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Nutrient acquisition and nitrate reductase activity of mycorrhizal *Retama sphaerocarpa* L. seedlings afforested in an amended semiarid soil under two water regimes

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Abstract. We studied the effect of 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 on root nitrate reductase (NR, EC 1.6.6.1.) activity, mycorrhizal colonization, plant growth and nutrient uptake in *Retama sphaerocarpa* L. seedlings afforested in a semiarid, degraded Mediterranean soil under well-watered and non-watered conditions. Six months after planting, the mycorrhizal inoculation and the irrigation of plants had a strong effect on the growth parameters. The effect on plant growth was a negative interaction between plant irrigation and mycorrhizal inoculation and a positive interaction between plant irrigation and composted sewage sludge addition. The latter treatment had a significant, but moderate, effect on the growth but conferred no additional benefit when combined with mycorrhizal inoculation. Mycorrhizal inoculation, composted sewage sludge and irrigation had a significant effect on NR activity in roots and on foliar nutrients. The irrigation significantly increased the positive effect of composted sewage sludge on NR activity and the concentrations of foliar N and K. The effect of mycorrhizal inoculation on NR activity did not depend on the water regime. The effectiveness of mycorrhizal inoculation on the establishment and growth of *R. sphaerocarpa* seedlings in these Mediterranean conditions was independent of water regime. The addition of composted sewage sludge was only effective when soil water was freely available. The combination of mycorrhizal inoculation and composted sewage sludge addition had no synergistic effect on plant growth.

Keywords: Mycorrhiza, composted sewage sludge, irrigation, nitrate reductase, nutrient uptake

INTRODUCTION

Nitrogen-fixing shrubs, mainly leguminous species, have been considered useful for revegetation of water-stressed ecosystems that are deficient in nitrogen (N), phosphorus (P) and other nutrients (Herrera *et al.* 1993). This is because of their ability to develop symbiotic associations with both rhizobial bacteria and mycorrhizal fungi (Requena *et al.* 2001; Caravaca *et al.* 2003a), which are of particular interest in desertified Mediterranean ecosystems. Leguminous shrubs such as *Retama sphaerocarpa* (L.) Boissier are native to the Iberian Peninsula and are common in a range of dry Mediterranean ecosystems (Valladares *et al.* 2002), where the annual precipitation can vary from 200–800 mm. *R. sphaerocarpa* has a remarkable capacity to withstand drought owing to its crown architecture and its deep root system, which can penetrate to depths of >25 m (Haase *et al.* 1996), providing access to deep water sources. However, the scarcity of available P in desertified ecosystems currently limits legume establishment (Herrera *et al.* 1993).

Inoculation with symbiotic microorganisms, especially arbuscular-mycorrhizal (AM) fungi, is an effective method of enhancing the ability of the host plants to establish and to cope in semiarid conditions (Alguacil *et al.* 2003a) and withstand stresses such as nutrient deficiency, drought and soil disturbance. In fact, several studies have demonstrated that mycorrhizal symbiosis can increase legume performance (Caravaca *et al.* 2003a). Various mechanisms have been proposed to explain the protection provided by AM symbiosis against the detrimental effects of drought.

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Several of these are not directly related to P nutrition or water uptake, such as: increased leaf gas exchange, photosynthetic rate and water use efficiency (Querejeta *et al.* 2003); changes in hormonal signalling (Goicoechea *et al.* 1996); increased antioxidant enzyme activities (Alguacil *et al.* 2003b); and enhanced activity of enzymes involved in nitrate assimilation (Ruíz-Lozano *et al.* 1996; Caravaca *et al.* 2003b).

Nitrate ion mobility in soils is severely restricted under drought due to its low concentration and diffusion rate. Under such conditions, AM colonization plays a key role in nitrate acquisition and assimilation in plants grown in neutral to alkaline soils (Azcón *et al.* 2001). Thus, nitrate reductase (NR, EC 1.6.6.1.) activity, which catalyses the rate-limiting step in the nitrate assimilation pathway, has been proposed as an index for assessing the effectiveness of fungus–host plant combinations for mitigation of drought stress (Caravaca *et al.* 2003b). Kaldorf *et al.* (1998) provided genetic evidence for the presence of this enzyme in a *Glomus* isolate. The effect of AM fungi on nitrate assimilation is dependent on the fungus involved in the symbiosis and the host plant species. However, there are no reports on the effect of mycorrhizal inoculation on the levels of nitrate reductase in *R. sphaerocarpa* plants under field conditions, nor on the effect of water regime.

