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Alteration in Rhizosphere Soil Properties of Afforested *Rhamnus lycioides* Seedlings in Short-Term Response to Mycorrhizal Inoculation with *Glomus intraradices* and Organic Amendment

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ABSTRACT / The reestablisment of autochthonous plant species is an essential strategy for recovering degraded areas under semiarid conditions. A field experiment was carried out to assess the short-term effect of two reafforestation methods involving mycorrhizal inoculation and compost addition on soil quality parameters and *Rhamnus lycioides* seedling growth. The nutrient content (NPK) and enzymatic activities (dehydrogenase, urease, protease-BAA, acid phosphatase and β-glucosidase) increased and bulk density decreased in the rhizosphere soil with the organic amendment. Biomass C of rhizosphere soil increased by at least 240% with respect to the control soil after mycorrhizal inoculation and the combination of compost addition + mycorrhizal inoculation. Both mycorrhizal inoculation and composted organic residue addition increased *R. lycioides* seedling growth in the same proportion. In the short term, we conclude that the application of both reafforestation methods not only enhances the establishment of *R. lycioides* seedlings, but also improves soil quality.

Reafforestation programs are necessary in semiarid areas where the main cause of soil degradation has been the loss of natural plant cover. In such areas, the resulting desertification disrupts plant–microbe symbioses, which represent a critical ecological factor in helping further plant growth in degraded ecosystems (Requena and others 2001).

The deterioration of the physical, chemical, and biological properties of soils is particularly troublesome in semiarid Mediterranean areas because of the gradual decrease in their organic matter content. The improvement of soil quality properties should be an essential step to establishing vegetation. To help in this task, the application of organic residues such as urban waste has gained renewed interest as a means of increasing soil organic matter content and improving soil quality. It has been extensively demonstrated that the addition of organic matter improves soil structure by decreasing its bulk density and increasing its aggregate stability and biochemical activity (Roldán and others 1996a; Zebarth and others 1999). García and others (2000) have demonstrated the beneficial effect of an uncomposted residue on the performance of *Pinus halepensis* seedlings inoculated with an ectomycorrhizal fungus. However, the effect of composted organic residue on the rhizosphere soil has not been studied during reafforestation projects.

Several shrub species such as *R. lycioides*, which form symbioses with arbuscular mycorrhizal fungi (AMF) are characteristic of these semiarid ecosystems. AMF may enhance plant growth by improving the supply of nutrients of low mobility in the soil, such as phosphorus (P) (Tarafdar and Marschner 1994), and scavenging in nutrient-poor conditions. Both the formation and the activity of arbuscular mycorrhizal fungi can be affected by the organic matter content of soil, mainly because of the saprotrophic capability of AMF (Hodge and others 2001) and alteration of the soil physical properties such as porosity, water-holding capacity, etc. (Andrade and others 1998).

In semiarid Mediterranean areas, the establishment of plants is made difficult by the severe climate, characterized by low and irregular precipitation and fre-

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quent drought periods. Under these conditions, the first stages of growth are most critical for predicting the success of soil reafforestation (Perry and others 1987). For this reason, we investigated the relationships between mycorrhizae and soil properties after only one year of planting. Furthermore, measurements carried out in the rhizosphere of soil provide more direct information concerning the interaction between mycorrhizal symbiosis and composted residue.

The objectives of this study were: (1) to determine the viability of *Rhamnus lycioides* seedlings for use in soil revegetation programs in a semiarid Mediterranean area, and (2) to test the short-term influence of mycorrhizal inoculation of the seedlings and the addition of composted organic residue on the chemical, physical, and biochemical parameters in the rhizosphere soil.

Materials and Methods

Study Site

The experimental area was located on El Picarcho range in the Province of Murcia (southeast Spain) (coordinates: 1°10′W and 38°23′N). The climate is semiarid Mediterranean with an average annual rainfall of 312 mm and a mean annual temperature of 15.3°C; the potential evaportranspiration reaches 813 mm/yr. The soil used was a Petrocalcic Xerosol (FAO 1988), developed from limestones with a silt loam texture. The plant cover is sparse (less than 20% canopy cover) and degraded due to ancient grazing and logging. In this area, dwarf shrubs (<1 m high) such as *Rosmarinus officinalis* and *Stipa tenacissima* grass are very common, constituting more than 98% of plant cover in this area.

