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Re-establishment of *Retama sphaerocarpa* as a target species for reclamation of soil physical and biological properties in a semi-arid Mediterranean area

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Abstract

Re-establishment of the indigenous shrub vegetation is a key step in the restoration of abandoned agricultural semi-arid lands. A field experiment was carried out to evaluate the effect of a composted residue and mycorrhizal inoculation with *Glomus intraradices* on the viability of *Retama sphaerocarpa* (L.) Boissier. The influence of the rhizosphere of this shrub legume on the physical–chemical and biological properties of an abandoned semi-arid agricultural area in south-eastern Spain was also assessed. Eighteen months after planting, the combined treatment of mycorrhizal inoculation and composted residue was the most effective for increasing the growth and the N and P contents in shoot tissues of *R. sphaerocarpa*. There was a highly positive significant correlation between shoot dry weight and the nutrient contents in shoot tissues. Water-soluble C, water-soluble carbohydrates, biomass C contents, and enzyme activities (dehydrogenase, urease, protease–*N*-α-benzoyl-L-argininamide (BAA), and acid phosphatase) measured in the rhizosphere of *R. sphaerocarpa* were higher than in the bare soil. Rhizosphere aggregate stability of *R. sphaerocarpa* was about 47% higher than that of bare soil. These improvements in the physical–chemical and biological properties of the rhizosphere soil of *R. sphaerocarpa* could facilitate the establishment and development of new plants in the surrounding area, which would aid the revegetation of semi-arid ecosystems.

Keywords: *Glomus intraradices*; Organic amendment; Bare soil; Legumes; Enzymatic activities and biomass C

1. Introduction

The re-establishment of the indigenous shrub vegetation in the Mediterranean basin, a practice generally encouraged by the agricultural policies of the European Union, is useful not only for restoring the characteristic biodiversity of these regions, but also for preventing the processes of erosion and desertification in their semi-arid and arid areas (Requena et al., 2001). Shrub communities, associated with other small woody plants, are characteristic of these semi-arid ecosystems, with nitrogen-fixing legumes such as *Retama sphaerocarpa* (L.) Boissier being key components of the natural succession (Herrera et al., 1993; Azcón and Barea, 1997). This shrub has a deep root system, which is functional at depths of >25 m (Haase et al., 1996)

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and provides access to deep water sources, and 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, where facilitation among plants is the dominant interaction (Callaway, 1997). These species are extremely important because their associated rhizobial symbioses constitute a source of N input for the ecosystem (Barea et al., 1992). Thus, re-establishing a shrubland is a key step in revegetation strategies. However, it is a difficult task due to both poor quality of the soil and water shortage. Moreover, the knowledge of re-afforestation strategies involving *R. sphaerocarpa* is still very scarce.

The ability of plants to establish in semi-arid and disturbed soils can be improved by colonisation with arbuscular mycorrhizal fungi (Allen, 1989). Disturbance of the vegetation cover and soil erosion generally result in the loss or reduction of mycorrhizal propagules present in the soil and thus in the subsequent reduction of the inoculum potential for mycorrhiza formation (Requena et al., 1996), which are key ecological factors governing the cycles of major plant nutrients, particularly in semi-arid Mediterranean environments. Hence, revegetation must include the reconstitution of an appropriate mycosymbiont population (Barea et al., 1990). However, there are few studies concerning mycorrhizal symbiosis with *R. sphaerocarpa* (Requena et al., 1996).

The application of organic amendments to soil is an effective method for facilitating the recovery of physical–chemical and microbiological properties of degraded soils, which in turn favours the establishment and viability of a stable plant cover (Roldán et al., 1994). The beneficial effects of organic amendments include decreased soil bulk density and increased water-holding capacity, aggregate stability, saturated hydraulic conductivity, water infiltration rate, and biochemical activity (Zebarth et al., 1999). The effectiveness of such amendments greatly depends on their chemical composition. For example, non-composted organic residues have been shown to be more effective than composted residue in activating the soil biomass which, in turn, can reactivate the biogeochemical cycles of the soil (Pascual et al., 1997). Nevertheless, some authors have suggested that organic amendments should be composted before they are applied to soil in order to achieve biological transformations of the organic matter and avoid the presence of organic substances with a low molecular weight, which can be considered phytotoxic (Gliotti et al., 1997).

