Establishment of *Retama sphaerocarpa* L. seedlings on a degraded semiarid soil as influenced by mycorrhizal inoculation and sewage-sludge amendment

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Summary—Zusammenfassung

A field experiment was carried out to evaluate the effectiveness of mycorrhizal inoculation with three arbuscular mycorrhizal (AM) fungi (*Glomus intraradices* Schenck & Smith, *Glomus deserticola* (Trappe, Bloss. & Menge), and *Glomus mosseae* (Nicol & Gerd.) Gerd. & Trappe) and the addition of composted sewage sludge (SS) with respect to the establishment of *Retama sphaerocarpa* L. seedlings, in a semiarid Mediterranean area. Associated changes in soil chemical (nutrient content and labile carbon fractions), biochemical (enzyme activities), and physical (aggregate stability) parameters were observed. Six months after planting, both the addition of composted SS and the mycorrhizal-inoculation treatments had increased total N content, available-P content, and aggregate stability of the soil. Values of water-soluble C and water-soluble carbohydrates were increased only in the mycorrhizal-inoculation treatments. Rhizosphere soil from the mycorrhizal-inoculation treatments had significantly higher enzyme activities (dehydrogenase, protease-BAA, acid phosphatase, and β-glucosidase) than the control soil. In the short-term, mycorrhizal inoculation with AM fungi was the most effective treatment for enhancement of shoot biomass, particularly with *G. mosseae* (about 146% higher with respect to control plants). The addition of the composted SS alone was sufficient to restore soil structural stability but was not effective with respect to improving the performance of *R. sphaerocarpa* plants.

Besiedlung eines degradierten semiariden Bodens mit *Retama sphaerocarpa* L.-Setzlingen, beeinflusst durch Mykorrhiza-Inokulation und Klärschlammzugabe

Ein Feldversuch wurde durchgeführt, um den Effekt einer Inokulation mit drei arbuskulären Mykorrhizapilzen (AM) (*Glomus intraradices* Schenck & Smith, *Glomus deserticola* (Trappe, Bloss. & Menge) und *Glomus mosseae* (Nicol & Gerd.) Gerd. & Trappe) einerseits und der Zugabe von kompostiertem Klärschlamm (SS) andererseits auf die Besiedlung von *Retama sphaerocarpa* L.-Setzlingen in einem mediterranen semiariden Gebiet zu untersuchen. Es wurden chemischer Nährstoffgehalt, labile C-Fraktion, biochemische Enzymaktivitäten und physikalische Bodenparameter (Aggregatstabilität) untersucht. Sechs Monate nach der Pflanzung erbrachten beide Behandlungen – die Zugabe von kompostiertem Klärschlamm und die Mykorrhiza-Inokulation – Steigerungen des Gesamtstickstoff-Gehaltes, des verfügbaren Phosphor-Gehaltes sowie der Aggregatstabilität des Bodens. Wasserlöslicher Kohlenstoff und wasserlösliche Kohlenhydrate waren nur nach Mykorrhiza-Inokulation erhöht. Boden aus der Rhizosphäre, der mit Mykorrhizapilzen inokuliert wurde, zeigte signifikant höhere Enzymaktivitäten (Dehydrogenase, Protease-BAA, saure Phosphatase und β-Glucosidase) als der Kontrollboden. In der kurzen Periode war die Inokulation mit AM-Pilzen die effektivste Behandlung bei der Bildung von Sprossbiomasse, speziell bei *G. mosseae* (eine um über 146 % höhere Biomasse im Vergleich zu den Kontrollpflanzen). Die Zugabe von kompostiertem Klärschlamm allein war ausreichend, die Stabilität der Bodenstruktur wiederherzustellen, aber sie war nicht effektiv hinsichtlich der Entwicklung der *R. sphaerocarpa*-Pflanzen.

