recorded. Plant tissues were ground before chemical analysis. Plant P was determined colorimetrically according to Murphy and Riley (1962) after digestion in nitric–perchloric acid (5:3) for 6 h at 210°C. Plant N was determined by combustion at 1,020°C in a carbon and nitrogen analyser and reduction, separation of N_2 in a chromatographic column and measurement in a thermic conductivity detector. Plant K was estimated by flame photometry (Schollemberger and Simon 1954).

Table 3 Soil physical–chemical properties of *R. sphaerocarpa* after 1 year of growth in response to mycorrhizal inoculation treatments and KOH extract of DOR addition (n=4)

	No mycorrhiza				*G. intraradices*				*G. deserticola*				*G. mosseae*			
	0[a]	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
pH (H_2O)	8.56b	8.58b	8.58b	8.60b	8.34a	8.31a	8.44ab	8.48ab	8.47ab	8.46ab	8.34a	8.34a	8.39a	8.40ab	8.43ab	8.47ab
EC (1:5, µs cm^{-1})	310abcd	287ab	294abc	278a	303abcd	315abcde	320abcde	374e	347bcde	357de	290ab	310abcd	300abcd	324abcde	322abcde	354cde
Water-soluble C (µg g^{-1})	183a	208bcd	188ab	174a	213bcd	228cde	184a	240cde	200bc	225cde	249cde	209bcd	270de	283e	254cde	248cde
Aggregate stability (%)	10.6a	13.5bc	13.1b	14.8bcde	10.8a	13.7bcd	14.6bcde	15.0bcdef	11.0a	18.5fg	17.0cdefg	19.2g	14.0bcd	17.2defg	16.9cdefg	17.7efg

Values in rows followed by the same letter are not significantly different (LSD, p<0.05)
DOR Dry olive residue
[a]0, 1, 2 or 3 indicates 0, 5.8, 11.7 or 35.1 ml amendment kg^{-1} soil/vermiculite mixture, respectively

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).

Soil analyses

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous solution. In soil aqueous extracts (1:5), water-soluble C was determined with an automatic carbon analyser for liquid samples (Shimadzu TOC-5050A).

Microbial biomass C was determined by the fumigation–extraction method (Vance et al. 1987). Ten grams of soil at 60% of field capacity was fumigated in a 125-ml Erlenmeyer flask with purified $CHCl_3$ for 24 h. After removal of residual $CHCl_3$, 40 ml of 0.5 M K_2SO_4 solution was added and the sample was shaken for 1 h before filtration of the mixture. The K_2SO_4-extracted C was measured as indicated for water-soluble C. Microbial biomass C was calculated as the difference between the C of fumigated and non-fumigated samples divided by the calibration factor K_{EC}=0.38.

Dehydrogenase activity was determined according to Trevors et al. (1982). For this, 1 g of soil at 60% of field capacity was exposed to 0.2 ml of 0.4% 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride (INT) in distilled water for 20 h at 22°C in the dark. The iodonitrotetrazolium formazan (INTF) formed was extracted with 10 ml of 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*-α-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 millilitres of buffer and 0.5 ml of substrate were added to 0.5 g of 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.

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 *p*-nitrophenol (PNP). Two millilitres of 0.1 M maleate buffer (pH 6.5) and 0.5 ml of substrate were added to 0.5 g of sample and incubated at 37°C for 90 min. The reaction was stopped with tris(hydroxymethyl)aminomethane (THAM) according to Tabatabai (1982). The amount of PNP was determined by spectrophotometry at 398 nm (Tabatabai and Bremner 1969).

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 J m^{-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 the following relationship (P1−P2)×100/(4−P2+T).

Statistical analysis

Data were log-transformed to achieve for normality. Amendment addition, mycorrhizal inoculation and their interaction effects on measured variables were tested by a two-way analysis of variance, and comparisons among means were made using 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.

Results

Soil parameters

Only the mycorrhizal treatments with *G. intraradices* and *G. mosseae* significantly decreased soil pH (Tables 3 and 4). However, neither amendment addition nor the interaction of amendment × mycorrhizal inoculation had any significant effect on soil pH and electrical conductivity (Table 4).