The objectives of this study were: (1) to determine the viability of using *R. sphaerocarpa* as a target species in soil revegetation programs for an abandoned, semi-arid agricultural Mediterranean area, following the mycorrhizal inoculation of the seedlings and the addition of a composted organic residue to the soil, and (2) to assess whether the establishment of *R. sphaerocarpa* improves the physical, biochemical and biological properties of degraded soil.

2. Materials and methods

2.1. Study sites

The experimental area was located on the El Picarcho range in the Province of Murcia (south-east Spain) (coordinates: 1°10′W and 38°23′N). The climate is semi-arid Mediterranean with an average annual rainfall of 312 mm and a mean annual temperature of 15.3 °C; the potential evapo-transpiration reaches 813 mm per year. The predominant soils are Petrocalcic Xerosol, Petric Calcisol and Haplic Calcisol types (FAO, 1988) developed from limestones with a silt loam texture.

2.2. Materials

The composted organic residue used was the organic fraction of a municipal solid waste obtained from a municipal waste treatment plant in Murcia. The composted residue was mechanically produced by fast fermentation (60 days), mixing the waste heap daily under aerobic conditions. The analytical characteristics of the composted residue, determined by standard methods (Page et al., 1982) are shown in Table 1.

The plant used for the reafforestation experiment was *R. sphaerocarpa*, which is 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 water stress conditions and, therefore, frequently used in the reafforestation of semi-arid disturbed lands.

Table 1
Analytical characteristics of the composted residue used in the experiment

Ash (%)	44.8
pH (1:10, H_2O)	6.7
Electrical conductivity (EC, 1:5 μS cm^{-1})	4700
Total organic C (g kg^{-1})	276.0
Water-soluble C (μg g^{-1})	1950
Water-soluble carbohydrates (μg g^{-1})	76
Total N (g kg^{-1})	14.5
N-NH_4 (μg g^{-1})	3350
Total P (g kg^{-1})	3.8
Total K (g kg^{-1})	12.0
Cu (μg g^{-1})	146
Zn (μg g^{-1})	261
Ni (μg g^{-1})	25
Cr (μg g^{-1})	62.9
Cd (μg g^{-1})	5
Pb (μg g^{-1})	98

2.3. Mycorrhizal inoculation of seedlings

The mycorrhizal fungus used in the experiment was *Glomus intraradices*, obtained from the collection of the experimental field station of Zaidín, Granada (EEZ1).

Arbuscular mycorrhizal inoculum consisted of a mixture of rhizospheric soil from pure pot culture containing spores, hyphae and mycorrhizal root fragments. Once germinated, seedlings were transplanted into the growing substrate, consisting of peat and cocopeat (1:1, v:v) mixed (5%) with *G. intraradices* inoculum. The same amount of the autoclaved mixture of the inoculum 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 fertilisation treatment. Nursery procedures were conducted at Paisajes del Sur Ltd. (Granada, Spain). At the end of the nursery period, inoculated seedlings were slightly larger than non-inoculated ones, although differences in size were not statistically significant.

2.4. Experimental design and layout

The experiment was a randomised block design with two factors and four replication blocks. The first factor was the addition or not of composted organic residue to the soil, and the second factor was the direct mycorrhizal inoculation or not of *R. sphareocarpa* plants with *G. intraradices* in the nursery. Thus, four treatments were established: *R. sphareocarpa* without mycorrhizal treatment and soil without composted residue addition (control soil, C), *R. sphareocarpa* without mycorrhizal treatment and soil with composted residue addition (R), *R. sphareocarpa* mycorrhized with *G. intraradices* and soil without composted residue addition (M) and, *R. sphareocarpa* inoculated with *G. intraradices* and soil with composted residue addition (RM). In September 1999 an area of 1200 m^2 was mechanically prepared with a subsoiler. Eight rows (1 m wide, 25 m long, 3 m apart) were established. In early December 1999 half of the rows were amended following the randomised design with compost (0–20 cm depth) at a rate of 6.7 kg m^{-2}, which is sufficient to raise the soil total organic carbon content by 1%. Three weeks after the addition of the compost *R. sphareocarpa* seedlings (inoculated and non-inoculated) were planted in individual holes, at least 1 m apart in a single row and 3 m between blocks. At least, 32 seedlings per replication block were planted (eight plants × four treatments in each block).