Material Description

The composted organic residue used was the organic fraction of municipal solid waste obtained from a municipal waste treatment plant in Murcia. The composted organic residue was mechanically produced by fast fermentation (60 days), mixing the waste heap daily under aerobic conditions (García and others 1990). The analytical characteristics of the composted organic residue, determined by standard methods (Page and others 1982), are shown in Table 1.

The plant used for the reafforestation experiment was *R. lycioides*, which is a low-growing shrub reaching a height of 1.5 m and widely distributed in the Mediterranean area. It is also well adapted to hydric stress conditions and, therefore, potentially could be used in the reafforestation of semiarid disturbed lands.

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

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

Ash (%)	44.8
pH (1:10)	6.7
Electrical conductivity EC (1:5, μS/cm)	4700
Total Organic C (g/kg)	276.0
Water-soluble C (μg/g)	1950
Water-soluble carbohydrates (μg/g)	76
Total N (g/kg)	14.5
C/N	19
$N\text{-}NH_3$ (μg/g)	3350
Total P (g/kg)	3.8
Total K (g/kg)	12.0
Cu (μg/g)	146
Zn (μg/g)	261
Ni (μg/g)	25
Cr (μg/g)	62.9
Cd (μg/g)	5
Pb (μg/g)	98

Mycorrhizal Inoculation of Seedlings

Arbuscular mycorrhizal inoculum consisted of a mixture of rhizospheric soil from trap cultures containing spores (20 spores/g), hyphae, and mycorrhizal root fragments. Once seeds of *R. lycioides* had germinated, seedlings were transplanted into the growth substrate consisting of peat and cocopeat (1:1, v/v) mixed (5% by volume) with *G. intraradices* inoculum. Control plants were treated with the same amount of autoclaved inoculum (100°C for 1 hour during three consecutive days), supplemented with a filtrate (<20 μm) of the inoculum to provide the microbial populations accompanying the mycorrhizal fungi (Hodge and others 2001). Inoculated plants were allowed to grow for 8 months under greenhouse conditions without any fertilization treatment. Greenhouse procedures were conducted at Paisajes del Sur Ltd. (Granada, Spain). At the end of the nursery period, inoculated seedlings were slightly larger than noninoculated ones, although differences in size were not statistically significant. The *G. intraradices*-inoculated *R. lycioides* seedlings had significantly higher percentages of root colonization (on average 38% of the root length was infected) than the noninoculated plants, whose roots showed negligible levels of AM colonization.

Experimental Design and Layout

The experiment was a randomized 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 mycorrhizal inoculation or not of *R. lycioides* plants with *G. intraradices* in the nursery. More specifically, four treatments were established: (1) *R. lycioides* without mycorrhizal treat-

ment and soil without composted residue addition (control soil, C), (2) *R. lycioides* without mycorrhizal treatment and soil with composted residue addition (R), (3) *R. lycioides* inoculated with *G. intraradices* and soil without composted residue addition (M), and (4) *Rhamnus lycioides* 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 the rows were amended following the randomized 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 (C) content by 1%. Three weeks after the addition of the compost, *R. lycioides* seedlings (inoculated and uninoculated) were planted in individual holes, at least 1 m apart in a single row and with 3 m between blocks. At least 32 seedlings per replication block were planted (8 plants × 4 treatments in each block).

Sampling Procedures

One year after planting, four soil samples from each treatment were collected (16 soil samples in total). Each sample consisted of five bulked subsamples (200 cm^3 soil cores) randomly collected at 0–20 cm in the rhizosphere of five individual plants. The sampling was carried out in early December after the autumn rainy season, when the highest microbial activity could be expected (Lax and others 1997). Field-moist soil samples were divided into two subsamples. One soil subsample was sieved at 2 mm and stored at 4°C for biochemical analysis and the soil subsample was allowed to dry at room temperature. An aliquot of air-dried soil was sieved at 2 mm for chemical analysis and another at 0.2–4 mm for aggregate stability measurements.

