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Medium-term effects of mycorrhizal inoculation and composted municipal waste addition on the establishment of two Mediterranean shrub species under semiarid field conditions

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Abstract

The development of appropriate revegetation techniques is essential to reduce and to remediate the processes of erosion and desertification in semiarid Mediterranean areas. A factorial field experiment was carried out in a degraded semiarid Mediterranean area to assess the effectiveness of composted municipal waste addition to soil, mycorrhizal inoculation with *Glomus intraradices* and the combination of both treatments on the viability, growth and nutrition of *Olea europaea* L. subsp. *sylvestris* and *Rhamnus lycioides* L., over a 2-year growth period. Six months after planting, only mycorrhizal inoculation of *O. europaea* subsp. *sylvestris* and *R. lycioides* seedlings grown in the soil, with or without addition of composted municipal waste, statistically and significantly increased the shoot biomass and contents of foliar nutrients (N, P and K). During the last 6 months of the growth period, both shrub species displayed sharp increases in the shoot biomass. Two years after planting, the highest increases in the shoot biomass of *O. europaea* plants were recorded in the combined treatment of composted municipal waste addition and mycorrhizal inoculation (about 12-fold greater than control plants). The shoot biomass of *R. lycioides* was increased by composted municipal waste addition (about 226%) to a greater extent than by mycorrhizal inoculation (about 87%), at the end of the 2-year growth period. For both shrub species, there was a positive statistically significant correlation between shoot biomass, foliar contents of N, P and K and soil aggregate stability. Composted municipal waste addition, mycorrhizal inoculation and, in some cases, the combination of both treatments can be employed as effective tools in programmes using shrub species for revegetation of semiarid areas.

Keywords: Arbuscular mycorrhizal fungi; *Olea europaea* subsp. *sylvestris*; *Rhamnus lycioides*; Organic amendments; *Glomus intraradices*; Semiarid Mediterranean area

1. Introduction

The Mediterranean area of Southeast Spain is one of those most affected by environmental degradation and erosive processes, due to its climatic characteristics such as a scarce and irregular rainfall and a long, dry and hot summer. This has a damaging effect on soil quality and stability, together with a negative effect on plant growth in this ecosystem. Therefore, degraded soils need development techniques to reduce erosion and to remediate the effects of degradation. Organic matter supplements and subsequent planting

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of native shrub plants has been proposed as a good restoration strategy to rehabilitate erosion-affected soils (Vallejo et al., 1999; Cox et al., 2001). The beneficial effects of organic amendments include decreased soil bulk density and increased water-holding capacity, aggregate stability, saturated hydraulic conductivity, water infiltration rate and microbiological activity (Zebarth et al., 1999; García et al., 2000). The vegetation cover helps avoid soil losses, together with improvements in soil physical properties. In this regard, the use of native shrub species, such as *Olea europaea* L. subsp. *sylvestris* and *Rhamnus lycioides* L., in revegetation programmes for the drier areas has been encouraged recently by the Common Agricultural Policy of the European Union.

The successful reestablishment of native plants in degraded soils such as semiarid ecosystems may be limited by the low density of mycorrhizal propagules, which represent a significant factor for soil fertility by governing the cycles of major plant nutrients (Requena et al., 2001). There is evidence that mycorrhizas help plants to thrive in arid conditions by increasing the supply of nutrients to the plant (particularly P) (Querejeta et al., 1998), improving soil aggregation in eroded soils (Requena et al., 2001), and reducing water stress (Augé, 2001). However, the arbuscular mycorrhizal (AM) symbiosis is influenced by various management practices, such as the degree and type of fertilisation, host plant species or cultivar, mycorrhizal species, type of host plant root system, and crop rotation or soil tillage (Kurle and Pfleger, 1994). In addition, many environmental factors, such as soil water and aeration (Augé, 2001) and soil micro-organisms (Abdel-Fattah and Mohamedin, 2000), which have considerable impact on AM distribution and effectiveness.

Mycorrhizal inoculation and the addition of organic amendments to soil are of particular importance in agroecosystems of Mediterranean semiarid areas. In revegetation programmes, it is essential to apply methods which improve soil quality and the ability of the planted species to resist the severe environmental conditions, and thus allow restoration of the biodiversity. However, as only a few drought-resistant indigenous plant species can be used successfully in such programmes under these field conditions, it is necessary to carry out medium-term studies to ascertain the role that these symbionts could play in the establishment of such plant species in a managed ecosystem. The objective of this study was to determine the effectiveness of composted municipal waste addition to soil, mycorrhizal inoculation with *Glomus intraradices* (Schenck and Smith) and the combination of both treatments on the viability, growth and nutrition of *O. europaea* subsp. *sylvestris* and *R. lycioides*, for a 2-year growth period in a semiarid Mediterranean environment.