2.5. Sampling procedures

Eighteen months after planting, four soil samples of each treatment were collected (one per block, 16 soil samples in total). Each sample consisting of five bulked subsamples (200 cm^3 soil cores) randomly collected at 0–20 cm in the rhizospheres of five individual plants. Non-rhizosphere soil samples (8 soil samples in total) were also collected from areas between plant rows and were defined as bare soil. Four plants (one per block) of each treatment were also harvested. The sampling was carried out in May 2001 before the dry season, when the highest microbial activity would be expected (Lax et al., 1997).

2.6. Physical–chemical, biological and biochemical analyses

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous extract. Total nitrogen was determined by the Kjeldhal method, and the total organic C according to Yeomans and Bremner

(1989). 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 soil aqueous extracts, water-soluble carbon (WSC) 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 by the method of Brink et al. (1960).

Microbial biomass C was determined using a fumigation–extraction method (Vance et al., 1987).

Dehydrogenase activity was determined according to García et al. (1997). For this, 1 g of soil at 60% of its field capacity was exposed to 0.2 ml of 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h at 22 °C in darkness. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with 10 ml of methanol by shaking vigorously for 1 min and filtration through a Whatman No 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

Urease and *N*-α-benzoyl-L-argininamide (BAA) hydrolysing protease activities were determined in 0.1 M phosphate buffer at pH 7; 1 M urea and 0.03 M BAA were used as substrates, respectively. Two milliliters 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 (Nannipieri et al., 1980).

Acid phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate. Two milliliters of 0.5 M sodium acetate buffer at pH 5.5 using acetic acid (Naseby and Lynch, 1997) and 0.5 ml of substrate were added to 0.5 g of 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 of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were added, and the mixture was centrifuged at 4000 rpm for 5 min. The *p*-nitrophenol (PNP) formed was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969). 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; Masciandaro et al., 1994) as substrate. This assay is based on the release and detection of PNP. Two millilitres of 0.1 M maleate buffer pH 6.5 and 0.5 ml of substrate was 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 in a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969).

2.7. Physical analysis

The percentage of stable aggregates was determined by the method described by Lax et al. (1994). A 4 g aliquot of sieved (0.2–4 mm) soil 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 put in a previously weighed capsule (T), dried at 105 °C and weighed (P_1). 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 (P_2). The percentage of stable aggregates with regard to the total aggregates was calculated by $(P_1 - P_2) \times 100/(4 - P_2 + T)$.

2.8. Percentage of colonised root, growth parameters and shoot nutrients

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

Fresh and dry (70 °C, 48 h) weights of shoots, basal stem diameters and heights of the seedlings were measured.

The concentrations of nitrogen and phosphorus in shoot tissues were calculated after digestion in nitric-perchloric acid (5:3), the P content was determined by colorimetry (Murphy and Riley, 1962) and the N was determined by Kjeldhal method.

2.9. Statistical analysis

Residue addition, mycorrhizal inoculation and their interactions effects on measured variables were tested

by a two-way analysis of variance. 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 for Windows.

3. Results

3.1. Physical–chemical properties

The pH of the *R. sphaerocarpa* rhizosphere was 0.1 unit higher than in the bare soil, reaching values similar to the bare soil after the combined treatment of mycorrhizal inoculation and addition of composted residue (Table 2). There were no significant differences between the values of electrical conductivity for the *R. sphaerocarpa* rhizosphere and the bare soil (Table 2). However, the application of organic residue significantly increased the electrical conductivity of the *R. sphaerocarpa* rhizosphere. The concentrations of total N, available P, total organic carbon (TOC), and total carbohydrates in soil did not vary with the introduction of *R. sphaerocarpa* (Table 2). Both composted residue and mycorrhizal inoculation significantly increased the available P, extractable K, TOC, water-soluble carbon (WSC), water-soluble carbohydrates (WSCH), and the percentage of stable aggregates of the *R. sphaerocarpa* rhizosphere (Tables 2 and 3), and these chemical properties were also higher in the rhizosphere soil of *R. sphaerocarpa* than in the bare soil, except for the available P. Particularly of note was the positive effect that the interaction composted residue *x* mycorrhizal inoculation had on the total N (Tables 2 and 3).