Table 4 Two-factor ANOVA (mycorrhizal inoculation treatments and amendment addition) for all parameters studied in the rhizosphere soil of *R. sphaerocarpa* seedlings 1 year after planting (*P* significance values)

	Amendment (A)	Mycorrhiza (M)	Interaction (A×M)
pH	0.700	<0.001	0.814
Electrical conductivity	0.669	0.079	0.169
Water-soluble C	0.615	0.003	0.987
Aggregate stability	<0.001	0.014	0.362
Microbial biomass C	<0.001	0.587	0.806
Dehydrogenase	0.002	0.026	0.685
Urease	<0.001	<0.001	0.001
Protease	0.643	<0.001	0.807
β-Glucosidase	0.818	0.293	0.384
Shoot	0.037	0.001	0.211
Root	0.604	0.353	0.163
N foliar	0.006	<0.001	0.018
P foliar	0.002	<0.001	0.003
K foliar	0.319	0.001	0.028
Colonization	0.861	<0.001	0.547

Table 5 Soil microbial biomass C and enzyme activities of *R. sphaerocarpa* after 1 year of growth in response to mycorrhizal inoculation treatments and KOH extract of DOR addition (n=4)

	No mycorrhiza				*G. intraradices*				*G. deserticola*				*G. mosseae*			
	0[a]	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Microbial biomass C (μg g^{-1})	281e	177d	70bc	23a	296e	167d	86c	35a	288e	149d	180d	81c	301e	73bc	46ab	42ab
Dehydrogenase (μg INTF g^{-1} soil)	26.1a	26.0a	40.6cd	40.7cd	34.8bc	30.0ab	40.9cd	47.5e	29.8ab	31.3ab	46.0de	43.8de	34.8bc	30.2ab	42.4de	53.4f
Urease (μmol NH_3 g^{-1} h^{-1})	0.05a	0.19ab	0.43c	0.56cd	0.14ab	0.16ab	0.56cd	0.66d	0.14ab	0.24b	0.83e	0.56cd	0.19ab	0.19ab	0.69d	0.61d
Protease-BAA (μmol NH_3 g^{-1} h^{-1})	0.44a	0.54ab	0.48ab	0.53ab	0.95de	0.96de	0.68bc	0.84cd	0.46a	0.75bc	0.69bc	0.57b	1.29fg	1.50g	1.15ef	1.15ef
β-Glucosidase (μmol PNP g^{-1} h^{-1})	0.26abc	0.24abc	0.23ab	0.25abc	0.25abc	0.21a	0.29bc	0.22ab	0.25abc	0.27abc	0.31c	0.26abc	0.28abc	0.29abc	0.32c	0.27abc

Values in rows followed by the same letter are not significantly different (LSD, p<0.05)
DOR Dry olive residue
[a]0, 1, 2 or 3 indicates 0, 5.8, 11.7 or 35.1 ml amendment kg^{-1} soil/vermiculite mixture, respectively

Water-soluble C concentrations were increased only with the mycorrhizal inoculation treatments (Tables 3 and 4), the greatest increases being observed in the inoculation treatment with *G. mosseae* (on average, about 40% greater than in the soil of non-inoculated plants).

Both amendment addition and inoculation with *G. mosseae* significantly increased soil aggregate stability, reaching similar values at the end of the growth period (Tables 3 and 4). In the amended soils, the percentage of stable aggregates did not vary with the dose of the amendment added. The highest aggregate stability was found in the amended soil of plants inoculated with *G. deserticola* or *G. mosseae*.

The addition of the amendment decreased soil microbial biomass C for both non-inoculated and inoculated plants, particularly for the plants inoculated with *G. mosseae* or *G. intraradices* (Table 5). In general, microbial biomass C decreased as the dose of amendment increased. Microbial biomass C was not affected by the mycorrhizal inoculation treatments (Table 4). With the exception of *G. desert icola*, the mycorrhizal inoculation alone produced significantly higher dehydrogenase and benzoyl arginina-mide hydrolysing activities than the control soil (Table 5). In general, neither amendment addition nor mycorrhizal inoculation modified β-glucosidase activity. The medium and high dose of amendment significantly increased dehydrogenase and urease activities. Only urease activity increased proportionally with the increase in the amendment dose. The interaction amendment × mycorrhizal inoculation had no significant effect on the biochemical properties (Table 4).