2. Materials and methods

2.1. Study sites

The experimental area was located on the El Picarcho range, in the province of Murcia (Southeast Spain) (co-ordinates: 1°10′W and 38°23′N). The climate is semiarid Mediterranean, with a mean annual rainfall of 233 mm and a mean annual temperature of 20 °C during the experiment (Fig. 1). The soil used was a Petrocalcic Xerosol (FAO, 1988), developed from limestones, with a silt-loam texture.

2.2. Composted material and plants

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

The plants used for the revegetation experiments were *O. europaea* subsp. *sylvestris* and *R. lycioides*, which belong to the natural succession in certain plant communities of semiarid Mediterranean ecosystems in Southeast Spain. They are typically low-growing shrubs reaching heights of 1.5 and 3 m, respectively. They are also well adapted to water stress conditions and could potentially be used in the reafforestation of semiarid disturbed lands.

2.3. Mycorrhizal inoculation of seedlings

The mycorrhizal fungus used in the experiment was *G. intraradices*, obtained from the collection of the

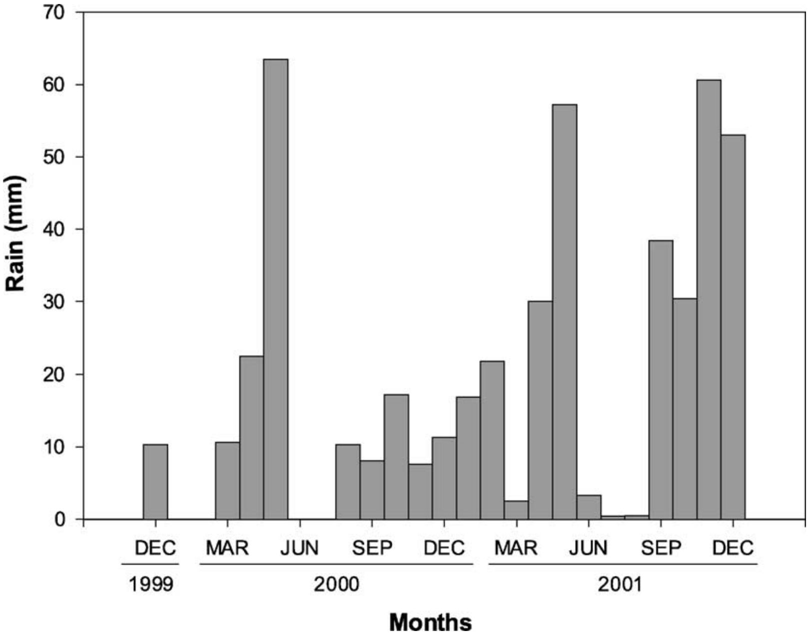

Fig. 1. Monthly rainfall amounts recorded during the period of the experiment in the El Picarcho experimental area.

experimental field station of Zaidín, Granada (EEZ1), Spain.

AM fungal inoculum consisted of a mixture of rhizospheric soil from trap cultures (*Sorghum* sp.) containing spores, hyphae and mycorrhizal root fragments. Once germinated, seedlings were transplanted into the growing substrate, consisting of peat and cocopeat (1:1, v/v) mixed with *G. intraradices* inoculum (5%). The same amount of the autoclaved mixture of the inoculum was added to control plants, supplemented with a filtrate ($<20\,\mu$m) 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 fertiliser treatment. Nursery procedures were conducted at Paisajes del Sur Ltd. (Granada, Spain).