3.2. Biological and biochemical properties

Biological and biochemical parameters were higher in the rhizosphere soil of *R. sphaerocarpa* than in the

Table 2
Physical–chemical properties changes in the *R. sphaerocarpa* plantation in response to mycorrhizal inoculation (M) and composted residue (R) addition ($n = 4$)

	Bare soil	C	R	M	RM
pH (H_2O)	7.6 (0.0)	7.7 (0.0)	7.7 (0.0)	7.7 (0.0)	7.6 (0.0)
EC (1:5, μS cm^{-1})	144 (3)	143 (0)	319 (20)	151 (1)	295 (33)
Total N (g kg^{-1})	1.1 (0.1)	1.0 (0.1)	1.2 (0.0)	1.2 (0.1)	1.9 (0.0)
Available P_2O_5 (μg g^{-1})	20 (4)	16 (1)	38 (3)	24 (2)	44 (4)
Extractable K_2O (μg g^{-1})	377 (54)	415 (4)	1445 (60)	888 (29)	1857 (146)
TOC (g kg^{-1})	20.8 (0.9)	21.4 (0.1)	26.7 (0.9)	26.3 (0.0)	28.9 (1.3)
Total CH (μg g^{-1})	1956 (82)	2063 (157)	2406 (102)	2113 (107)	2214 (125)
Water-soluble C (μg g^{-1})	134 (6)	178 (6)	266 (10)	204 (6)	296 (26)
Water-soluble CH (μg g^{-1})	1 (0)	2 (0)	6 (1)	2 (0)	8 (1)
Aggregate stability (%)	19.5 (3.0)	28.7 (0.8)	37.1 (0.9)	37.5 (2.6)	37.1 (0.6)

Standard error for each measure is given in parenthesis. TOC: total organic carbon; Total CH: total carbohydrates; Water-soluble C: water-soluble carbon; Water-soluble CH: water-soluble carbohydrates; Total N: total nitrogen. C: control soil, without mycorrhizal inoculation and without composted residue addition and RM: composted residue addition + mycorrhizal inoculation.

Table 3
Two factors ANOVA (mycorrhizal inoculation and residue addition) for physical and chemical properties studied*

Source of variation	Total N	Available P	Extractable K	TOC	TCH	WSC	WSCH	AS
Residue (R)	0.0000	0.0000	0.0000	0.0003	0.1007	0.0000	0.0000	0.0195
Mycorrhiza (M)	0.0000	0.0290	0.0001	0.0008	0.5874	0.0780	0.0706	0.0126
R × M	0.0022	0.7347	0.7135	0.1252	0.3606	0.8933	0.0565	0.0126

TOC: total organic carbon; Total CH: total carbohydrates; Water-soluble C: water-soluble carbon; Water-soluble CH: water-soluble carbohydrates; Total N: total nitrogen; AS: aggregate stability.

* *P* significance values.

Table 4
Biochemical properties changes in the *R. sphaerocarpa* plantation in response to mycorrhizal inoculation (M) and composted residue (R) addition ($n = 4$)

	Bare soil	C	R	M	RM
Microbial biomass C (μg g^{-1})	413 (7)	529 (25)	458 (17)	604 (22)	743 (39)
Dehydrogenase (μg INTF g^{-1}soil)	101 (11)	119 (3)	159 (1)	140 (5)	146 (7)
Urease (μmol NH_3 g^{-1} h^{-1})	0.26 (0.03)	0.56 (0.02)	1.29 (0.08)	0.61 (0.07)	1.29 (0.24)
Protease–BAA (μmol NH_3 g^{-1} h^{-1})	0.40 (0.05)	0.58 (0.01)	0.79 (0.02)	1.01 (0.08)	1.14 (0.05)
Acid phosphatase (μmol PNP g^{-1} h^{-1})	43 (7)	77 (4)	113 (12)	89 (3)	82 (8)
β-Glucosidase (μmol PNP g^{-1} h^{-1})	66 (2)	66 (0)	112 (4)	92 (1)	127 (3)

Standard error for each measure is given in parenthesis. C: control soil, without mycorrhizal inoculation and without composted residue addition and RM: composted residue addition + mycorrhizal inoculation.