Chemical and Biochemical Analyses

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous solution. Total nitrogen was determined by the Kjeldhal method, and 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). Soil potassium (K) was extracted with ammonium acetate, and the extract was determined by flame photometry (Schollemberger and Simon 1954). 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 and others (1960) and polyphenol-soluble compounds by the method of Kuwatsuka and Shindo (1973).

Microbial biomass C was determined using a fumigation-extraction method (Vance and others 1987). Ten grams of soil at 60% of its field capacity are fumigated in a 125-ml Erlenmeyer flask with purified $CHCl_3$ for 48 hours. After removal of residual $CHCl_3$, 40 ml of 0.5M K_2SO_4 solution is added and the sample is shaken for 1 hour before filtration of the mixture. The K_2SO_4-extracted C was measured as indicated for WSC and microbial biomass C is calculated as the difference between fumigated and nonfumigated samples.

Dehydrogenase activity was determined according to García and others (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 hours at 22°C in darkness. The INTF (iodonitrotetrazolium formazan) formed was extracted with 10 ml of methanol by shaking vigorously for 1 min and filtering through a Whatman No. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

Urease and *N*-α-benzoyl-L-argininamide (BAA) hydrolyzing 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. Buffer (2 ml) and substrate (0.5 ml) 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 and others 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 by spectrophotometry 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 and others 1994) as substrate. This assay is based on the release and detection of PNP. Two milliliters 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 trishydroxymethyl aminomethano (THAM) according to Tabatabai (1982). The amount of PNP was determined by spectrophotometry at 398 nm (Tabatabai and Bremner 1969).

Table 2. Changes in chemical properties of rhizosphere soil in response to mycorrhizal inoculation and composted residue addition[a]

	C	R	M	RM
pH (H_2O)	8.60 ± 0.05	8.39 ± 0.05	8.62 ± 0.04	8.43 ± 0.05
EC (1:5, μS/cm)	220 ± 5	341 ± 36	220 ± 10	313 ± 5
Total N (g/kg)	1.2 ± 0.1	1.7 ± 0.3	0.9 ± 0.1	1.5 ± 0.1
Available P_2O_5 (μg/g)	8 ± 2	49 ± 0	8 ± 2	32 ± 2
Extractable K_2O (μg/g)	311 ± 66	989 ± 75	386 ± 18	980 ± 66
TOC (g/kg)	24.7 ± 0.9	28.8 ± 1.0	23.9 ± 0.1	30.6 ± 0.9
Total CH (μg/g)	1969 ± 118	3090 ± 363	2353 ± 158	2488 ± 118
Water-soluble C (μg/g)	210 ± 25	290 ± 16	201 ± 5	287 ± 25
Water-soluble CH (μg/g)	8 ± 2	16 ± 2	8 ± 1	11 ± 2
Water-soluble PP (μg/g)	6 ± 2	15 ± 3	5 ± 0	18 ± 2

[a]Values are mean ± standard error ($N = 4$). EC, electrical conductivity; TOC, total organic carbon; CH, carbohydrate; PP, polyphenols. C, control soil; R, compost residue alone; M, mycorrhizal inoculation alone; RM, combination of compost residue and mycorrhizal inoculation.

Physical Analysis

The percentage of water-stable aggregates was determined by the method described by Lax and others (1994). A 4-g aliquot of sieved (0.2–4 mm) soil was placed on a 0.250-mm sieve and wetted by spray. After 15 min, the soil was subjected to an artificial rainfall of 150 ml with an energy of 270 J/m^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 hours, 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 $(P_1 - P_2) \times 100/(4 - P_2 + T)$. Bulk density was determined by the paraffin method described by Barahona and Santos (1981) after maintaining soil moisture at 60% of field capacity for one month.

One year after planting, four plants (one per block) from each treatment were harvested. Basal stem diameter and height of plants were measured with callipers and rulers. Plant tissues were ground before chemical analysis. The foliar concentration of phosphorus was determined after digestion in nitric-perchloric acid (5:3) for 6 hours. The P was determined by colorimetry (Murphy and Riley 1962). The percentage of root length colonized by *Glomus intraradices* was calculated by the gridline intersect method (Giovannetti and Mosse 1980) after staining with trypan blue (Phillips and Hayman 1970).