Table 1
Analytical characteristics of the composted municipal waste used in the experiment

Parameter	Quantity (±S.E.) ($n = 8$)
Ash (%)	44.8 (0.5)
pH (1:10)	6.7 (0.6)
Electrical conductivity, EC (1:5, μS cm^{-1})	4700 (90)
Total organic C (g kg^{-1})	276.0 (15)
Water soluble C (μg g^{-1})	1950 (589)
Water soluble carbohydrates (μg g^{-1})	76 (12)
Total N (g kg^{-1})	14.5 (0.9)
N–NH$_3$ (μg g^{-1})	3350 (469)
Total P (g kg^{-1})	3.8 (0.6)
Total K (g kg^{-1})	12.0 (0.8)
Cu (μg g^{-1})	146 (17)
Zn (μg g^{-1})	261 (19)
Ni (μg g^{-1})	25 (3)
Cr (μg g^{-1})	62.9 (4.3)
Cd (μg g^{-1})	5 (0)
Pb (μg g^{-1})	98 (13)

2.4. Experimental design and layout

The experiments were arranged in a randomised block design, with two factors and six replication blocks. The first factor was the addition or not of composted municipal waste to the soil, and the second was the direct inoculation of either *O. europaea* or *R. lycioides* plants with *G. intraradices* in the nursery. A no mycorrhizal treatment was included as control. Thus, four treatments in each experiment were established: (1) seedlings without mycorrhizal treatment and soil without composted municipal waste addition (control, C), (2) seedlings without mycorrhizal treatment and soil with composted municipal waste addition (W), (3) seedlings inoculated with *G. intraradices* and soil without composted municipal waste addition (M), and (4) seedlings inoculated with *G. intraradices* and soil with composted municipal waste addition (WM). In September 1999, two adjacent plots of 1200 m^2 were prepared mechanically with a subsoiler prior to planting with a maximum tillage depth of 25 cm. In both plots, eight rows (1 m wide, 25 m long, 3 m apart) were established. In early December 1999, composted municipal waste was added to half of the rows (0–20 cm depth) at a rate of 6.7 kg m^{-2}, following the randomised design. This is sufficient to raise the soil total organic carbon content by 10 g kg^{-1}. Three weeks after the addition of the compost, *O. europaea* and *R. lycioides* seedlings (inoculated and non-inoculated) were planted in individual holes, at least 1 m apart in a single row, with 3 m between blocks. At least 32 seedlings per replication block were planted (eight plants × four treatments in each block).

2.5. Sampling procedures

Immediately prior to planting, 16 soil samples were collected randomly, 8 samples from the rows with composted municipal waste and 8 from the rows without composted municipal waste. Eighteen months after planting, 6 soil samples of each treatment were collected (24 soil samples per species and 48 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. The sampling was carried out in June 2001, before the dry season, when the highest microbial activity would be expected (Lax et al., 1997). Every 6 months after planting, six plants (one per block) of each treatment were also harvested.

2.6. Plant analyses

Basal stem diameters and heights of plants were measured with callipers and rules (24 plants per plot). Fresh and dry (105 °C, 5 h) weights of shoots and roots were recorded. Plant tissues were ground before chemical analysis. The foliar concentrations of N, P and K were determined after digestion in nitric-perchloric acid (5:3) for 6 h (Plank, 1992). The foliar P was determined by colorimetry (Murphy and Riley, 1962), foliar N was colorimetrically measured after Kjeldhal digestion (Page et al., 1982) and foliar K was estimated by flame photometry (Schollenberger and Simon, 1954).

The percentage of root length colonised by AM 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 analyses

Total nitrogen was determined by the Kjeldhal method, and the total organic C was determined by oxidation with potassium dichromate in a sulphuric medium and excess dichromate evaluated using Mohr’s salt (Yeomans and Bremner, 1988). In soil aqueous extracts, water soluble carbon was determined by wet oxidation with $K_2Cr_2O_7$ and measurement of the absorbance at 590 nm (Sims and Haby, 1971). Available P, extracted with 0.5 M $NaHCO_3$, was determined by colorimetry according to Murphy and Riley (1962). Extractable (with ammonium acetate) K was determined by flame photometry.

Soil bulk density was determined as described by Barahona and Santos (1981). Oven-dried soil clods were coated with paraffin and weighed in water (Pw) and in air (Pa). Bulk density was calculated by Pa/(Pa − Pw).

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 $(P1 - P2) \times 100/(4 - P2 + T)$.

2.8. Statistical analysis

Aggregate stability and percentage colonisation were arcsin-transformed, and the other parameters were log-transformed to compensate for variance heterogeneity before analysis of variance. The effects of composted municipal waste addition and mycorrhizal inoculation, and their interactions, 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 $P < 0.05$. Correlation analysis between all the soil parameters measured was carried out using Pearson's rank correlation coefficients. Statistical procedures were carried out with the software package SPSS 10.0 for Windows.