The incorporation of organic amendments, such as sewage sludge, can favour seedling establishment under semiarid conditions. Such materials can improve the soil fertility and, in turn, activate the microbial biomass, improve soil structure and increase water-holding capacity (Turner *et al.* 1994; Zebarth *et al.* 1999). One problem with sewage sludge is the presence of heavy metals, organic toxins and pathogens (Linden *et al.* 1995). Some authors have suggested that sewage sludge should be composted before application to soil, in order to minimize these contaminants (Pascual *et al.* 1999). This type of material is used widely in agriculture but there is little information available on its use in revegetation programmes (Navas *et al.* 1999).

The objective of this study was to compare the effectiveness of three different AM fungi to inoculate *R. sphaerocarpa* seedlings, afforested in a semiarid degraded soil under wet and dry conditions. The effect of adding composted sewage sludge to the soil on root nitrate reductase and acid phosphatase activities, mycorrhizal colonization, plant growth and nutrient uptake were also investigated.

MATERIALS AND METHODS

Study sites

The experimental area was located in Los Cuadros in the Province of Murcia, southeast Spain (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 loam soil used was a Typic Haplocalcid (Soil Survey Staff 1999) developed from Quaternary sediments (Table 1).

Table 1. Chemical, biochemical, microbiological and physical characteristics of the soil used in the experiment.

pH (H_2O)	8.5 ± 0.0[a]
EC (1:5, μS cm^{-1})	225 ± 2
Texture	Loam[b]
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[c] (%)	11.5 ± 0.4
Bulk density[d] (g cm^{-3})	1.10 ± 0.02

[a] Mean ± standard error ($n = 6$); [b]sand 50%; silt 35%; clay 15%; [c]Lax *et al.* 1994; [d]Barahona & Santos (1981).
EC = electrical conductivity; INTF = iodonitrotetrazolium formazan; PNP = *p*-nitrophenyl phosphate.

Materials

The compost used in this experiment was produced from a mixture of wood-shavings and an aerobically digested sewage sludge (1:1 v/v), the latter being obtained from a water treatment plant in Murcia. The composting process involved a first stage lasting two months, during which the waste heaps were turned nine times in the open air, and a second maturation stage, in which the products were allowed to stabilize standing on boards for two months. The analytical characteristics of the composted sewage sludge (Table 2) were determined by standard methods (Page *et al.* 1982).

The reafforestation experiment used *Retama sphaerocarpa*, a shrub reaching a height of 1.3 to 2.5 m that is widely distributed in the Mediterranean. It is well adapted to drought and frequently used in the reafforestation of semiarid disturbed land.

Table 2. Analysis of the composted sewage sludge used in the experiment.

Ash (%)	18.6 ± 0.1[a]
pH (1:5)	6.1 ± 0.0
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
NH_3-N (μg g^{-1})	312 ± 13
NO_3-N (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

[a] Mean ± standard error ($n = 6$).

Seeds of *R. sphaerocarpa* were collected at random from wild plants growing in the experimental area. The seeds were treated with concentrated sulphuric acid for 5 min followed by immersion in water at 90 °C to aid germination.

Mycorrhizal inoculation of seedlings

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

AM fungal inoculum consisted of rhizospheric soil from trap cultures (*Sorghum* sp.) containing spores, hyphae and mycorrhizal root fragments. Once germinated, the *R. sphaerocarpa* seedlings were transplanted into a mixture of peat and cocopeat (1:1, v/v). The corresponding arbuscular mycorrhizal inoculum was applied at a rate of 5% (v/v). The same amount of an autoclaved mixture of the inocula was added to control plants supplemented with a filtrate (< 20 μm) of culture to provide the microbial populations accompanying the mycorrhizal fungi. Inoculated and non-inoculated seedlings were grown for 8 months under nursery conditions without addition of fertilizer.

Experimental design and layout

A factorial design in randomized blocks was established with three factors and fivefold replication. The first factor had two levels: soil with or without addition of composted sewage sludge. The second factor had four levels: no inoculation, or inoculation of *R. sphaerocarpa* with three AM fungi (*G. intraradices*, *G. deserticola* or *G. mosseae*) in the nursery. The third factor had two levels: no irrigation or irrigated.

Each replicate plot was 180 m^2. Planting holes (40 cm wide × 40 cm long × 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 uninoculated) were planted at least 1 m apart, between holes, with 3 m between blocks. At least 128 seedlings per block were planted (8 plants × 16 treatments in each block). Half of the plants received no irrigation, except for natural precipitation (about 130 mm) during the 6 months of the growth. The remaining plants were irrigated regularly (once a month) with tap water (30 mm each time) in order to apply a total precipitation of 310 mm. With this irrigation, we intended to simulate conditions in a zone with high soil moisture after the rainy season in spring.