Key words: enzyme activity / aggregate stability / *Glomus intraradices* / *Glomus deserticola* / *Glomus mosseae*

1 Introduction

Arbuscular mycorrhizal (AM) fungi play an important role in rehabilitation processes of disturbed arid soils (*Caravaca* et al., 2003b). The symbioses between AM fungi and their host plants improve the uptake of nutrients and water by plants and enhance soil aggregation (*Augé*, 2001; *Caravaca* et al., 2002; *Jeffries* et al., 2003). In this way, the importance of mycorrhizal symbioses in plant establishment in semiarid soils has been stressed by many authors (*Roldán* et al., 1992; *Jeffries* and *Barea*, 2000). Legumes are among the most effective species in re-vegetation programs because of their ability to form symbiotic associations with both N-fixing bacteria and AM fungi (*Requena* et al., 2001). Previous work has demonstrated the benefits of AM inoculation of these species in arid soils (*Caravaca* et al., 2003a). The selection of efficient AM fungi is a major topic in inoculation programs, especially in disturbed soils where the indigenous mycorrhizal fungi may not be adequate for plant establishment (*Requena* et al., 1996; *Azcón-Aguilar* et al., 2003).

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Mediterranean soils are often subjected to severe degradation processes accompanied by a decline of the soil organic matter content, which contributes to a loss of soil fertility. One method to reverse this degradation in soil quality is the addition of organic residues, such as sewage sludge (*Roldán* et al., 1996). To ensure the efficient use of sewage sludge as a soil amendment, it is important to select the properties of soil most sensitive to changes in soil quality following the addition of such residues. There is growing evidence that soil biological and biochemical parameters may play a potential role as early and sensitive indicators of soil ecological stress and restoration (*García* et al., 2000). In particular, enzyme activities are very significant because of their major contribution to the ability of the soil to degrade organic matter. Several investigations have been performed regarding to the effects of sewage sludge on soil microorganisms. In response to sewage-sludge amendments, both increases (*Fliessbach* et al., 1994) and decreases (*Chander* and *Brookes*, 1993) in microbiological activity have been shown, as well as a lack of effects (*García-Gil* et al., 2004). This type of material has been used widely on agricultural lands. However, little information is available on the use of such materials in re-vegetation programs (*Navas* et al., 1999).

The objectives of this study were: 1) to compare the effectiveness of mycorrhizal inoculation with that of the addition of composted sewage sludge, with respect to increasing mycorrhizal colonization, plant growth, and nutrient uptake in *Retama sphaerocarpa* seedlings afforested in a degraded Mediterranean semiarid soil and 2) to ascertain the short-term changes in physical, biochemical, and biological parameters related to soil microbial activity induced by the re-afforestation practices.

Table 1: Chemical, microbiological, biochemical, and physical characteristics of the soil used in the experiment.
Tabelle 1: Chemische, mikrobiologische, biochemische und physikalische Eigenschaften des verwendeten Bodens.

pH (H_2O)	8.5±0.0*
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
Bulk density (g cm^{-3})	1.10±0.02

* Mean ± standard error (N = 6).

2 Materials and methods

2.1 Study sites

The experimental area is located in Los Cuadros in the Province of Murcia (SE Spain) (coordinates: 1°05′ W and 38°10′ N). The climate is semiarid Mediterranean with an average annual rainfall of 300 mm and a mean annual temperature of 19.2°C; the potential evapotranspiration reaches 1000 mm yr^{-1}. The loamy soil used was a Typic Haplocalcid (*Soil Survey Staff*, 1999) developed from Quaternary sediments (Tab. 1).

2.2 Materials

The compost used in this experiment was produced from a mixture of wood-shaving and an aerobically digested sewage sludge at a rate 1:1 (v:v). The sewage sludge was obtained from a water-treatment plant in Murcia. The composting process involved a first stage lasting 2 months, during which the waste heaps were turned in open air nine times and a second maturation stage, in which the products were allowed to stand on boards for 2 months so that they could stabilize. The analytical characteristics of the composted sewage sludge, determined by standard methods (*Page* et al., 1982) are shown in Tab. 2. Total contents of heavy metals are below the limits imposed by the Spanish legislation for sewage sludges (*BOE*, 1990).

Table 2: Analytical characteristics of the composted sewage sludge used in the experiment.
Tabelle 2: Analytische Kennwerte des kompostierten Klärschlamms.