3. Results

3.1. Changes in soil physical–chemical properties

Composted municipal waste addition significantly increased aggregate stability of the soil used for the revegetation experiments (Table 2). Eighteen months after planting *O. europaea* seedlings, composted municipal waste addition had increased the percentage of stable aggregates of rhizosphere soil, by about 26% compared to the control soil. However, the increases produced by mycorrhizal inoculation and the combined treatment of composted municipal waste addition and mycorrhizal inoculation were not statistically significant (Tables 2 and 3). Similarly, the addition of composted municipal waste was the only treatment which increased the aggregate stability of the rhizosphere soil of *R. lycioides* (Tables 2 and 3).

The bulk density of soil selected for revegetating with *O. europaea* and *R. lycioides* was decreased by the addition of composted municipal waste (Table 2). Eighteen months after planting, mycorrhizal inoculation, addition of composted municipal waste, or a combination of both treatments had no effect on the bulk density of rhizosphere soil of *O. europaea* (Tables 2 and 3). However, with *R. lycioides* the addition of composted municipal waste statistically significant decreased the bulk density of the rhizosphere soil (Table 2).

The addition of composted municipal waste significantly increased the concentrations of total N, available P, extractable K, total organic C and water soluble C in the soil (Table 2). The increase in total organic C, water soluble C and nutrients (N, P and K) was also statistically significant in the rhizosphere soil 18 months after planting of *O. europaea* and *R. lycioides* seedlings (Tables 2 and 3). In the revegetation experiment with *O. europaea*

Table 2

Analytical characteristics of the soil previous planting and changes in physical–chemical properties of rhizosphere soil of *O. europaea* subsp. *sylvestris* and *R. lycioides* in response to mycorrhizal inoculation and composted municipal waste addition 18 months after planting ($n = 6$)

	Aggregate stability (%)	Bulk density (g cm^{-3})	Total N (g kg^{-1})	Available P (µg g^{-1})	Extracted K (µg g^{-1})	Total organic C (g kg^{-1})	Water soluble C (µg g^{-1})
Soil prior planting							
C	44.5a	1.2b	0.7a	3a	702a	22.2a	108a
W	53.5b	1.1a	1.2b	90b	1474b	31.7b	268b
O. europaea subsp. *sylvestris*							
C	40.2a	1.1a	1.3b	12a	582a	22.2a	178a
W	50.8b	1.1a	2.9c	38b	1291b	27.9b	287b
M	47.2ab	1.2a	0.9a	27b	405a	18.3a	171a
WM	49.2ab	1.1a	1.8c	33b	1470b	25.4b	305b
R. lycioides							
C	37.0a	1.2b	1.2b	26a	876b	22.6a	170a
W	49.8b	1.0a	1.7c	43b	1505c	25.7b	260b
M	44.7ab	1.1b	0.9a	19a	384a	22.4a	172a
WM	46.7ab	1.1b	1.5bc	49b	1312c	28.0b	314b

C: control; W: composted municipal waste addition; M: mycorrhizal inoculation; WM: composted municipal waste addition + mycorrhizal inoculation. Values in columns sharing the same letter do not differ significantly ($P < 0.05$) as determined by the LSD test.

Table 3
Two-way ANOVA (mycorrhizal inoculation and composted municipal waste addition) for all parameters studied in *O. europaea* subsp. *sylvestris* and *R. lycioides* seedlings 18 months after planting