Table 5
Two factors ANOVA (mycorrhizal inoculation and residue addition) for biological and biochemical properties studied*

Source of variation	Microbial C-biomass	Dehydrogenase	Urease	Protease–BAA	Phosphatase	Glucosidase
Residue (R)	0.2385	0.0005	0.0002	0.0061	0.0722	0.0000
Mycorrhiza (M)	0.0000	0.4094	0.8533	0.0000	0.2322	0.0000
R × M	0.0023	0.0043	0.8388	0.4835	0.0147	0.0903

* *P* significance values.

bare soil, except for the β-glucosidase activity (Table 4). The rhizosphere of the selected target species particularly stimulated the enzymatic activities involved in the N (urease) and P (acid phosphatase) cycles, by about 115 and 79%, respectively, compared to bare soil. The addition of composted residue had a much greater effect on the enzymatic activities in the rhizosphere soil of *R. sphaerocarpa* than mycorrhizal inoculation (Tables 4 and 5). In fact, dehydrogenase, urease, protease–BAA, and β-glucosidase activities were significantly increased by the composted residue addition to soil. Only the mycorrhizal inoculation and the mycorrhizal inoculation in combination with the composted residue had a significant effect ($P < 0.01$) on the soil microbial biomass C.

3.3. Mycorrhizal colonisation, plant growth, and nutrients in shoot tissues

The percentage of plant survival was about 60% in each treatment and there were no significant differences between treatments. The percentage of mycorrhizal root length was only increased significantly by the mycorrhizal inoculation treatment (Fig. 1 and Table 6). The natural colonisation observed in the uninoculated seedlings was less than 10% in both control soil and soil treated with composted residue. Composted residue and mycorrhizal inoculation treatments increased the growth parameters and nutrient (N and P) contents in shoot tissues of *R. sphaerocarpa* plants (Fig. 1). The highest increases in basal diameter (about 67% above control), shoot dry weight (about

Table 6
Two factors ANOVA (mycorrhizal inoculation and residue addition) for root colonisation, growth parameters and nutrient contents in shoot tissues of *R. sphaerocarpa**

Source of variation	Root colonisation	Height	Shoot dry weight	Basal diameter	Shoot N	Shoot P
Residue (R)	0.3663	0.6182	0.0024	0.0051	0.0001	0.0000
Mycorrhiza (M)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
R × M	0.0553	0.1669	0.7901	0.4568	0.1999	0.0144

* *P* significance values.