Ash (%)	18.6±0.1
pH (1:5)	6.1±0.0
Electrical conductivity EC (1:5, μS cm^{-1})	3095±48
Total Organic C (g kg^{-1})	380±4
Water-soluble C (μg g^{-1})	7245±22
Water-soluble carbohydrates (μg g^{-1})	590±53
Total N (g kg^{-1})	14.5±0.1
N-NH_4^+ (μg g^{-1})	312±13
N-NO_3^- (μg g^{-1})	1967±49
Total P (g kg^{-1})	4.5±0.1
Total K (g kg^{-1})	2.3±0.1
Fe (μg g^{-1})	6562±165
Cu (μg g^{-1})	212±8
Zn (μg g^{-1})	588±30
Ni (μg g^{-1})	44±3
B (μg g^{-1})	85±2
Cd (μg g^{-1})	9±1
Pb (μg g^{-1})	180±28
Porosity (%)	78±1

* Mean ± standard error (N = 6).

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

2.3 Mycorrhizal inoculation of seedlings

The mycorrhizal fungi used in the experiment, *Glomus intraradices* Schenck & Smith (EEZ 1), *Glomus deserticola* (Trappe, Bloss. & Menge) (EEZ 45), and *Glomus mosseae* (Nicol & Gerd.) Gerd. & Trappe (EEZ 43), were obtained from the collection of the experimental field station of Zaidín, Granada. The acronym EEZ refers to Estación Experimental del Zaidín.

AM fungal inoculum consisted of a mixture of rhizospheric soil from trap cultures (*Sorghum* sp.) containing spores, hyphae, and mycorrhizal root fragments. Once germinated, the *R. sphaerocarpa* seedlings were transplanted into the growth substrate, consisting of peat and cocopeat (1:1, v:v). The corresponding arbuscular mycorrhizal inoculum was applied at a rate of 5% (v/v). The same amount of an autoclaved mixture of the 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 fertilizer treatment.

2.4 Experimental design and layout

A factorial design in randomized blocks was established with two factors and five-fold replication. The first factor had two levels: addition or not of composted sewage sludge to the soil, and the second had four levels: non-inoculation, inoculation of *R. sphaerocarpa* plants with three AM fungi (*G. intraradices*, *G. deserticola*, or *G. mosseae*) in the nursery.

Each replication plot was 180 m^2. Planting holes (40 cm wide, 40 cm long, and 30 cm deep) were dug manually. In early February 2003, composted sewage sludge was added to half of the holes (0–20 cm depth) and mixed manually with the soil, at a rate of 1%. The seedlings (inoculated and non-inoculated) were planted at least 1 m apart, between holes, with 3 m between blocks. At least 64 seedlings per block were planted (8 plants × 8 treatments in each block).

2.5 Sampling procedures

Six months after planting, five soil samples (one per block) were collected from each treatment (40 soil samples in total). Each sample consisted of eight bulked subsamples (200 cm^3 soil cores), collected randomly at 0–20 cm in the rhizospheres of eight individual plants. Each root system was extracted excavating manually a hole 40 cm wide, 40 cm long, and 20 cm deep. To collect the rhizosphere soil, the root system with rhizosphere soil adhered was introduced into a plastic bag, shaken, and the rhizosphere soil was separated from the root system. Five plants of each treatment (one per block) were harvested 6 months after planting.

2.6 Plant analyses

Fresh and dry (105°C, 5 h) mass 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 NH_3 distillation after Kjeldahl digestion.

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

2.7 Soil physical-chemical, chemical, and biochemical analyses

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous solution. In soil aqueous extracts, water-soluble C was determined by wet oxidation with $K_2Cr_2O_7$ and measurement of the absorbance at 590 nm (*Sims* and *Haby*, 1971). Water-soluble carbohydrates were determined by the method of *Brink* et al. (1960). Total N was determined by NH_3 distillation after Kjeldahl digestion. Available P, extracted with 0.5M $NaHCO_3$, was determined by colorimetry according to *Murphy* and *Riley* (1962).

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 the dark. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with 10 ml of methanol by shaking vigorously for 1 min and filtering through Whatman N° 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. 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 2287 *g* 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) 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 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).

2.8 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 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 (P1 – P2) × 100 / (4 – P2 + T).

2.9 Statistical analysis

Data were log transformed to compensate for variance heterogeneity before analysis of variance. Composted-sewage-sludge 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.

3 Results

3.1 Chemical parameters

The soils amended with SS had lower pH and higher electrical-conductivity values than the control soil (Tab. 3). The mycorrhizal-inoculation treatments did not have significant effects on soil pH and electrical conductivity (Tab. 5).