Statistical Analysis

Effects of residue addition and mycorrhizal inoculation, and their interaction on measured variables were tested by a two-way analysis of variance and comparisons among means were made using the least significant difference (LSD) multiple-range test, calculated at $P < 0.05$. Pearson's correlation coefficients between all the soil parameters were assessed. Statistical procedures were carried out with the software package Statgraphics for Windows 7.0.

Results and Discussion

Chemical Parameters

The addition of composted residue lowered the pH by at least 0.17 unit and increased electrical conductivity by at least 93 μS/cm in the rhizosphere soil (Table 2). However, no changes were found after the mycorrhizal inoculation treatment.

The NPK content of the rhizosphere soil significantly increased with organic amendment, the highest concentrations of these nutrients being reached in the soil treated with composted residue (R) and in the soil treated with composted residue and mycorrhizal inoculation (RM) (Table 2). Of particular note was the fact that the combination of mycorrhizal inoculation and composted residue addition increased the extractable P content to a lesser extent than the addition of composted residue alone (R). That might be due to the capacity of the fungus to increase available P uptake from composted residue (Nadian and others 1996, Linderman and Davis 2001) or to the drop in rhizosphere pH from 8.60 to 8.43 with the combined treatment of composted residue addition and mycorrhizal inoculation (also reported by Gahoonia and Nielsen 1992). The fact that the highest content of P in leaves occurred for seedlings inoculated with *G. intraradices* demonstrated a higher accumulation of P as a consequence of mycorrhizal inoculation (Figure 1).