Plant analysis

NR activity was assayed *in vivo* by measuring NO_2^- production in tissue that had been vacuum-infiltrated with buffered NO_3^- solutions (Downs *et al.* 1993). Acid phosphatase activity was determined by the method of Tabatabai & Bremner (1969).

Six months after planting, five plants from each treatment were harvested. Basal stem diameter and height of plants were measured; fresh and dry (105 °C, 5 h) weights of shoots and roots were recorded. Plants were harvested, and the roots washed free from soil under cold tap water. Fresh and dry (105 °C, 5 h) weights of shoots and roots were recorded. Plant tissue was ground before chemical analysis. The foliar concentrations of phosphorus (P) and potassium (K) were determined after digestion in nitric–perchloric acid (5:3) for 6 h. The P concentration was determined by colorimetry (Murphy & Riley 1962), the N concentration by the Kjeldahl method, and the K concentration was estimated by flame photometry (Schollemberger & Simon 1954).

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

Statistical analysis

Data were log transformed to achieve normality. Composted sewage sludge addition, mycorrhizal inoculation, water regime and their interaction effects on measured variables were tested by a three-way analysis of variance, and comparisons among means were made using Least Significant Difference (LSD) test calculated at $P < 0.05$. Statistical procedures were carried out with the software package SPSS 10.0 for Windows.

RESULTS

Growth parameters of R. sphaerocarpa

At the end of the nursery period (zero time), shoot and root dry weights of *R. sphaerocarpa* plants colonized by *G. intraradices* or *G. deserticola* were greater than for uninoculated plants and plants inoculated with *G. mosseae* (Table 3). Six months after planting, survival was about 90% for all treatments, without significant differences among treatments. The water regime affected shoot and root biomass and basal diameter of the plants to a very significant degree ($P < 0.001$, Table 4), with the growth parameters determined in non-watered plants being significantly smaller than those in well-watered plants. The three mycorrhizal inoculation treatments also had a strong effect on shoot biomass (Table 4). The inoculation with *G. mosseae* was the most effective for increasing shoot biomass (about 146% greater than the control) (Table 3). Shoot biomass was increased by mycorrhizal inoculation but only in the absence of irrigation. The composted sewage sludge seemed to have a significant but moderate effect on growth, shoot biomass being increased by about 20% over control, but it conferred no additional benefit when combined with mycorrhiza. Composted sewage sludge significantly increased the growth of irrigated plants but not in the absence of irrigation.

Foliar nutrients and mycorrhizal infection of R. sphaerocarpa

Foliar N, P and K concentrations were significantly greater in the inoculated than in the uninoculated *R. sphaeroma* seedlings before planting in the field (Table 5). Six months after planting, all treatments had increased concentrations of foliar nutrients over the controls. As observed for the

Table 3. Growth parameters of *R. sphaerocarpa* seedlings in response to mycorrhizal inoculation treatments, composted sewage sludge addition and water regime previous to planting and 6 months after planting.