Both the addition of composted SS and the mycorrhizal-inoculation treatments increased significantly total N and available P in the soil (Tab. 3). The greatest increase in response to the addition of the amendment was observed in the soil available-P content (about three-fold higher than in the control soil).

Water-soluble C and water-soluble carbohydrate values increased only with the mycorrhizal-inoculation treatments (Tabs. 4 and 5), the greatest increases being observed in the water-soluble carbohydrates fraction (on average, about 80% greater than for non-amended soil). Mycorrhizal-inoculation treatments and composted-SS addition significantly enhanced the structural stability of the rhizosphere soil of *R. sphaerocarpa* (Tab. 4), similar increases being achieved by the mycorrhizal-inoculation treatment with *G. deserticola* and by the addition of composted SS (about 90% greater, compared to the control soil).

Table 3: Chemical properties of rhizosphere soil of *R. sphaerocarpa* in response to mycorrhizal-inoculation treatments and composted-sewage-sludge addition 6 months after planting (n = 5).
Tabelle 3: Chemische Eigenschaften des Rhizsphären-Bodens von *R. sphaerocarpa* 6 Monate nach Pflanzung (n = 5) nach Mykorrhiza-Inokulation und Klärschlammzugabe.

	pH (H_2O)	EC (1:5, μS cm^{-1})	Total N (g kg^{-1})	Available P (μg g^{-1})
C	8.44±0.01	254±6	0.80±0.01	1.2±0.0
SS	8.26±0.02	338±6	1.04±0.03	3.3±0.2
G1	8.43±0.02	267±4	1.04±0.03	2.1±0.1
SSG1	8.28±0.01	305±3	1.06±0.02	3.2±0.2
G2	8.34±0.02	301±8	1.26±0.04	2.7±0.1
SSG2	8.09±0.03	288±5	1.45±0.07	3.8±0.1
G3	8.41±0.01	284±1	1.04±0.04	2.3±0.0
SSG3	8.01±0.03	314±10	1.42±0.09	4.6±0.4

C = control; SS = composted-sewage-sludge addition; G1 = inoculation with *G. intraradices*; SSG1 = composted-sewage-sludge addition and inoculation with *G. intraradices*; G2 = inoculation with *G. deserticola*; SSG2 = composted-sewage-sludge addition and inoculation with *G. deserticola*; G3 = inoculation with *G. mosseae*; SSG3 = composted-sewage-sludge addition and inoculation with *G. mosseae*. *Mean ± standard error.

Table 4: Carbon fractions and structural stability of rhizosphere soil of *R. sphaerocarpa* in response to mycorrhizal-inoculation treatments and composted-sewage-sludge addition 6 months after planting (n = 5).
Tabelle 4: Kohlenstofffraktionen und Strukturstabilität des Rhizosphären-Bodens von *R. sphaerocarpa* 6 Monate nach Pflanzung (n = 5) nach Mykorrhizainokulation und Klärschlammzugabe.

	Water-soluble C (μg g^{-1})	Water-soluble CH (μg g^{-1})	Aggregate stability (%)
C	84±2	10±1	16.6±0.2
SS	93±2	10±0	31.1±2.8
G1	107±4	23±0	23.9±0.9
SSG1	91±1	9±0	30.8±0.6
G2	107±3	17±1	32.1±1.9
SSG2	116±6	22±2	35.3±2.0
G3	108±2	14±0	26.8±0.2
SSG3	132±6	15±1	37.9±1.2

C = control; SS = composted-sewage-sludge addition; G1 = inoculation with *G. intraradices*; SSG1 = composted-sewage-sludge addition and inoculation with *G. intraradices*; G2 = inoculation with *G. deserticola*; SSG2 = composted-sewage-sludge addition and inoculation with *G. deserticola*; G3 = inoculation with *G. mosseae*; SSG3 = composted-sewage-sludge addition and inoculation with *G. mosseae*. CH = carbohydrates. *Mean ± standard error.