Table 6 Growth parameters, foliar nutrient content and root infection of *R. sphaerocarpa* in response to mycorrhizal inoculation treatments and KOH extract of DOR addition previous to planting (n=4)

	No mycorrhiza	*G. intraradices*	*G. deserticola*	*G. mosseae*
Shoot (g dry wt. $plant^{-1}$)	0.5a	0.7a	0.7a	0.5a
Root (g dry wt. $plant^{-1}$)	0.6ab	0.8b	0.7ab	0.5a
Nitrogen (mg $plant^{-1}$)	8a	13b	10ab	9a
Phosphorus (mg $plant^{-1}$)	0.6a	0.8ab	1.2b	1.0ab
Potassium (mg $plant^{-1}$)	7a	9a	9a	8a
Colonization (%)	0.4a	61.9b	67.7b	51.8b

Values in rows followed by the same letter are not significantly different (LSD, p<0.05)
[a]0, 1, 2 or 3 indicates 0, 5.8, 11.7 or 35.1 ml amendment kg^{-1} soil/vermiculite mixture, respectively

Table 7 Growth parameters, foliar nutrient content and root infection of *R. sphaerocarpa* in response to mycorrhizal inoculation treatments and KOH extract of DOR addition after 1-year growth (n=4)

	No mycorrhiza				*G. intraradices*				*G. deserticola*				*G. mosseae*			
	0[a]	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Shoot (g dry wt. plant^{-1})	2.8a	4.1bc	3.5bc	3.5bc	4.0bc	3.3ab	6.1e	5.3de	3.6bc	4.5cd	6.2e	3.3ab	5.0de	3.9bc	4.5cd	4.3bcd
Root (g dry wt. plant^{-1})	2.9ab	2.8ab	2.5a	2.3a	2.6a	2.3a	4.0abc	3.7ab	2.7a	2.9ab	5.4c	3.0ab	2.9ab	2.8ab	2.9ab	4.5bc
Nitrogen (mg plant^{-1})	39a	64bc	63bc	72bc	66bc	78bc	114d	91bcd	58ab	66bc	93bcd	63bc	99cd	67bc	65bc	63bc
Phosphorus (mg plant^{-1})	4.8a	9.4bcd	9.8bcd	7.3b	8.1bc	7.5b	12.7de	12.2cde	8.8bcd	9.1bcd	14.6e	9.0bcd	14.8e	9.7bcd	9.2bcd	11.5bcde
Potassium (mg plant^{-1})	51a	79bcd	74bcd	63ab	95bcde	68bc	111de	102cde	82bcd	67bc	112e	58ab	103cde	89bcd	80bcd	59ab
Colonization (%)	3.3a	1.5a	10.5a	10.0a	70.5cde	44.5b	74.5de	57.3bcd	70.0cde	72.0de	79.8e	67.0cde	52.8bcd	60.5bcde	56.0bcd	48.3bc

Values in rows followed by the same letter are not significantly different (LSD, p<0.05)
[a]0, 1, 2 or 3 indicates 0, 5.8, 11.7 or 35.1 ml amendment kg^{-1} soil/vermiculite mixture, respectively

Growth and mycorrhizal infection of *R. sphaerocarpa*

One year after planting, plant survival was about 100% for all treatments. At the time of planting, there were no significant differences in the shoot dry weight of non-inoculated and inoculated *R. sphaerocarpa* plants (Table 6). Only the *G. intraradices*- or *G. deserticola*-colonized *R. sphaerocarpa* plants had higher foliar N and P contents, respectively, than non-inoculated plants. One year after planting, both the addition of amendment and the mycorrhizal inoculation treatments had significantly stimulated the shoot biomass of *R. sphaerocarpa* with respect to the control plants (Tables 4 and 7). There were no significant differences in the growth of plants treated with different amendment dose. Plant root biomass was not affected by mycorrhizal inoculation or by the addition of amendment. The inoculation with *G. mosseae* increased plant growth to a greater extent than the addition of the amendment (about 35% greater than that of plants grown in the amended soil and about 79% greater than for control plants) (Table 7). The medium dose of amendment had the strongest effect on shoot biomass of *G. intraradices*- or *G. deserticola*-colonized *R. sphaerocarpa* plants (about 77% greater than plants grown in the amended soil and about 63% greater than inoculated plants), nearly doubling the shoot biomass compared to inoculated plants without amendment.