	Composted waste (W)	Mycorrhiza (M)	Interaction (WM)
O. europaea subsp. *sylvestris*			
Aggregate stability	8.869 (0.012)	1.204 (0.294)	3.737 (0.077)
Bulk density	1.946 (0.188)	0.486 (0.506)	1.946 (0.188)
Total organic C	13.863 (0.003)	4.279 (0.061)	0.748 (0.413)
Water soluble C	83.390 (<0.001)	0.006 (0.938)	0.918 (0.367)
Total N	20.234 (<0.001)	7.858 (0.016)	0.086 (0.773)
Available P	131.204 (<0.001)	33.364 (<0.001)	59.818 (<0.001)
Extractable K	52.002 (<0.001)	0.712 (0.424)	3.113 (0.103)
Colonisation	0.783 (0.403)	97.074 (<0.001)	0.588 (0.466)
Shoot dry biomass	14.514 (0.003)	11.601 (0.005)	22.738 (<0.001)
Root dry biomass	10.851 (0.006)	10.648 (0.007)	0.260 (0.624)
Foliar N	40.496 (<0.001)	40.496 (<0.001)	34.917 (<0.001)
Foliar P	38.761 (<0.001)	6.252 (0.028)	13.261 (0.003)
Foliar K	44.687 (<0.001)	28.600 (<0.001)	44.093 (<0.001)
R. lycioides			
Aggregate stability	29.860 (<0.001)	2.401 (0.147)	13.470 (0.003)
Bulk density	8.721 (0.012)	0.349 (0.572)	6.153 (0.029)
Total organic C	25.606 (<0.001)	1.090 (0.317)	1.865 (0.197)
Water soluble C	41.044 (<0.001)	1.816 (0.203)	1.623 (0.227)
Total N	55.783 (<0.001)	13.202 (0.003)	1.722 (0.214)
Available P	20.189 (<0.001)	1.125 (0.310)	1.476 (0.248)
Extractable K	313.215 (<0.001)	91.667 (<0.001)	46.091 (<0.001)
Colonisation	0.877 (0.377)	9.673 (0.009)	1.350 (0.723)
Shoot dry biomass	14.392 (0.003)	8.516 (0.013)	3.660 (0.080)
Root dry biomass	10.715 (0.007)	3.288 (0.095)	0.043 (0.842)
Foliar N	31.758 (<0.001)	18.211 (0.001)	10.031 (0.008)
Foliar P	18.510 (0.001)	3.929 (0.071)	3.412 (0.089)
Foliar K	25.301 (<0.001)	20.324 (<0.001)	17.308 (0.001)

The values shown in the parenthesis represent *P*-values.

seedlings, the mycorrhizal inoculation treatment also significantly increased the level of available P (by about 125%) in the rhizosphere soil, but decreased the level of total N (by about 31%) compared to control plants. In the case of *R. lycioides*, mycorrhizal inoculation did not affect significantly the concentration of available P in the rhizosphere soil. The concentrations of total N and extractable K significantly decreased in the rhizosphere soil of *G. intraradices*-colonised *R. lycioides* plants.

3.2. Changes in root infection

At the time of planting, the *G. intraradices*-inoculated *O. europaea* and *R. lycioides* seedlings had significantly higher percentages of root colonisation (on average 62 and 38% of the root length was infected, respectively) than the non-inoculated plants, whose roots showed negligible levels of AM colonisation (Table 4). The degree of mycorrhizal colonisation of the non-inoculated *O. europaea* seedlings increased to an average of 10% as a result of natural infection, while that of inoculated seedlings hardly varied during the 18-month growth period. In the case of *R. lycioides*, the seedlings inoculated with *G. intraradices* also showed statistically significant higher levels of colonisation than those infected naturally, although they were always lower than those of inoculated *O. europaea* seedlings.

3.3. Changes in shoot and root biomass and foliar nutrient contents

At the time of transplanting, there were no statistically significant differences in shoot and root dry biomass between treatments for both species (Fig. 2).

Table 4
Foliar nutrients and root infection evolution of *O. europaea* subsp. *sylvestris* and *R. lycioides* seedlings in response to mycorrhizal inoculation and composted municipal waste addition ($n = 6$)

Parameter	*O. europaea* subsp. *sylvestris*			*R. lycioides*		
	0 months	6 months	18 months	0 months	6 months	18 months
Nitrogen (mg per plant)						
C	18.8a	16.6a	26.1a	9.8a	8.9a	7.7a
W	18.8a	17.0a	125.6b	9.8a	9.6a	43.6bc
M	19.2a	64.6c	121.9b	7.6a	27.5b	36.4b
WM	19.2a	43.5b	134.7b	7.6a	35.7b	58.2c
Phosphorus (mg per plant)						
C	1.5a	1.9a	1.1a	1.0a	0.7a	0.5a
W	1.5a	1.4a	5.5b	1.0a	0.8a	2.6b
M	3.7b	8.3b	3.1b	0.9a	2.8b	1.4b
WM	3.7b	9.9b	4.5b	0.9a	3.6b	2.5b
Potassium (mg per plant)						
C	19.5a	17.3a	9.6a	7.5a	9.7a	3.9a
W	19.5a	22.6a	56.8b	7.5a	9.3a	26.4b
M	23.8b	64.8b	49.2b	6.5a	24.7b	23.9b
WM	23.8b	63.7b	46.5b	6.5a	31.8b	29.1b
Colonisation (%)						
C	0.4a	14.8a	9.5a	0.0a	0.0a	6.2a
W	0.4a	13.6a	10.3a	0.0a	1.8a	15.8a
M	62b	64.6b	61.6b	37.9b	48c	31.0b
WM	62b	65.4b	69.5b	37.9b	28bc	35.6b

C: control; W: composted municipal waste addition; M: mycorrhizal inoculation; WM: composted municipal waste addition + mycorrhizal inoculation. Values in columns sharing the same letter do not differ significantly ($P < 0.05$) as determined by the LSD test.