	0 months	6 months	
		Unirrigated	Irrigated
Shoot (g dry weight)			
C	0.47 ± 0.03[a]	0.70 ± 0.03	1.34 ± 0.05
SS	0.47 ± 0.03	0.84 ± 0.07	2.01 ± 0.06
G1	0.71 ± 0.03	1.32 ± 0.03	2.63 ± 0.10
SSG1	0.71 ± 0.03	1.50 ± 0.12	2.44 ± 0.13
G2	0.67 ± 0.03	1.27 ± 0.06	2.72 ± 0.12
SSG2	0.67 ± 0.03	1.23 ± 0.07	3.24 ± 0.24
G3	0.51 ± 0.04	1.72 ± 0.06	2.53 ± 0.06
SSG3	0.51 ± 0.04	1.46 ± 0.04	3.95 ± 0.32
Root (g dry weight)			
C	0.63 ± 0.03	0.56 ± 0.03	0.79 ± 0.04
SS	0.63 ± 0.03	0.51 ± 0.03	1.34 ± 0.09
G1	0.81 ± 0.04	0.59 ± 0.03	1.29 ± 0.09
SSG1	0.81 ± 0.04	0.82 ± 0.03	1.40 ± 0.07
G2	0.68 ± 0.04	0.55 ± 0.03	2.18 ± 0.14
SSG2	0.68 ± 0.04	0.54 ± 0.01	1.47 ± 0.07
G3	0.45 ± 0.01	0.80 ± 0.03	1.10 ± 0.06
SSG3	0.45 ± 0.01	0.60 ± 0.02	1.83 ± 0.16
Height (cm)			
C	26.8 ± 1.0	34.8 ± 0.9	25.4 ± 1.2
SS	26.8 ± 1.0	30.6 ± 0.6	33.5 ± 1.6
G1	36.9 ± 1.2	43.1 ± 0.9	36.3 ± 0.8
SSG1	36.9 ± 1.2	36.8 ± 1.0	35.1 ± 2.7
G2	40.5 ± 0.8	35.2 ± 1.1	34.1 ± 1.4
SSG2	40.5 ± 0.8	34.1 ± 1.1	43.3 ± 1.1
G3	32.2 ± 1.1	33.3 ± 0.6	35.0 ± 1.4
SSG3	32.2 ± 1.1	36.5 ± 0.5	37.6 ± 1.1
Basal diameter (mm)			
C	2.81 ± 0.03	3.06 ± 0.18	3.99 ± 0.28
SS	2.81 ± 0.03	2.36 ± 0.07	3.77 ± 0.33
G1	2.99 ± 0.09	3.13 ± 0.10	5.61 ± 0.40
SSG1	2.99 ± 0.09	2.85 ± 0.15	4.51 ± 0.12
G2	2.60 ± 0.05	2.88 ± 0.19	5.69 ± 0.35
SSG2	2.60 ± 0.05	2.94 ± 0.05	5.01 ± 0.26
G3	2.53 ± 0.06	3.11 ± 0.08	5.55 ± 0.24
SSG3	2.53 ± 0.06	2.91 ± 0.01	5.23 ± 0.28

[a] Mean ± standard error ($n = 5$).
Treatment key: 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*). For statistical differences between treatments, see Table 4.

growth parameters, the beneficial effect of the composted sewage sludge on the concentrations of foliar N and K was increased only in the presence of irrigation (Tables 4 and 5). However, the effect of all binary combinations of the treatments on foliar P concentration exceeded the additive effect of each treatment when applied individually, as shown by the significant sewage sludge × mycorrhization, sewage sludge × water regime and mycorrhization × water regime interactions.

At the time of planting, the *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 uninoculated plants, whose roots showed negligible levels of AM colonization (Table 5). Six months after planting, the largest levels of mycorrhizal colonization were recorded in the inoculated seedlings. The percentages of root colonization were enhanced greatly by irrigation and, to a lesser extent, by the composted sewage sludge. The results of the factorial analysis (Table 4) show that colonization of roots due to the mycorrhizal inoculation was enhanced by both the composted sewage sludge and the irrigation.

Nitrate reductase and acid phosphatase activities

Most of the NR activity was in the roots of the *R. sphaerocarpa* seedlings, since no activity was detected in the shoots. Mycorrhizal inoculation, composted sewage sludge addition, irrigation and the interaction of all treatments had significant effects on NR activity in roots (Table 4). The effect of mycorrhizal inoculation did not depend on the water regime. The composted sewage sludge strongly increased the positive effect of mycorrhizal inoculation on NR activity ($P = 0.001$). Likewise, irrigation significantly increased the positive effect of the addition of composted sewage sludge on NR activity.

Mycorrhizal inoculation treatments, composted sewage sludge and water regime had no significant effect on acid phosphatase in the roots of *R. sphaerocarpa* seedlings (Tables 4 and 6).

DISCUSSION

In this study we determined how the mycorrhizal inoculation of *R. sphaerocarpa* seedlings with *G. intraradices*,

Table 4. Three factors ANOVA (sewage sludge addition, mycorrhizal inoculation and water regime) for all parameters studied in the *R. sphaerocarpa* seedlings 6 months after planting, expressed as *P* significance values.

Source of variation	Sewage sludge (SS)	Mycorrhiza (M)	Water regime (WR)	Interactions			
				SS × M	SS × WR	M × WR	SS × M × WR
Shoot biomass	0.015	<0.001	<0.001	0.633	0.036	0.030	0.861
Root biomass	0.121	0.001	<0.001	0.201	0.075	0.043	0.114
Height	0.252	<0.001	0.274	0.734	0.001	0.163	0.159
Basal diameter	0.032	<0.001	<0.001	0.924	0.924	0.006	0.201
Colonization	0.013	<0.001	<0.001	<0.001	0.574	0.002	0.403
Foliar N	<0.001	<0.001	<0.001	0.062	<0.001	0.562	0.439
Foliar P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
Foliar K	<0.001	<0.001	<0.001	0.604	0.001	0.199	0.670
Nitrate reductase	<0.001	<0.001	<0.001	0.001	<0.001	0.236	0.001
Acid phosphatase	0.349	0.117	0.492	0.563	0.983	0.597	0.667

Table 5. Foliar nutrients and root infection of *R. sphaerocarpa* seedlings in response to mycorrhizal inoculation treatments, composted sewage sludge addition and water regime previous to planting and 6 months after planting.