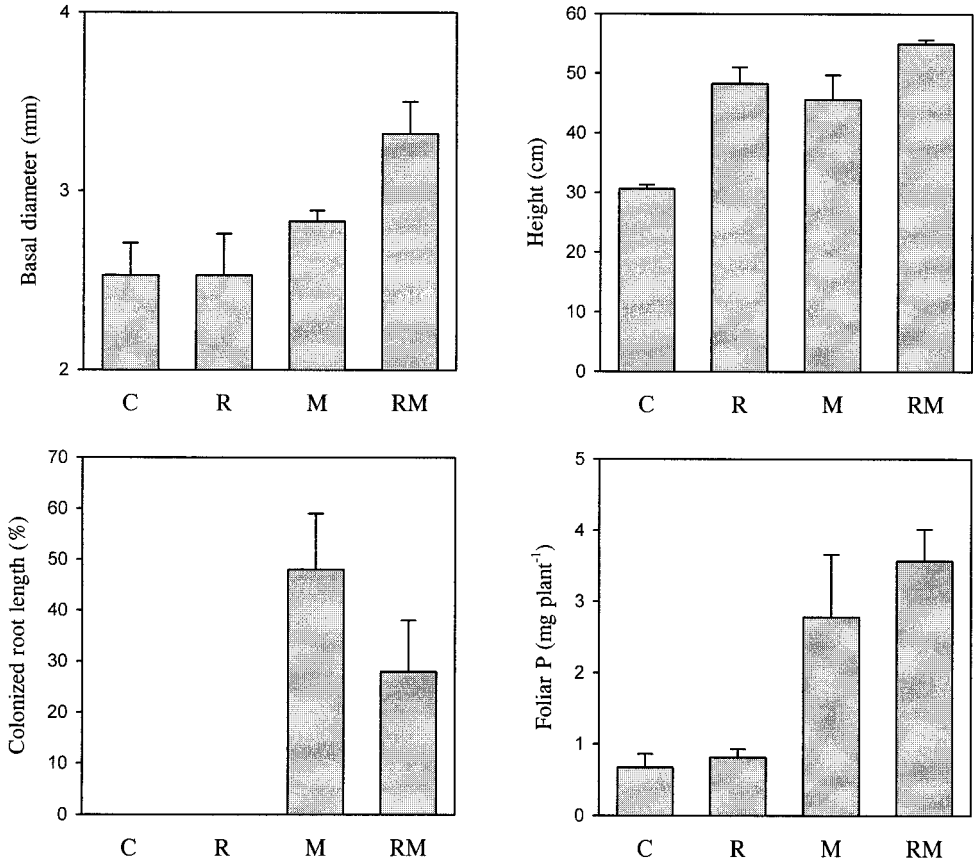

Figure 1. Effect of composted residue addition and mycorrhizal inoculation on growth, root mycorrhizal colonization, and foliar P content of *Rhamnus lycioides* under field conditions. C, control soil; R, compost residue alone; M, mycorrhizal inoculation alone; RM, combination of compost residue and mycorrhizal inoculation. Bars represent standard error for each measure ($N = 4$).

The highest increases in total organic carbon (TOC) and all C fractions (total carbohydrates, water-soluble C, water-soluble carbohydrates, and water-soluble polyphenols) were observed in R- and RM-treated soils due to composted residue addition (Tables 2 and 3). One year after planting, at least 40% of the TOC added remained in the amended soils. The water-soluble C fractions, which are considered good indicators of a soil potential microbial activity (Ceccanti and García 1994) and soil quality (Bolinder and others 1999), represented a small proportion of the TOC in all treatments (≤1%). Hence, the increases observed in the water-soluble fractions may indicate an improvement of rhizosphere soil microbial activity due to organic amendment.

Biochemical Parameters

Over short periods, changes in microbial biomass C can be a sensitive index of changes in the organic matter content of a soil (Piao and others 2001). In our experiment the highest increases in biomass C occurred with mycorrhizal inoculation and the combined treatment of composted residue addition and mycorrhizal inoculation (Tables 3 and 4), with increases of 349% and 242%, respectively, over the control soil. The positive effect of mycorrhizal inoculation on microbial biomass has also been described by García and others (2000), although these authors used an ectomycorrhizal fungus, *Pisolithus arhizus*, in the reafforestation of degraded zones with *Pinus halepensis* seedlings.

The addition of organic materials to soil may encourage microbial activity to an extent that is closely related to the amount and nature of the organic matter added (Roldán and others 1996). Thus, uncomposted organic amendment, rich in easily biodegradable compounds, is more effective in stimulating the microbial activity of soil than composted organic amendment. In addition, the promoting effect of organic amendment on soil microbial biomass declines rapidly with time, especially in soils of degraded semiarid zones. In our experiment, the addition of composted residue had no effect on the soil microbial biomass C (Tables 3 and 4).

Table 3. Two factorial ANOVA for mycorrhizal inoculation and composted residue addition

	F values[a]		
	Composted residue (R)	Mycorrhiza (M)	Interaction (R × M)
pH (H_2O)	19.865***	0.458	0.115
EC	99.266***	0.766	1.438
Total N	53.388***	11.550**	0.195
Available P	550.245***	33.205***	37.853***
Extractable K	140.851***	1.067	0.119
TOC	112.047***	0.104	0.723
Total CH	8.421*	0.253	5.184*
Water-soluble C	29.502***	0.142	0.033
Water-soluble CH	11.455**	2.104	1.662
Water-soluble PP	36.644***	0.473	0.927
Biomass C	0.013	49.749***	8.783*
Dehydrogenase	35.108***	0.011	0.297
Urease	59.460***	10.431**	9.538**
Protease-BAA	33.100***	4.222	1.613
Acid phosphatase	18.980***	3.902	0.264
β-Glucosidase	16.328**	1.485	0.498
Aggregate stability	0.056	0.566	1.673
Bulk density	6.422*	0.682	1.110
Mycorrhizal root	0.409	33.854***	0.409
Foliar P	0.829	23.129***	0.415
Basal diameter	1.024	5.126*	1.024
Height	9.940**	6.434*	0.937

[a]*, **, *** Significant at $P < 0.05$, <0.01, and <0.001, respectively.