Two years after planting, there were no statistically significant differences in plant survival between treatments for both shrub species and about 90% of the plants survived, irrespective of the treatment (data not shown). Six months after planting, mycorrhizal inoculation of *O. europaea* seedlings, grown in the soil with or without addition of composted municipal waste, significantly increased the shoot dry biomass (on average by 173%) and root dry biomass (on average by 47%) (Fig. 2). This effect on the shoot biomass of *O. europaea* plants, due to the treatments assayed was still observed after 1 year. The effects of composted municipal waste addition, mycorrhizal inoculation and the combination of both treatments on shoot and root dry biomass of *O. europaea* were similar at 18 months after planting in the field, all producing about 150% more biomass than the control plants. In the last 6 months of the growth period, the shoot dry biomass of *O. europaea* and *R. lycioides* plants sharply increased in response to composted municipal waste addition, mycorrhizal inoculation and the combination of both treatments. At the end of the 2-year growth period, the highest increases in the shoot dry biomass of *O. europaea* plants were recorded in the combined treatment of composted municipal waste addition and mycorrhizal inoculation (about 12-fold greater than control plants), followed by the composted municipal waste alone and mycorrhizal inoculation treatments (about 5- and 3-fold greater than control plants, respectively).

R. lycioides seedlings responded to mycorrhizal inoculation and the combined treatment of composted municipal waste addition and mycorrhizal inoculation had a greater shoot dry biomass (on average 163% more) than non-inoculated plants at 6 months (Fig. 2). Eighteen months after planting, the addition of composted municipal waste and mycorrhizal inoculation considerably increased the shoot and root dry biomass of *R. lycioides* plants, but no statistically significant differences were observed between these two treatments. *R. lycioides* plants that had received composted municipal waste, mycorrhizal inoculation