Table 5: Two-factor ANOVA (mycorrhizal-inoculation treatments and composted-SS addition) for all parameters studied in the rhizosphere soil of *R. sphaerocarpa* seedlings 6 months after planting. P significance values.
Tabelle 5: Zweifaktorielle ANOVA (Mykorrhiza-Inokulation und Zugabe von kompostiertem Klärschlamm) für alle Parameter im Rhizosphärenboden von *R. sphaerocarpa*-Setzlingen 6 Monate nach Pflanzung. Signifikante Werte P.

	Amendment (A)	Mycorrhiza (M)	Interaction (A × M)
pH	<0.001	0.071	0.481
Electrical conductivity	<0.001	0.727	<0.001
Total N	0.006	<0.001	0.786
Available P	<0.001	0.002	0.252
Water-soluble C	0.217	0.001	0.759
Water-soluble carbohydrates	0.448	0.001	0.499
Aggregate stability	<0.001	<0.001	0.052
Dehydrogenase	0.178	<0.001	0.332
Urease	0.218	0.003	0.568
Protease	0.046	0.001	0.188
Phosphatase	0.010	<0.001	0.185
β-glucosidase	0.237	<0.001	0.182
Shoot	0.681	<0.001	0.461
Colonization	0.239	<0.001	0.031
N foliar	0.848	<0.001	0.029
P foliar	0.045	<0.001	0.261

3.2 Biochemical parameters

Rhizosphere soil from the mycorrhizal-inoculation treatments had significantly higher enzyme activities (dehydrogenase, protease-BAA, acid phosphatase, and β-glucosidase) than the control soil (Tab. 6). The addition of composted SS only had a significant effect on protease and phosphatase activities (Tab. 5). However, protease-BAA and phosphatase activities were higher in the soils from the mycorrhizal-inoculation treatments, compared to sludge-amended soil. The combined treatments, involving mycorrhizal inoculation of seedlings with *G. deserticola* or *G. mosseae* and the addition of composted SS to soil, increased the values of the biochemical parameters of the rhizosphere soil to a higher extent than each treatment applied separately.

3.3 Growth and mycorrhizal infection of *R. sphaerocarpa*

At the time of planting, the shoot dry weight of inoculated *R. sphaerocarpa* plants was slightly greater than for non-inoculated plants (Fig. 1). Six months after planting, only mycorrhizal inoculation and the combined treatment of addition of composted SS and mycorrhizal inoculation had stimulated significantly the shoot-biomass production of *R. sphaerocarpa* with respect to the control plants (Tab. 5 and Fig. 1). The inoculation with *G. mosseae* was the most effective one with respect to increasing shoot biomass (about 146% higher with respect to control plants).

Table 6: Enzyme activities of rhizosphere soil of *R. sphaerocarpa* in response to mycorrhizal-inoculation treatments and composted-sewage-sludge addition 6 months after planting (n = 5).
Tabelle 6: Enzymaktivitäten des Rhizosphären-Bodens von *R. sphaerocarpa* 6 Monate nach Pflanzung (n = 5) nach Mykorrhiza-Inokulation und Klärschlammzugabe.

	Dehydrogenase (μg INTF (g soil)$^{-1}$)	Urease (μmol NH_3 g^{-1} h^{-1})	Protease (μmol NH_3 g^{-1} h^{-1})	Phosphatase (μmol PNP g^{-1} h^{-1})	β-glucosidase (μmol PNP g^{-1} h^{-1})
C	52.5±1.0	0.41±0.03	0.25±0.01	0.31±0.01	0.36±0.01
SS	55.2±4.0	0.47±0.02	0.33±0.01	0.40±0.01	0.34±0.01
G1	72.8±1.6	0.60±0.01	0.68±0.04	0.57±0.02	0.79±0.05
SSG1	71.2±2.8	0.58±0.01	0.59±0.07	0.50±0.02	0.63±0.05
G2	95.9±4.7	0.75±0.05	0.68±0.01	0.55±0.01	0.87±0.02
SSG2	113.0±4.4	0.78±0.04	1.16±0.08	0.83±0.03	1.23±0.10
G3	77.5±4.1	0.54±0.02	0.73±0.05	0.44±0.01	0.71±0.06
SSG3	111.4±7.1	1.04±0.14	2.08±0.25	0.94±0.07	1.47±0.17

C = control; SS = composted-sewage-sludge addition; G1 = inoculation with *G. intraradices*; SSG1 = composted-sewage-sludge addition and inoculation with *G. intraradices*; G2 = inoculation with *G. deserticola*; SSG2 = composted-sewage-sludge addition and inoculation with *G. deserticola*; G3 = inoculation with *G. mosseae*; SSG3 = composted-sewage-sludge addition and inoculation with *G. mosseae*. INTF = iodo-nitrotetrazolium formazan; PNP = p-nitrophenol. *Mean ± standard error.