	0 months	6 months	
		Unirrigated	Irrigated
Nitrogen (mg plant $^{-1}$)			
C	8.1 ± 0.3[a]	9.9 ± 0.6	13.8 ± 0.3
SS	8.1 ± 0.3	14.5 ± 1.6	31.4 ± 1.5
G1	12.9 ± 0.5	21.9 ± 0.6	31.6 ± 1.2
SSG1	12.9 ± 0.5	22.7 ± 1.7	33.6 ± 2.0
G2	10.1 ± 0.6	24.9 ± 1.4	29.9 ± 1.3
SSG2	10.1 ± 0.6	19.4 ± 1.0	45.6 ± 3.7
G3	8.5 ± 0.4	31.6 ± 1.8	28.5 ± 1.3
SSG3	8.5 ± 0.4	21.7 ± 0.7	51.0 ± 3.2
Phosphorus (mg plant $^{-1}$)			
C	0.59 ± 0.05	0.37 ± 0.02	0.70 ± 0.05
SS	0.59 ± 0.05	0.48 ± 0.03	3.26 ± 0.11
G1	0.81 ± 0.04	1.10 ± 0.02	2.26 ± 0.11
SSG1	0.81 ± 0.04	1.25 ± 0.08	6.47 ± 0.11
G2	1.18 ± 0.08	1.07 ± 0.05	2.14 ± 0.05
SSG2	1.18 ± 0.08	1.61 ± 0.10	8.88 ± 0.65
G3	0.98 ± 0.05	1.34 ± 0.07	2.22 ± 0.10
SSG3	0.98 ± 0.05	1.73 ± 0.06	9.52 ± 0.40
Potassium (mg plant $^{-1}$)			
C	7.1 ± 0.5	10.7 ± 0.9	20.0 ± 0.8
SS	7.1 ± 0.5	15.8 ± 1.7	41.6 ± 1.8
G1	8.6 ± 0.5	28.5 ± 1.4	50.7 ± 1.3
SSG1	8.6 ± 0.5	31.6 ± 2.6	52.6 ± 2.2
G2	8.9 ± 0.4	29.3 ± 2.1	42.3 ± 2.2
SSG2	8.9 ± 0.4	26.7 ± 1.1	70.0 ± 6.9
G3	8.1 ± 0.7	30.7 ± 0.8	36.9 ± 2.6
SSG3	8.1 ± 0.7	30.6 ± 1.2	70.6 ± 4.0
Colonization (%)			
C	0.4 ± 0.2	3.3 ± 0.2	18.8 ± 1.4
SS	0.4 ± 0.2	15.7 ± 2.2	37.3 ± 1.3
G1	61.9 ± 1.7	82.6 ± 1.4	87.5 ± 1.1
SSG1	61.9 ± 1.7	82.8 ± 1.9	83.0 ± 1.0
G2	67.7 ± 3.1	79.6 ± 2.3	91.0 ± 1.2
SSG2	67.7 ± 3.1	81.8 ± 2.7	80.3 ± 0.9
G3	51.8 ± 3.7	77.4 ± 1.0	75.8 ± 1.2
SSG3	51.8 ± 3.7	63.6 ± 1.5	76.0 ± 2.5

[a] Mean ± standard error ($n = 5$).
[b] Treatment key: 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*. For statistical differences between treatments, see Table 4.

Table 6. Nitrate reductase (NR) and acid phosphatase activities in roots of *R. sphaerocarpa* seedlings in response to mycorrhizal inoculation treatments, composted sewage sludge addition and water regime 6 months after planting.

Treatments	NR activity (nmol NO_2^- g FW^{-1} h^{-1})		Acid phosphatase (μmol PNP g^{-1} h^{-1})	
	Unirrigated	Irrigated	Unirrigated	Irrigated
C	31 ± 1[a]	49 ± 2	18.6 ± 0.5	18.4 ± 0.4
SS	36 ± 2	58 ± 5	19.4 ± 0.1	19.9 ± 0.4
G1	37 ± 1	42 ± 1	18.8 ± 0.3	20.3 ± 0.6
SSG1	38 ± 1	119 ± 14	23.2 ± 0.9	23.8 ± 1.5
G2	68 ± 2	65 ± 6	18.8 ± 0.6	19.1 ± 1.2
SSG2	61 ± 1	136 ± 7	18.2 ± 0.4	18.2 ± 0.4
G3	59 ± 1	58 ± 1	21.0 ± 0.3	23.0 ± 0.4
SSG3	76 ± 3	153 ± 14	19.0 ± 0.4	20.2 ± 0.9

[a] Mean ± standard error ($n = 5$).
Treatment key: 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*. For statistical differences between treatments, see Table 4. PNP = *p*-nitrophenyl phosphate; FW = fresh weight.