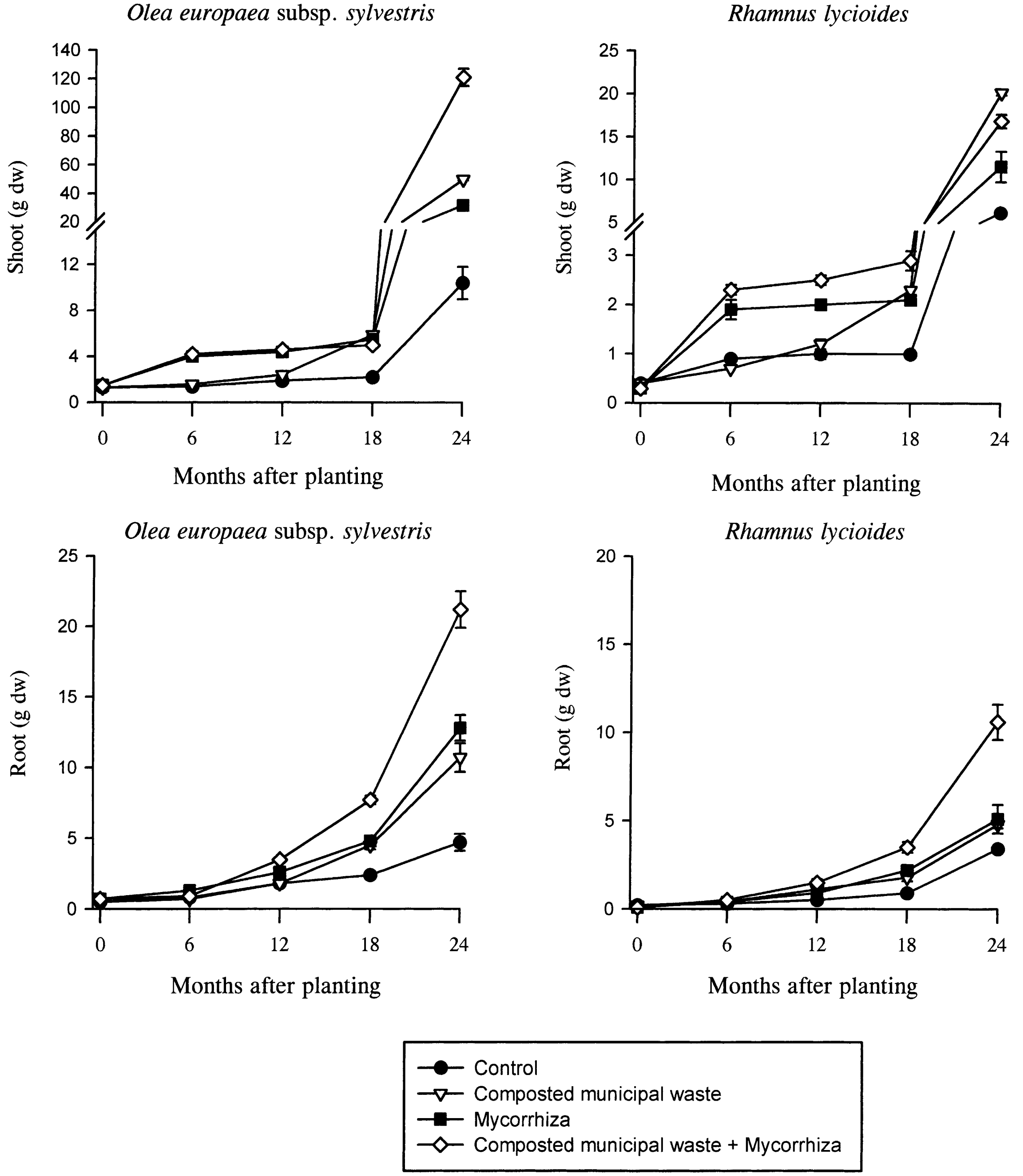

Fig. 2. Shoot and root dry biomass evolution of *O. europaea* subsp. *sylvestris* and *R. lycioides* seedlings in response to mycorrhizal inoculation and composted municipal waste addition during a 2-year growth period ($n = 6$). Bars represent standard error.

or a combination of the two treatments registered an increased shoot biomass more pronounced during the last 6 months than in the rest of the growth period. Two years after planting, the increase in shoot dry biomass of *R. lycioides* associated with composted municipal waste addition (about 226%) was greater than for mycorrhizal inoculation (about 87%).

As observed for the growth parameters, the highest N, P and K contents were seen 6 months after planting of *O. europaea* and *R. lycioides* seedlings inoculated with *G. intraradices* and grown in the soil with or without composted municipal waste addition (Table 4). Eighteen months after planting, the N, P and K contents in shoot tissues of both shrub species were significantly increased by composted municipal waste addition and mycorrhizal inoculation, being higher in shoot tissue of *O. europaea* than in that of *R. lycioides*.

For both shrub species, there was a positive statistically significant correlation between shoot dry biomass, foliar N, P and K contents and aggregate stability, although the significance level was higher for *R. lycioides* ($P < 0.01$) than for *O. europaea* ($P < 0.05$).

4. Discussion

4.1. Composted municipal waste addition and soil conditions

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, P and K (Cox et al., 2001). However, the benefits of organic amendments are due also to the improvement of the physical characteristics of the soil, which in turn favours the establishment and viability of a stable plant cover (Roldán et al., 1996; Cox et al., 2001; Caravaca et al., 2002). In this regard, decreased soil bulk density was observed in the revegetation experiment with *R. lycioides* following the addition of composted municipal waste. Likewise, composted municipal waste statistically and significantly improved the structural stability of rhizosphere soil of *O. europaea and R. lycioides*. Such organic material has a cementing effect, due to the polysaccharides present (Lax and García-Orenes, 1993), and enhances microbial populations (García et al., 2000; Borken et al., 2002). In the present study, the addition of composted municipal waste to soil increased the level of water soluble C, which can be used as carbon and energy sources for soil microflora and may also have a structural function (Haynes and Swift, 1990). The enhanced soil microflora, particularly bacteria and fungi are principally responsible for the formation of aggregates larger than 0.2 mm (Lax et al., 1997; Andrade et al., 1998). Statistically significant correlations were found between shoot dry biomass of *O. europaea and R. lycioides* plants and aggregate stability of the rhizosphere soil of both shrub species.