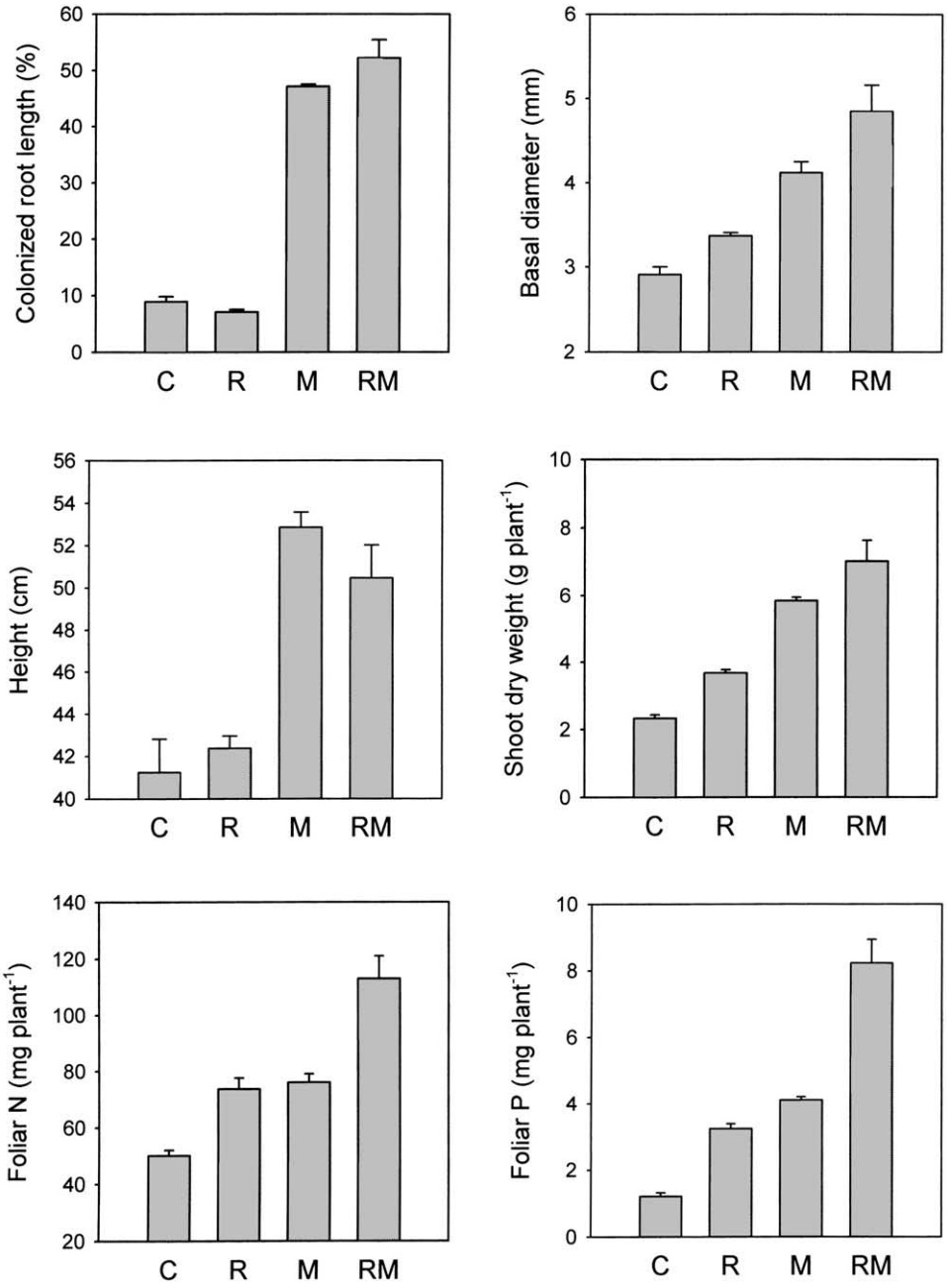

Fig. 1. Root colonisation, growth parameters and nutrient contents in shoot tissues changes in the *R. sphaerocarpa* plantation following the different treatments (C: control soil, without mycorrhizal inoculation and without composted residue addition; R: composted residue addition; M: mycorrhizal inoculation; RM: composted residue addition + mycorrhizal inoculation). Bars represent standard deviation for each measure ($n = 4$).

200% above control), and N (about 125% above control) and P (about 575% above control) assimilated in shoot tissues were recorded in the *R. sphaerocarpa* plants inoculated with *G. intraradices* and grown in amended soil.

4. Discussion

Our results showed an additive positive effect of composted residue and mycorrhizal inoculation on the growth of *R. sphaerocarpa* in semi-arid conditions. Arbuscular mycorrhiza was more effective for improving the performance of this legume in an amended soil than in an unamended soil. This result disagrees with the widely accepted idea that mycorrhizae present little advantage to seedlings grown in amended soils (Yanai et al., 1995). The fact that the highest contents of P and N in shoot tissues occurred for seedlings grown in amended soil and inoculated with *G. intraradices* might explain why the growth of *R. sphaerocarpa* was greatest in this treatment. There was a highly positive significant correlation between shoot dry weight and nutrient contents in shoot tissues (Table 7). The increased plant N content found in the mycorrhizal plants may be due to the ability of AM fungi to enhance the decomposition of organic material and increase nitrogen capture from organic material (Hodge et al., 2001), and to increase P uptake, which strongly promotes biological N_2 fixation (Azcón and Barea, 1992). It is worth noting that the combined treatment of mycorrhizal inoculation and composted residue caused the greatest increase in the level of total N in the rhizosphere of *R. sphaerocarpa*. The rapid growth of seedlings inoculated with *G. intraradices,* as compared with the uninoculated seedlings, might be related to the high dependence of woody legumes on mycorrhizae, especially in stressed ecosystems (Herrera et al., 1993). It is important to emphasise that mycorrhizal inoculation on its own was even more effective than the addition of composted residue alone to soil for improving the performance of *R. sphaerocarpa* plants, even though the available P in the rhizosphere soil treated with composted residue was higher than in the rhizosphere soil of plants inoculated with *G. intraradices*.