Table 4. Changes in biochemical and physical properties in response to mycorrhizal inoculation and composted residue addition[a]

	C	R	M	RM
Microbial biomass C (μg/g)	213 ± 27	424 ± 14	957 ± 143	728 ± 27
Dehydrogenase (μg INTF/g soil)	103 ± 20	161 ± 4	96 ± 6	166 ± 20
Urease (μmol NH_3/g/h)	0.63 ± 0.19	1.20 ± 0.14	0.65 ± 0.07	1.98 ± 0.19
Protease-BAA (μmol NH_3/g/h)	0.89 ± 0.03	1.36 ± 0.10	1.11 ± 0.02	1.41 ± 0.03
Acid phosphatase (μmol PNP/g/h)	66 ± 5	84 ± 3	55 ± 1	77 ± 8
β-glucosidase (μmol PNP/g/h)	87 ± 5	113 ± 11	72 ± 8	109 ± 5
Aggregate stability (%)	57.1 ± 4.2	52.6 ± 2.6	55.5 ± 2.5	58.6 ± 4.2
Bulk density (g/liter[1])	1130 ± 18	1060 ± 16	1120 ± 6	1100 ± 18

[a]Mean ± standard error ($N = 4$). C, control soil; R, compost residue alone; M, mycorrhizal inoculation alone; RM, combination of compost residue and mycorrhizal inoculation.

Dehydrogenase activity, which some consider as a marker of soil microbial activity (García and others 1997), was significantly increased with the addition of composted residue (Tables 3 and 4). It seems, then, that application of composted residue may repress or induce the synthesis of enzymes without affecting overall microbial activity, as suggested by Nannipieri and others (1990).

Application of the composted residue increased the activity of hydrolases in the rhizosphere soil (Table 4), probably because it contains endo- or exocellular enzymes and substrates able to promote enzyme synthesis (Giusquiani and others 1995, García and others 2000). The phosphatase activity of rhizosphere soil may be stimulated in the presence of easily hydrolyzed substrates (Tarafdar and Claassen 1988), but repressed by nonhydrolyzable forms of organic P (Azcón and others 1982). In our experiment, phosphatase activity at pH 5.5 increased as a result of amendment with composted organic residue (Table 4). In contrast, mycorrhizal inoculation led to a decrease in acid phosphatase activity in the soil not receiving organic amendment. Studies on the influence of arbuscular mycorrhizal fungi on acid phosphatase have shown variable responses, which

Table 5. Correlation coefficients between chemical, biochemical, and physical parameters of rhizosphere soil ($N = 4$)

	TOC	TCH	WSC	WSCH	WSPP
Bulk density	NS	−0.953*	NS	−0.995**	−0.944*
Dehydrogenase	0.999***	NS	0.997**	NS	0.991**
Urease	NS	NS	NS	NS	0.942*
Protease-BAA	NS	NS	NS	NS	NS
Acid phosphatase	0.924*	NS	0.937*	NS	NS
β-Glucosidase	0.963*	NS	0.968*	NS	0.929*

[a]TOC, total organic carbon; CH, carbohydrate; PP, polyphenols. *, **, *** Significant at $P < 0.05$, <0.01, and <0.001, respectively. NS: not significant.

could be related to the use of different host-endophyte combinations (Rao and Tarafdar 1993). Joner and Jakobsen (1995) concluded that acid phosphatase activity is not altered by the presence of AMF, but the quantity exuded by the mycorrhizal roots is reduced, although their results were based on *Cucumis sativus* L., cv. Amin-ex–*Glomus invermaium* combination.

Physical Parameters

It is generally recognized that the benefits of organic amendment are not only due to the supply of nutrients, but also to the improvement of the soil physical characteristics (Roldán and others 1996, McCoy 1998). Changes in the aggregate stability of rhizosphere soil have often been attributed to organic compounds released by roots, such as polysaccharides (Oades 1984). Furthermore, mycorrhizal inoculation favors the proliferation of fungal hyphae, which, in turn, have a beneficial effect on soil aggregate stability (Andrade and others 1998). However, neither of the reafforestation methods used in the present study had any effect on rhizosphere soil aggregate stability (Tables 3 and 4); the percentage of stable aggregates remained constant in all treated soils one year after planting. This lack of change in soil aggregate stability may be attributed to the short duration of the experiment. On the other hand, the biological transformations that a compost undergoes in the waste treatment plant reduce the quantity of chemical aggregate-stabilizing agents, such as polysaccharides or water-soluble organic matter and increase the number of carbon fractions most resistant to rapid decomposition (Roldán and others 1996). Furthermore, the dose applied was not high enough to result in significant differences in the aggregate stability with respect to the untreated soil (C).