This type of composted municipal waste presumably can be less effective than uncomposted municipal waste for improving soil structure (Caravaca et al., 2002). However, we have proved that the addition of composted municipal waste significantly improved the structural stability of rhizosphere soil of *O. europaea and R. lycioides*. In addition, the use of composted municipal waste leads to fewer problems related to toxic substances, which are eliminated during the composting process (Pascual et al., 1999). The heavy metal contents of the composted municipal waste used in our study did not exceed the maximum levels authorised by EU law (Directive of European Communities Council, 86/278/CEE).

4.2. Effectiveness of mycorrhizal inoculation on growth of O. europaea and R. lycioides

The role of AM in the stimulation of growth and nutrient uptake of many host plants is well documented (Smith and Read, 1997). AM have been shown to enhance the uptake of P and N (Toro et al., 1997; Vázquez et al., 2001). It is worth noting that mycorrhizas played a key role in the first stages of the reestablishment of *O. europaea* and *R. lycioides* seedlings in semiarid conditions, because the only increases in the shoot biomass were observed in inoculated plants grown in the soil, with or without composted municipal waste addition. The fact that the shoot biomass in plants that received composted municipal waste alone did not differ from control plants demonstrates that the improvements in shoot biomass of both shrub species subjected to the combined treatment were solely attributable to the symbiotic fungus. The lowest concentrations of total N in the rhizosphere soil of *O. europaea* and of total N and extractable K in the rhizosphere soil of *R. lycioides* occurred for seedlings inoculated with *G. intraradices* and grown in the soil without composted municipal waste addition. This could be related to the well-established activity of external mycelium in the uptake and translocation of nutrients from soil. In addition, *G. intraradices*-colonised *O. europaea* and *R. lycioides* exhibited higher foliar N and K contents than control plants.

It has been demonstrated that different plant species, and even cultivars or ecotypes of the same species, show differenting degrees of susceptibility to colonisation by an AM fungus (Roldán et al., 1992). In the present study, arbuscular mycorrhizal fungal colonisation differed significantly between the two shrub species. Thus, the level of colonisation by *G. intraradices* was higher in roots of *O. europaea* than for *R. lycioides*. As suggested by Xueli et al. (2002), the host plant species apparently have direct effects on the abundance and colonisation of AM fungi.

The extent of mycorrhizal infection is of importance when studying the influence of AM fungi on the host plant. High 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 colonising a relatively large percentage of the roots. In the present revegetation experiment, the effect of AM inoculation on shoot biomass was related positively to the colonisation level of the AM fungi tested in the roots of both shrub species. At the end of the growth period, mycorrhizal inoculation had increased the shoot biomass of *O. europaea* to a greater extent (about 205% with respect to control plant) than that of *R. lycioides* (about 87% with respect to control plant).

4.3. Effectiveness of the combination of composted municipal waste addition and mycorrhizal inoculation on growth of O. europaea and R. lycioides

The revegetation experiment with *O. europaea* showed that the combination of composted municipal waste addition and mycorrhizal inoculation can considerably stimulate the shoot biomass in semiarid conditions. The synergistic effect of the two treatments, when combined, contrasts with the widely accepted idea that mycorrhizae present little advantage to seedlings grown in fertilised soils (Yanai et al., 1995). The rapid growth of seedlings inoculated with *G. intraradices*, as compared with non-inoculated seedlings, in the amended soil might be related to the capacity of the fungus to increase available P uptake from the composted municipal waste (Roldán et al., 1996). On the other hand, mycorrhizal fungi are also known to enhance water absorption by plants grown under water-deficit conditions, owing to altered root morphology or contributions by soil hyphae (Augé, 2001). Since the revegetation experiment was carried out in semiarid conditions (during the first 12 months of the experiment, only 151 mm of rainfall were recorded), where water is by far the most limiting resource for plant growth, the increased shoot biomass of inoculated seedlings could be related to the increase in water uptake that a high level of root mycorrhizal infection provides under these conditions.

5. Conclusions

On the basis of medium-term effects on the physiological parameters tested, the study affirmed that composted municipal waste addition and mycorrhizal inoculation, and in some cases the combination of both treatments, can be effective tools in revegetation of semiarid areas with shrub species. In particular, the effectiveness of mycorrhizal symbiosis can be critical in the first stages of growth of shrub species.

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