Foliar-N and -P contents in inoculated *R. sphaerocarpa* seedlings were significantly higher than in non-inoculated plants prior to planting in the field (Fig. 1). Six months after planting, all treatments had increased foliar-P contents with respect to the control plants, but foliar N was only increased by the mycorrhizal-inoculation treatments.

At the time of planting, *R. sphaerocarpa* seedlings inoculated with any of the three AM fungi had significantly higher percentages of root colonization (particularly those inoculated with *G. deserticola* or *G. intraradices*) than the non-inoculated plants, whose roots showed negligible levels of AM colonization (Fig. 1). Six months after planting, the highest levels of mycorrhizal colonization were recorded in the inoculated seedlings, without significant differences among AM fungi. The results of the factorial analysis (Tab. 5) show that, with respect to colonization of roots, the mycorrhizal inoculation had a positive interaction with the addition of composted SS.

4 Discussion

4.1 Effectiveness of the mycorrhizal-inoculation treatments

Inoculation with any of the AM fungi tested proved to be an effective means of promoting *R. sphaerocarpa* seedling growth. Mycorrhizae increase nutrient uptake, especially of P and N, by providing a larger absorbing surface, favor root-system development and produce substances that promote seedling growth (*Jeffries* et al., 2003). The mycorrhizal-inoculation treatments showed different levels of effectiveness with