The application of organic materials reduces the soil bulk density and automatically increases total porosity, which has a positive effect on plant growth. In a compacted soil, mycorrhizal symbiosis can enhance the uptake of nutrients by plant roots since external hyphae of an extensive mycelium penetrates small pores more easily than roots (Nadian and others 1996). Soil bulk density was significantly lower in the amended soil (R) than unamended soils (C and M) (Table 4). There was a highly significant correlation ($P < 0.05$) between soil bulk density and the total carbohydrate and water-soluble carbohydrate contents, which indicates the contribution of the polysaccharides in the rhizosphere soil to improvement of soil structure (Table 5). A decrease in soil bulk density caused by organic residue addition is commonly found (Giusquiani and others 1995, Zebarth and others 1999), although the decreases observed are generally greater than those observed in the present study. The small differences found between the bulk density of R treatment and the control soil were attributed to the same causes already mentioned to explain the observed unchanging soil aggregate stability.

It has been suggested that improvement in soil physical properties, particularly soil porosity, may affect its biological and biochemical activities, including enzymatic activities (Giusquiani and others 1995). However, we found no significant correlation between soil enzyme activities and soil bulk density.

Mycorrhizal Infection and Growth of *Rhamnus lycioides*

Figure 1 shows data on the percentages of root mycorrhizal colonization corresponding to the plants randomly sampled one year after planting. The absence of mycorrhizal colonization of the noninoculated seedlings indicated that indigenous AMF had not colonized the control plants (C) and the plants grown in the compost amended soil (R). The inoculated seedlings grown in amended and nonamended soils shows the highest percentages of root colonization, and there were no significant differences among them.

Only the combined treatment of composted residue addition and mycorrhizal inoculation increased the basal diameter growth of *R. lycioides* seedlings (31%; Figure 1). Linderman and Davis (2001) also found that

inoculation with *G. intraradices* in combination with composted grape pomace had growth-stimulating effects on *Allium cepa* plants under P-limiting conditions. Both reafforestation methods increased seedling height growth by at least 49% with respect to noninoculated seedlings grown in the unamended soil. It is important to emphasize this effect of mycorrhizal inoculation on *R. lycioides* seedling growth, which was similar to that observed in the soil amended with composted organic residue. Furthermore, the combined effect of both methods was not significantly different from each method applied alone (Table 3).

Inoculation with *G. intraradices* proved to be an effective means of encouraging *R. lycioides* seedling growth. Mycorrhizae increase nutrient uptake, especially of P and N (Tarafdar and Marschner 1994, Hodge and others 2001) by providing a larger absorbing surface, favor root system development, and produce substances that promote seedling growth (Álvarez 1991). In our experiment, the highest contents of foliar P were recorded in the *G. intraradices*-colonized *R. lycioides* plants (as mentioned above). N levels in the rhizosphere soil of seedlings inoculated with *G. intraradices* were even lower than in the control soil, which may be explained by the positive effect of fungus on the assimilation of N. Likewise, mycorrhizal inoculation increased soil microbial activity and in particular enhanced the protease-BAA activity, which is involved in the N cycle.

The addition of composted residue decreased soil bulk density and increased the level of nutrients in the rhizosphere soil. The significantly enhanced growth of *R. lycioides* seedlings could be explained by this structural improvement and increase in fertility observed in the rhizosphere of amended soil.

It may be concluded that the success of the reafforestation carried out with *R. lycioides* seedlings under semiarid conditions was due to mycorrhizal inoculation with *G. intraradices* and the addition of composted organic residue. In the short term, both reafforestation methods increased various chemical and biochemical parameters of the rhizosphere soil, thus contributing to afforested soil quality.

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