Inoculation with the AM fungi stimulated foliar N, P and K contents of *R. sphaerocarpa* plants (Table 7). In general, the addition of amendment also increased significantly the contents of foliar nutrients, but these increases were not proportional to the amendment dose (Tables 4 and 7). As observed for the growth parameters, the highest N, P and K contents were seen in the plants inoculated with *G. mosseae* and in the plants inoculated with *G. intraradices* or *G. deserticola* and grown in the soil amended with the medium dose.

At the time of planting, the inoculated seedlings had significantly higher percentages of root colonization (on average, 60% of the root length was infected) than the non-inoculated plants, whose roots showed negligible levels of AM colonization (Table 6). The degree of mycorrhizal colonization of the non-inoculated seedlings increased to an average of 6% as a result of natural infection, while that of inoculated seedlings hardly varied during the 1-year growth period (Table 7). The amendment addition alone had no effect on the mycorrhizal infection of *R. sphaerocarpa* plants (Table 4). The percentages of root colonization were significantly (P<0.01) correlated with the shoot biomass and foliar N, P and K contents.

Discussion

Effect of the mycorrhizal inoculation treatments

This experiment shows that the inoculation of seedlings with AM fungi significantly stimulated the production of shoot biomass by the shrub species *R. sphaerocarpa*. The mycorrhizal inoculation treatments showed different levels

of effectiveness with respect to improving the performance of seedlings. *G. mosseae* was the most effective for increasing plant growth. It is important to emphasise that mycorrhizal inoculation with *G. mosseae* on its own was even more effective than the addition of amendment alone in improving plant growth. These results confirm the importance of mycorrhizal symbiosis for the successful growth of plants in a degraded soil, where the mycorrhizal inoculum potential is low. 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 our study, mycorrhizal inoculation appeared effective in improving nutrient content, particularly in plants inoculated with *G. mosseae*. The increased plant N content found in the mycorrhizal plants may be due to the ability of AM fungi to enhance N capture from soil and to increase P uptake, which strongly promotes biological N_2 fixation (Azcón and Barea 1992).

Arbuscular mycorrhizal fungi can affect rhizodeposition and thus the quantity and quality of organic C delivered to the soil via fungal hyphae, which in turn affects the composition, activity and size of the rhizosphere soil microflora (Wamberg et al. 2003; Alguacil et al. 2005). It should also be mentioned that mycorrhizal inoculation also has a beneficial effect on root and aboveground growth in *R. sphaerocarpa* and this greater growth is translated into a greater presence of root exudates and increased microbial activity in the rhizosphere. Indeed, the concentration of water-soluble C was higher in the soil of plants inoculated with AM fungi, particularly with *G. mosseae*. A positive correlation between the soluble C fractions and microbial activity exists in soil (Ghani et al. 2003). The soluble C fractions can be used as C and energy sources for soil microflora and may also participate in soil aggregation (Haynes and Swift 1990). Dehydrogenase activity, which has been frequently used as an indicator of soil microbial activity (García et al. 1997), responded to the treatments in a similar manner to the water-soluble C fractions, i.e., increasing with the mycorrhizal inoculation treatments. Increased microbiological activity was also revealed by the increases in urease and benzoyl argininamide hydrolysing activities. The measurement of these hydrolase activities can provide an early indication of changes in soil fertility since they are related to the mineralization of important nutrient elements such as N (Ceccanti and Garcia 1994). In our study, no significant changes were found in the content of microbial biomass C in the soil of mycorrhized plants.

Roots and associated mycorrhizal hyphae may form a three-dimensional network that aggregates small soil particles (Roldán et al. 1994; Lax et al. 1997). In our study, the percentage of stable aggregates was significantly increased by mycorrhizal inoculation with *G. mosseae*. As suggested by Bearden and Petersen (2000), the symbiosis between AM fungi and plants would have increased the stability of the soil aggregates. In fact, the percentage of colonized root length in plants inoculated with *G. mosseae* was significantly higher than for non-inoculated plants. Recent studies have indicated also that AM fungi produce a glycoprotein, glomalin, that acts as an insoluble glue to stabilise aggregates (Wright and Anderson 2000).