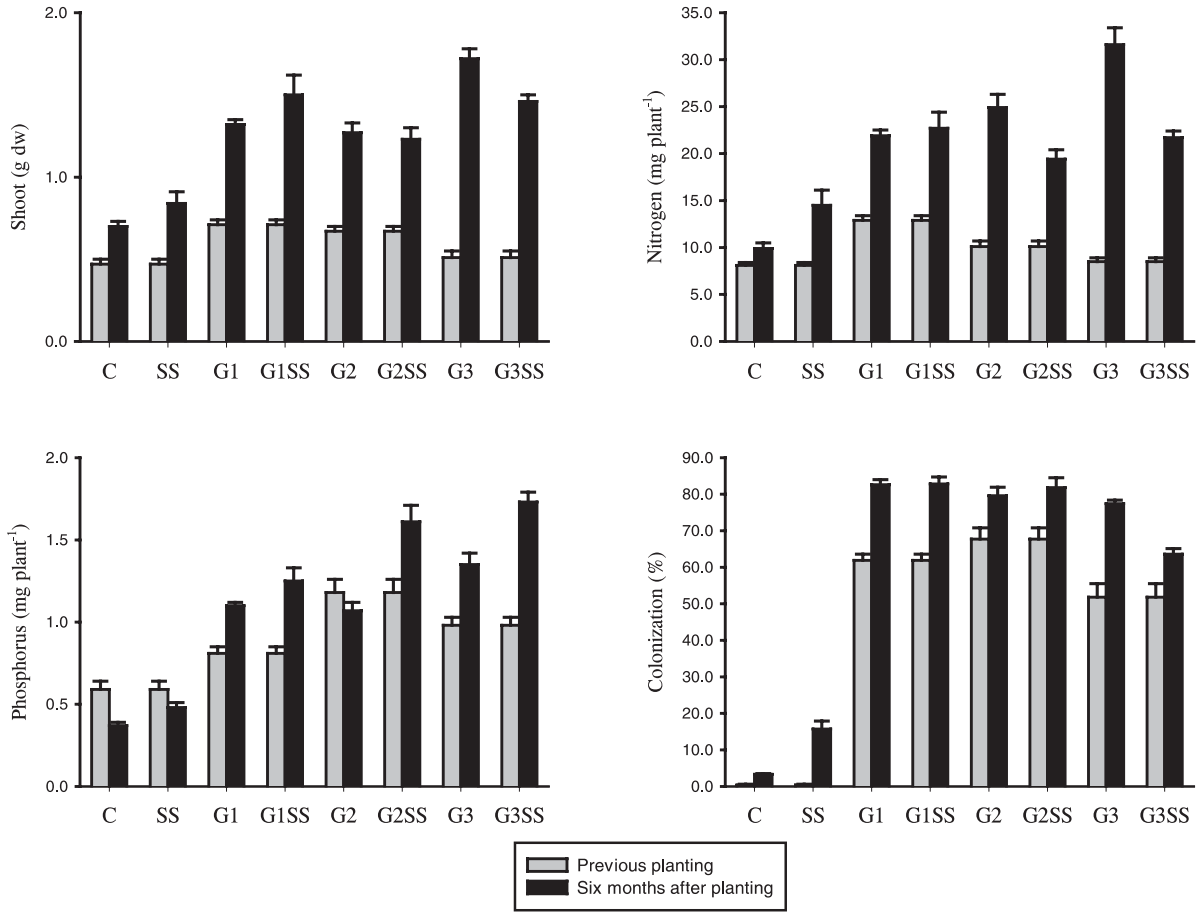

Figure 1: Shoot dry weight, foliar nutrients, and root infection of *R. sphaerocarpa* seedlings in response to mycorrhizal-inoculation treatments, composted-sewage-sludge addition previous planting and 6 months after planting (n = 5).
Abbildung 1: Sprosstrockengewicht, Blattspiegelwerte und Mykorrhizierungsrate von *R. sphaerocarpa*-Setzlingen vor Pflanzung und 6 Monate nach Pflanzung (n = 5) nach Mykorrhiza-Inokulation und Klärschlammzugabe.

respect to improving the performance of *R. sphaerocarpa*. *G. mosseae* was most effective at increasing plant growth. It is worth noting that mycorrhizas played a key role in the first stage of the re-establishment of *R. sphaerocarpa* seedlings (6 months after planting), which is the most critical period for re-vegetation, particularly in Mediterranean semiarid areas. Furthermore, the mycorrhization produced the same effects on the shoot biomass with or without composted-SS addition.

Soil microbial activities influence directly site quality and soil fertility, because microorganisms play a fundamental role in establishing biogeochemical cycles (*Jeffries* et al., 2003). AM fungi are key components of the soil microbiota and interact with other microorganisms in the rhizosphere, hence affecting soil microbiological properties. Formation of AM considerably alters root biomass and exudation which, in turn, influences the quantity and quality of C delivered to the soil *via* fungal hyphae (*Marschner* et al., 1997). In fact, the concentrations of water-soluble carbohydrates and water-soluble C were higher in the rhizosphere soil of the plants inoculated with AM fungi. A higher rhizodeposition of soluble-C fractions is expected to stimulate microbial activity in the rhizosphere (*Wamberg* et al., 2003). This is clearly demonstrated in our case, since the mycorrhizal-inoculation treatments increased the levels of dehydrogenase activity, which is strongly related to microbial activity (*Nannipieri*, 1994). Oxidoreductases, such as dehydrogenase, are involved in oxidative processes in soils, and their activity mainly depends on the metabolic state of soil biota, thus they are considered as good indicators of the soil microbial activity in semiarid areas (*García* et al., 1997). The re-activation of microbial populations depended on the assayed mycorrhizal-inoculation treatment. *G. deserticola*-inoculated *R. sphaerocarpa* was most effective at increasing dehydrogenase activity (by about 83% with respect to the control). Increased biological activity was also revealed by the variations in activities of hydrolases such as urease, protease-BAA, acid phosphatase, and β-glucosidase. The measurement of these hydrolases provides an early indication of changes in soil fertility, since they are related to the mineralization of such important nutrient elements as N, P, and C (*Ceccanti* et al., 1994). Enzyme activities also are sufficiently sensitive to indicate perturbations caused by microbial inoculation (*Naseby* and *Lynch*, 1997). They give an indication of ecosystem function rather than just a measurement of perturbation. The increases observed in urease, protease-BAA, and β-glucosidase activities may be related mainly to re-activation of the rhizosphere microbial population as a consequence of the inoculation treatments.