G. deserticola or *G. mosseae*, the addition of composted sewage sludge to the soil and changes in water regime, stimulated plant growth in a semiarid Mediterranean area. The mycorrhizal inoculation treatments all improved the performance of this legume, with *G. mosseae* being the most effective. Total plant nutrient contents were improved by all the mycorrhizal inoculants and can be taken as an indicator of mycorrhizal effectiveness, because they take into account the well-balanced effects of nutrient acquisition and biomass production (Jeffries *et al.* 2003). The greatest growth of *R. sphaerocarpa* seedlings inoculated with *G. mosseae* was related to the concentrations of N and P in shoot tissue. The extent of mycorrhizal infection is of importance when studying the influence of arbuscular-mycorrhizal (AM) fungi on the host plant. A high degree of infection may not be a prerequisite for growth responses in all plants inoculated with AM fungi. Thus, Requena *et al.* (1996) observed that native fungi were ineffective at promoting growth of *Anthyllis cytisoides*, despite colonizing a relatively large percentage of the roots. In our experiments, the fact that the highest effectiveness occurred with *G. mosseae* was not related to the extent of mycorrhizal infection, because all inoculated plants exhibited high infection rates in their roots, under both water regimes. It is worth noting that the mycorrhizal inoculation treatments were very effective at increasing plant growth in the absence of irrigation. These results confirm the effectiveness of mycorrhizal symbiosis in the successful establishment and growth of plants in semiarid conditions, where water is by far the most limiting factor for plant growth.

The addition of composted sewage sludge to soil alone had little effect on increasing the growth of *R. sphaerocarpa*. Thus, the effectiveness of composted sewage sludge was increased greatly by the irrigation of the plants. This could be due to an improvement in the supply of available nutrients to the soil, from the composted sewage sludge. Thus, irrigated plants grown in the amended soil had higher nutrient (NPK) concentrations in their tissues than non-irrigated plants.

The lack of a response to sewage sludge in the absence of water agrees with the widely accepted idea that mycorrhizas present little advantage to seedlings grown in amended soils (Jeffries 1987). However, we have found a positive synergism between the inoculation of an AM fungus and the addition of composted urban waste for the establishment of *Pistacia lentiscus* L. in a semiarid Mediterranean area (Caravaca *et al.* 2002). Therefore, the effect of a combined treatment (amendment and mycorrhizal inoculation) on plant growth may depend on the type of waste material used.

Nitrate reductase (NR) activity can be considered an indicator of the effectiveness of AM fungi in water-deficit environments (Ruíz-Lozano & Azcón 1996; Subramanian & Charest 1999). In this study, we found that mycorrhizal inoculation increased NR activity in the roots of *R. sphaerocarpa* seedlings, which was independent of irrigation of the plants. The increase in N-assimilating enzymes, such as NR, may be attributed to the contribution of hyphal transport of N (Subramanian & Charest 1999). In fact, as water stress impedes the mobility of nitrate (Azcón *et al.* 1996), mycorrhizal plants may have access, through the extraradical mycelium, to the forms of N that are usually unavailable to the non-inoculated plants.

Some authors have indicated that the increase in NR activity of mycorrhizal plants compared with non-mycorrhizal ones can be related to the phosphate requirement of this enzyme (Ruíz-Lozano & Azcón 1996). In this sense, the mycorrhizal effect could be interpreted as an indirect response to the improved nutrient status, particularly of phosphorus (P). Thus, the fact that the highest contents of P in shoots was in irrigated plants inoculated with AM fungi and growing in the amended soil could explain how these plants had the highest NR activity.

It is worth noting that the effect of the mycorrhizal treatments on NR activity in roots depended on the associated fungus. Thus, inoculation with *G. deserticola* EEZ 45 was the most effective mycorrhizal treatment with respect to N assimilation in *R. sphaerocarpa*. Recently, we have reported the effectiveness of *G. deserticola* EEZ 45 in increasing the root NR activity and N uptake in shoot tissues of *Dorycnium pentaphyllum* L. seedlings under water-stressed conditions (Caravaca *et al.* 2003b). This means that NR activity was regulated not only by the P content in the host plant, but also by the colonizing AM fungus, indicating specific physiological behaviour in different AM fungi. Likewise, the differing effects of AM fungi on this enzyme activity could be a consequence of fungal NR activity.