Effect of amendment

The results of this study demonstrate that the soluble organic fraction extracted from DOR with KOH can improve the growth and (NPK) nutrient status of *R. sphaerocarpa*. The effectiveness of amendment for stimulating the plant growth did not depend on the doses added. Phenolic compounds contained in the amendment can have toxic effects on plant growth (Leadir et al. 1997). Most phenolic acids began to manifest their phytotoxicity at a concentration of 60 mg of phenolic content per kilogram soil in lettuce and soybean plants (Martín et al. 2002). The highest dose of our amendment corresponded to application of 55 mg kg^{-1} of phenolic compounds, and this concentration was not enough to inhibit the growth of *R. sphaerocarpa* plants. A strong hydrophobic interaction between the phenols and soil particles can explain the lack of effect on the plants (Wang et al. 1967).

The effectiveness of the amendment with respect to enhancing the performance of *R. sphaerocarpa* plants relies on the improvement of soil physical properties such as aggregate stability, which in turn favours the establishment and viability of plants (Cox et al. 2001; Caravaca et al. 2002). Such organic material has persistent cementing agents such as humic polymers (Lax et al. 1997) and transient cementing agents such as polysaccharides (Lax and Garcia-Orenes 1993). The polysaccharides, water-soluble C fraction and micro-organisms seem to be particularly involved in the formation and stability of macro-aggregates larger than 0.2 mm, and humic polymers are responsible for soil micro-aggregation. In our particular case, the addition of amendment to soil significantly decreased microbial biomass C. Some authors have suggested that bacterial communities, which restrict their action to the stabilisation of aggregates measuring less than 500 μm, are inhibited by the addition of DOR due to its content of phenols (Martínez et al. 1998). The improved aggregate stability could depend on the addition of hydrophobic organic material of the KOH extract. The decrease in microbial biomass C was accompanied by an increase in soil microbial activity. These results could indicate the presence of micro-organisms more active in the amended soil than in the non-amended soil. Roldán et al. (1996) found that the restoration of soil structure may depend on the amount and nature of the organic matter added. However, we did not see variations in aggregate stability with the dose of amendment. This could be due to a low concentration of polysaccharides in this amendment in comparison with other amendments used in the recovery of soils in semi-arid areas (Roldán et al. 1996). On the other hand, this liquid amendment was equally as effective for improving aggregate stability as a composted solid urban residue used in a previous study (Caravaca et al. 2003b). In addition, this type of liquid residue has the advantage that it is easier to apply to soil than a solid residue.

Effect of the combined treatment

The benefit of the combined treatment (addition of amendment and mycorrhizal inoculation) with respect to the growth of *R. sphaerocarpa* seedlings was similar to that from each treatment individually. Only the addition of a medium dose of amendment together with mycorrhizal inoculation with *G. intraradices* or *G. deserticola* produced an additive effect on the plant growth (about 77% greater than plants grown in the amended soil and about 63% greater than inoculated plants). The extent of mycorrhizal infection is of importance when studying the influence of AM fungi on the host plant. In our experiment, shoot biomass was positively related to the colonization level of the AM fungi tested in the roots of *R. sphaerocarpa*. It is worth noting that the highest colonization levels were recorded in the combinations medium amendment dose–*G. intraradices* or *G. deserticola*, which could explain the greatest effect of these combined treatments on shoot biomass of plants. Arbuscular mycorrhizal fungi can increase the sensitivity of colonized plants to the phytotoxicity of phenolic compounds, facilitating the action or transfer of these toxic substances to plant (Martín et al. 2002). Thus, these authors observed that growth reduction of mycorrhizal plants due to the soil application of DOR was higher than in non-mycorrhizal plants. In our particular case, no negative effect of the amendment on the colonization or on the growth of the AM-inoculated plants was observed, thus confirming the hypothesis that phenolic compounds are tightly associated to soil colloids.

Conclusions

Both mycorrhizal inoculation and the addition of the KOH-soluble organic fraction extracted from composted DOR were effective in improving the performance of *R. sphaerocarpa* seedlings under our experimental conditions. The effectiveness of amendment for improving plant growth did not depend on dose. The inoculation with *G. mosseae* increased plant growth to a greater extent than the addition of the amendment, and both treatments produced similar increases in soil aggregate stability. The addition of amendment to soil significantly decreased microbial biomass C with a strong increase in soil microbial activity, which were both proportional to the dose of the amendment added. Only two of the amendment dose–fungus combinations produced an additive effect on the plant growth. It would be of interest to carry out further surveys to select synergistic combinations of amendment dose–fungus with high levels of mycorrhizal colonization, which in turn would have a major beneficial effect for the plant.

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