To our knowledge, there is no evidence regarding the secretion of these enzymes by AM fungi. In contrast, increased acid phosphatase activity in the rhizosphere of mycorrhizal plants may be due to a direct fungal secretion or an induced secretion by the plant roots, as pointed out by *Joner* et al. (2000). Phosphatases are enzymes with a relatively broad specificity, capable of hydrolyzing various organic and inorganic phosphate esters, and are involved in the P cycle. The highest increase in phosphatase activity was recorded in the rhizosphere soil of mycorrhizal *R. sphaerocarpa*. On the other hand, the fact that the concentrations of available P in the soil rhizosphere were increased by the mycorrhizal-inoculation treatments suggests also the involvement of extracellular fungal phosphatase in the hydrolysis of organic P compounds in the soil. However, the quantitative contribution of extracellular enzymes to the P nutrition of AM plants is estimated to be insignificant (*Joner* et al., 2000).

Soil structure largely determines soil quality and fertility, which, in turn, favor the establishment and viability of a stable plant cover. The present study confirms the influence of mycorrhizal-inoculation treatments on soil aggregate stability. With the exception of *G. intraradices*, the mycorrhizal-inoculation treatments produced increases in aggregate stability similar to the addition of composted SS alone. The mechanisms involved in aggregate stabilization are based on the enmeshment of soil particles by hyphae and roots and on the exudation of polysaccharides (*Bearden* and *Petersen*, 2000). The water-soluble C fraction is also regarded as one of the key labile components of organic matter responsible for soil aggregation (*Puget* et al., 1999). The increased levels of stable aggregates resulting from mycorrhizal-inoculation treatments can be attributed also to the stimulation of the rhizosphere microbial population and, particularly, to the proliferation of fungal hyphae (*Roldán* et al., 1994; *Jeffries* and *Barea*, 2000). According to *Roldán* et al. (1994), the binding effect of roots and hyphae is long-lived, while that of polysaccharides is transient because they are decomposed rapidly by microbes.

4.2 Effectiveness of composted-sewage-sludge addition

Research published on the use of organic soil amendments in eroded soils shows that organic amendments can improve soil productivity, increasing the soil nutrient status for several potentially limiting nutrients, such as N and P (*Cox* et al., 2001). In our experiment, the addition of composted SS increased the total N and available-P contents of the soil, the greatest increase being observed for available-P. The benefits of composted SS were also due to improved physical properties of the soil, such as aggregate stability. The increase in structural stability of the amended soil could be related to the total polysaccharides fraction added with the SS, because the labile organic-matter fraction of the soil did not vary with the addition of the amendment.

The addition of organic materials to soil may promote microbial activity to an extent which is closely related to the amount and nature of the organic matter added (*Roldán* et al., 1996). Thus, an uncomposted organic amendment rich in easily biodegradable compounds is more effective at stimulating the microbial activity of a soil than a composted organic amendment. In addition, the promoting effect of an organic amendment on soil microbial biomass declines rapidly with time, especially in soils of degraded semiarid zones. In our experiment, the addition of composted SS only had an effect on the protease-BAA and phosphatase activities. The composted-SS addition led to a decrease in soil pH, which may explain the higher acid-phosphatase activity in this treatment. As discussed by *Johansson* et al. (1999), a decrease in pH would favor the acid-phosphatase activity. These results can also, presumably, be ascribed to the organic-P sources provided with the SS, which can lead to an increased synthesis of these enzymes. The significant increase in protease activity in the amended soil may have been due to the residual source of N substrates added with the SS and/or to enhanced root exudation, that can stimulate microbial activity and, in turn, intracellular enzyme activity. Root exudates can be a source of easily degradable N compounds, such as amino acids and small peptides, which are able to induce protease synthesis (*García-Gil* et al., 2000).

The results of this study have demonstrated the limited effectiveness of the addition of composted SS to soil with respect to improving the growth of the target shrub legume species, *R. sphaerocarpa*, selected for re-vegetation of a semiarid Mediterranean area.

It may be concluded that, in the short-term, mycorrhizal inoculation with AM fungi is the most effective treatment for improving soil physical (aggregate stability), chemical (nutrient content and labile-carbon fractions), and biochemical (enzyme activities) quality, leading to enhanced plant growth, particularly with *G. mosseae*. The addition of the composted SS alone was sufficient to restore soil structural stability but it was not effective with respect to improving the performance of *R. sphaerocarpa* plants.

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