Phosphatase is a hydrolase that is involved in the P-cycle, whereby organic P is transformed into plant-available P. Phosphatases are inhibited by the final product of the enzymatic reaction, viz. inorganic P, representing a feedback inhibition. Composted sewage sludge and mycorrhizal inoculation treatments had no effect on the acid phosphatase activity in roots of *R. sphaerocarpa*. Khalil *et al.* (1994) found a positive correlation between root phosphatase and AM formation, but the level of the correlation depended on the plant species tested. The phosphatase activity in roots of *R. sphaerocarpa* was not related to plant growth. This agrees with the finding of Azcón & Barea (1997), suggesting that a high phosphatase activity does not compensate for an inadequate supply of assimilable P to the plant. In this regard, high values of phosphatase activity were recorded in control plants, which grew in a soil having a low concentration of available inorganic P.

In conclusion, the effectiveness of mycorrhizal inoculation on the establishment and growth of *R. sphaerocarpa* seedlings in a semiarid Mediterranean area was independent of the water regime. The capacity of AM fungi for increasing plant growth in drought conditions may be related to improved nutrient uptake and to an increase in N assimilation through NR activity. The addition of composted sewage sludge was only effective when soil water was highly available. The combination of mycorrhizal inoculation and composted sewage sludge addition had no synergistic effect on plant growth.

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REFERENCES

Alguacil MM Caravaca F Azcón R Pera J Díaz G & Roldán A 2003a. Improvements in soil quality and performance of mycorrhizal *Cistus albidus* L. seedlings resulting from addition of microbially treated sugar beet residue to a degraded semiarid Mediterranean soil. Soil Use and Management 19, 277–283.

Alguacil MM Hernández JA Caravaca F Portillo B & Roldán A 2003b. Antioxidant enzyme activities in shoots from three mycorrhizal shrub species afforestated in a degraded semi-arid soil. Physiologia Plantarum 118, 562–570.

Azcón R & Barea JM 1997. Mycorrhizal dependency of a representative plant species in Mediterranean shrublands (*Lavandula spica* L.) as a key factor to its use for revegetation strategies in desertification-threatened areas. Applied Soil Ecology 7, 83–92.

Azcón R Ruíz-Lozano JM & Rodríguez R 2001. Differential contribution of arbuscular mycorrhizal fungi to plant nitrate uptake (^{15}N) under increasing N supply to the soil. Canadian Journal of Botany 79, 1175–1180.

Azcón R Gómez M & Tobar RM 1996. Physiological and nutritional responses by *Lactuca sativa* L. to nitrogen sources and mycorrhizal fungi under drought conditions. Biology and Fertility of Soils 22, 156–161.

Barahona E & Santos F 1981. Un nuevo método para la determinación de densidades aparentes y del coeficiente de extensividad lineal (COLE) por método de parafina. Anales de Edafología y Agrobiología 40, 721–725.

Caravaca F Alguacil MM Figueroa D Barea JM & Roldán A 2003a. Re-establishment of *Retama sphaerocarpa* as a target species for reclamation of soil physical and biological properties in a semi-arid Mediterranean area. Forest Ecology and Management 182, 49–58.

Caravaca F Alguacil MM Díaz G & Roldán A 2003b. Use of nitrate reductase activity for assessing the effectiveness of mycorrhizal symbiosis in *Dorycnium pentaphyllum* under induced water deficit. Communications in Soil Science and Plant Analysis 34, 2291–2302.

Caravaca F Barea JM & Roldán A 2002b. Synergistic influence of an arbuscular mycorrhizal fungus and organic amendment on *Pistacia lentiscus* L. seedlings afforested in a degraded semiarid soil. Soil Biology and Biochemistry 34, 1139–1145.

Downs MR Nadelhoffer KJ Melillo JM & Aber JD 1993. Foliar and fine root nitrate reductase activity in seedlings of four forest tree species in relation to nitrogen availability. Trees (Berlin) 7, 233–236.

Giovannetti M & Mosse B 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytologist 84, 489–499.

Goicoechea N Antolín MC Strnad M & Sánchez-Díaz M 1996. Root cytokinins, acid phosphatase and nodule activity in drought stressed mycorrhizal or nitrogen fixing alfalfa plants. Journal of Experimental Botany 47, 683–686.