Table 7
Pearson rank correlation between physical, chemical and biochemical parameters ($n = 4$)[a]

	Aggregate stability	Shoot dry weight
TOC	0.5922*	0.8338***
Total CH	0.5238*	ns
Total N	ns	0.7703***
Available P	0.5440*	0.6068*
Extractable K	0.5320*	0.7010**
C-Biomass	ns	0.8178***
Dehydrogenase	0.7421**	ns
Urease	ns	ns
Protease–BAA	0.7298**	0.9323***
Acid phosphatase	ns	ns
β-Glucosidase	0.6944**	0.7008**
Root colonisation	ns	0.9296***
Shoot N	ns	0.8802***
Shoot P	0.5330*	0.8501***

*, **, *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. ns = not significant.

[a] Correlation coefficient (significance level).

Restoration of the physical properties of degraded soils is a precondition for the control of desertification. The percentage of stable aggregates in the rhizosphere soil of *R. sphaerocarpa* was significantly higher than in the bare soil. Plant roots increase the stability of surrounding aggregates through several interacting mechanisms. Roots and associated mycorrhizal hyphae may form a three-dimensional network that enmeshes fine particles of soil into aggregates. In addition, the organic C released by roots promotes a dense microbial community in the immediate environment of the root, which, in turn, produces exocellular mucilaginous polysaccharide material that has the capacity to stabilise soil aggregates (Jastrow et al., 1998). In our experiment, the effect of rhizosphere soil of *R. sphaerocarpa* on the stability of aggregates might be attributed to a greater degree of biological activity in the rhizosphere soil of *R. sphaerocarpa*. In fact, biomass C, dehydrogenase activity, WSC and WSCH contents were higher in the rhizosphere soil of *R. sphaerocarpa*, and these parameters have been used frequently as indicators of soil microbial activity (De Luca and Keeney, 1993; García et al., 1997).

It is generally recognised that the benefits of organic amendment are not only due to the supply of nutrient elements, but also to the improvement of the soil's physical characteristics (Roldán et al., 1996; McCoy, 1998). Such material has a cementing effect, due to the polysaccharides present (Lax and García-Orenes, 1993), and reactivates microbial populations (Roldán

et al., 1994) to such an extent that it has been used as an amendment in the recovery of soils in semi-arid areas (Roldán et al., 1996). Composted residue significantly improved the structural stability of rhizosphere soil of *R. sphaerocarpa*. In our particular case, there was a very significant increase in the levels of TOC, soluble C fractions, biomass C, and dehydrogenase activity, indicating that the agents responsible for aggregate stabilisation were mainly of biological origin. In this regard, significant correlations were found between soil stable aggregates, TOC, total carbohydrates, dehydrogenase, protease–BAA, and β-glucosidase (Table 7). On the other hand, this type of composted residue is less effective than uncomposted residue for improving soil structure (Caravaca et al., 2001), although, on the positive side, its use leads to fewer problems related to toxic substances, which are eliminated during the composting process (Pascual et al., 1999). Thus, the biological transformations that a compost undergoes in the waste treatment plant reduce the quantity of chemical aggregate-stabilising agents, such as polysaccharides or water-soluble organic matter and increase the number of carbon fractions more resistant to rapid decomposition.

Fungal populations are principally responsible for the formation of aggregates larger than 0.2 mm (Lax et al., 1997; Andrade et al., 1998). In our study the percentage of stable aggregates was significantly increased by mycorrhizal inoculation. As suggested by Bearden and Petersen (2000), the symbiosis between arbuscular mycorrhizal fungi and plants would have increased the stability of the soil aggregates. In fact, the percentage of colonised root length in plants inoculated with *G. intraradices* was significantly higher than for non-inoculated plants. Recent studies have indicated also that arbuscular mycorrhizal fungi produce a glycoprotein, glomalin, that acts as an insoluble glue to stabilise aggregates (Wright and Anderson, 2000).

In conclusion, the most effective treatment for the re-establishment of *R. sphaerocarpa* in a semi-arid Mediterranean area was that which involved the joint treatment of soil and plant, i.e. the combined treatment of mycorrhizal inoculation of seedlings and composted residue addition to soil. Finally, the improvements which occurred in the physical–chemical and biological properties of the rhizosphere soil of *R. sphaerocarpa* could facilitate the establishment and development of new plants in the surrounding area, which would aid the revegetation of semi-arid ecosystems.

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