Haase P Pugnaire FI Fernández EM Puigdefábregas J Clark SC & Incoll LD 1996. Investigation of rooting depth in the semi-arid shrub *Retama sphaerocarpa* (L.) Boiss. by labelling of ground water with a chemical tracer. Journal of Hydrology 177, 23–31.

Herrera MA Salamanca CP & Barea JM 1993. Inoculation of woody legumes with selected arbuscular mycorrhizal fungi and rhizobia to

recover desertified Mediterranean ecosystems. Applied and Environmental Microbiology 59, 129–133.

Jeffries P Gianinazzi S Perotto S Turnau K & Barea JM 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biology and Fertility of Soils 37, 1–16.

Jeffries P 1987. Use of mycorrhizae in agriculture. CRC Critical Reviews in Biotechnology 5, 319–357.

Kaldorf M Schmelzer E & Bothe H 1998. Expression of maize and fungal nitrate reductase genes in arbuscular mycorrhiza. Molecular Plant–Microbe Interactions 11, 439–448.

Khalil S Loynachan TE & Tabatabai MA 1994. Mycorrhizal dependency and nutrient uptake by improved and unimproved corn and soybean cultivars. Agronomy Journal 86, 949–958.

Lax A Díaz E Castillo V & Albaladejo J 1994. Reclamation of physical and chemical properties of a salinized soil by organic amendment. Arid Soil Research and Rehabilitation 8, 9–17.

Linden DR Larson WE Dowdy RH & Clapp CE 1995. Agricultural utilization of sewage sludge. University of Minnesota Agricultural Experiment Station Bulletin 606. University of Minnesota St Paul MN.

Murphy J & Riley JP 1962. A modified single solution method for determination of phosphate in natural waters. Analytica Chimica Acta 27, 31–36.

Navas A Machín J & Navas B 1999. Use of biosolids to restore the natural vegetation cover on degraded soils in the badlands of Zaragoza (NE Spain). Bioresource Technology 69, 199–205.

Page AL Miller RH & Keeney OR 1982. Methods of soil analysis. American Society of Agronomy and Soil Science Society of America Madison WI.

Pascual JA García C & Hernández T 1999. Comparison of fresh and composted organic waste in their efficacy for the improvement of arid soil quality. Bioresource Technology 68, 255–264.

Phillips JM & Hayman DS 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55, 158–161.

Querejeta JI Barea JM Allen MF Caravaca F & Roldán A 2003. Differential response of $\delta^{13}C$ and water use efficiency to arbuscular mycorrhizal infection in two aridland woody plant species. Oecologia 135, 510–515.

Requena N Jeffries P & Barea JM 1996. Assessment of natural mycorrhizal potential in a desertified semi-arid ecosystem. Applied Environmental Microbiology 62, 842–847.

Requena N Pérez-Solís E Azcón-Aguilar C Jeffries P & Barea JM 2001. Management of indigenous plant–microbe symbioses aids restoration of desertified ecosystems. Applied Environmental Microbiology 67, 495–498.

Ruíz-Lozano JM & Azcón R 1996. Mycorrhizal colonization and drought stress as factors affecting nitrate reductase activity in lettuce plants. Agriculture Ecosystems and Environment 60, 175–181.

Schollemberger CJ & Simon RH 1954. Determination of exchange capacity and exchangeable bases in soils. Soil Science 59, 13–24.

Soil Survey Staff 1999. Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys. USDA Natural Resources Conservation Service. Agricultural Handbook 436. US Government Printing Office Washington DC.

Subramanian KS & Charest C 1999. Acquisition of N by external hyphae of an arbuscular mycorrhizal fungus and its impact on physiological responses in maize under drought-stressed and well-watered conditions. Mycorrhiza 9, 69–75.

Tabatabai MA & Bremner JM 1969. Use of *p*-nitrophenol phosphate in assay of soil phosphatase activity. Soil Biology and Biochemistry 1, 301–307.

Turner MS Clark GA Stanley CD & Smajstrla AG 1994. Physical characteristics of a sandy soil amended with municipal solid waste compost. Soil Crop Science Society of Florida Proceedings 53, 24–26.

Valladares F Villar-Salvador P Domínguez S Fernández-Pascual M Peñuelas JL & Pugnaire FI 2002. Enhancing the early performance of the leguminous shrub *Retama sphaerocarpa* (L.) Boiss: fertilisation versus *Rhizobium* inoculation. Plant and Soil 240, 253–262.

Zebarth BJ Neilsen GH Hogue E & Neilsen D 1999. Influence of organic waste amendments on selected soil physical and chemical properties. Canadian Journal of Soil Science 79, 501–504.

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