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Assessing the effectiveness of mycorrhizal inoculation and soil compost addition for enhancing reafforestation with *Olea europaea* subsp. *sylvestris* through changes in soil biological and physical parameters

F. Caravaca [a,*], J.M. Barea [b], D. Figueroa [a], A. Roldán [a]

[a] *Department of Soil and Water Conservation, CSIC–Centro de Edafología y Biología Aplicada del Segura (CEBAS), Campus de Espinardo, P.O. Box 4195, 30100-Murcia, Spain*

[b] *Microbiology Department, CSIC–Estación Experimental del Zaídin, Professor Albareda 1, 18008-Granada, Spain*

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Abstract

A field experiment was carried out in a semiarid area to assess the influence of mycorrhizal inoculation and soil compost addition on establishment of *Olea europaea* seedlings. Associated changes in soil biological and physical parameters were observed. One year after planting, both reafforestation methods had significantly improved the soil structure. Thus, mycorrhizal inoculation increased soil aggregate stability (AS) and composted residue addition decreased soil bulk density (BD). A significant correlation ($P < 0.05$) was found between BD and several biochemical parameters (dehydrogenase, protease and β-glucosidase activities), indicating that soil biological agents play an important role in improving soil structure. The growth of *O. europaea* was significantly enhanced by both composted organic residue addition and mycorrhizal inoculation treatment. The increase in mycorrhizal *O. europaea* seedling growth may be due to the positive influence of mycorrhiza on soil AS. The combination of high fertility levels and low BD also favoured the growth of *O. europaea* in compost-amended soils. Finally, the positive interaction between the two methods in relation to seedling height growth could be related to the capacity of the fungus to increase nutrient uptake from the composted residue.

Keywords: *Olea europaea* subsp. *sylvestris*; *Glomus intraradices*; Composted residue; Reafforestation; Semiarid soils

1. Introduction

The establishment of autochthonous plant species is a widely used practice for reclaiming degraded lands in Mediterranean semiarid areas. However, in these areas the unproductiveness of the soil and the water deficit seriously limit plant growth. To carry out successful reafforestation programmes, then, it is necessary to apply methods which improve soil quality and the ability of the planted species to resist semiarid environmental conditions.

There is evidence that mycorrhizas help plants to thrive in arid conditions (Nelson and Safir, 1982) by increasing the supply of nutrients to the plant (particularly P) (Abbott et al., 1983; Querejeta et al., 1998), improving soil aggregation in eroded soils (Tisdall, 1991), and reducing hydric stress (Boyle and Hellenbrand, 1991). In degraded zones the

* Corresponding author. Tel.: +34-968-396337; fax: +34-968-396213.
E-mail address: fcb@cebas.csic.es (F. Caravaca).

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mycorrhizal component may disappear or, at least, be severely depleted and so it may be necessary to reinforce or replace it by appropriate inoculation (Barea et al., 1990).

The quality and productivity of degraded soils can be improved by the addition of organic amendments to soil (Roldán et al., 1994; García et al., 1998). The beneficial effects of organic amendments include decreased soil bulk density (BD) and increased water-holding capacity, aggregate stability (AS), saturated hydraulic conductivity, water infiltration rate, and biochemical activity (Turner et al., 1994; Zebarth et al., 1999). The effectiveness of such amendments greatly depends on their stability. For example, non-composted organic residues have been shown to be more effective than composted residue in activating the soil biomass which, in turn, can reactivate the soil's biogeochemical cycles (Pascual et al., 1997). Nevertheless, some authors have suggested organic amendments should be composted before they are applied to soil in order to achieve biological transformations of the organic matter and avoid the presence of organic substances with a low molecular weight which can be considered phytotoxic (Gliotti et al., 1997).

There is a need to select soil properties that rapidly respond to changes in soil quality while a particular conservation practice is being carried out in order to ascertain whether that practice is recommendable or not. Soil organic matter influences a wide range of physical, chemical and biological properties of soil and is considered by some authors as the most important indicator of soil quality (Bolinder et al., 1999). Changes in total soil organic matter may be difficult to monitor and detect in the short-term because of the large amount of soil C and the natural variability of soils. Recently, there has been widespread agreement on the importance of measuring the soil biochemical and biophysical parameters related to microbial activity in order to evaluate soil quality (Nannipieri et al., 1990; García et al., 1998). However, there are relatively few studies regarding the use of such parameters as indicators of revegetated soil quality (García et al., 2000).

The objectives of this study were: (1) to determine the viability of *Olea europaea* subsp. *sylvestris* seedlings for use in soil revegetation programmes in a semiarid Mediterranean area, and (2) to assess the effectiveness of mycorrhizal inoculation of seedlings and the addition of composted organic residue through measuring the physical, biochemical and biological parameters related to soil microbial activity in the establishment of *O. europaea*.

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 an average annual rainfall of 312 mm and a mean annual temperature of 15.3 °C; the potential evapo-transpiration reaches 813 mm per year. The predominant soils are Petrocalcic Xerosol, Petric Calcisol and Haplic Calcisol types (FAO, 1988) developed from limestones with a silt loam texture.

2.2. Materials

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

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

Ash (%)	44.8
pH (1:10)	6.7
EC (1:5, $\mu S\,cm^{-1}$)	4700
Total organic C ($g\,kg^{-1}$)	276.0
WSC ($\mu g\,g^{-1}$)	1950
WSCH ($\mu g\,g^{-1}$)	76
TN ($g\,kg^{-1}$)	14.5
$N–NH_3$ ($\mu g\,g^{-1}$)	3350
Total P ($g\,kg^{-1}$)	3.8
Total K ($g\,kg^{-1}$)	12.0
Cu ($\mu g\,g^{-1}$)	146
Zn ($\mu g\,g^{-1}$)	261
Ni ($\mu g\,g^{-1}$)	25
Cr ($\mu g\,g^{-1}$)	62.9
Cd ($\mu g\,g^{-1}$)	5
Pb ($\mu g\,g^{-1}$)	98

The plant used for the reafforestation experiment was *O. europaea* L. subsp. *sylvestris*, which is a low-growing shrub reaching a height of 3 m and widely distributed in the Mediterranean area. It is also well adapted to water stress conditions and, therefore, frequently used in the reafforestation of semiarid disturbed lands.

2.3. Mycorrhizal inoculation of seedlings

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

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

2.4. Experimental design and layout

The experiment was a randomised block design with two factors and four replication blocks. The first factor was the addition or not of composted organic residue to the soil, and the second factor was the direct mycorrhizal inoculation or not of *O. europaea* subsp. *sylvestris* plants with *G. intraradices* in the nursery. Thus, four treatments were established—control soil (C): −mycorrhiza, −compost; composted residue (CR): −mycorrhiza, +compost; mycorrhiza (M): +mycorrhiza, −compost; (CRM): +mycorrhiza, +compost. In September 1999, an area of $1200\,m^2$ was mechanically prepared with a subsoiler. Eight rows (1 m wide, 25 m long, 3 m apart) were established. In early December 1999, half of the rows were amended following the randomised design with compost (0–20 cm depth) at a rate of $6.7\,kg\,m^{-2}$, which is sufficient to raise the soil total organic carbon (TOC) content by 1%. Three weeks after the addition of the compost *O. europaea* seedlings (inoculated and non-inoculated) were planted in individual holes, at least 1 m apart in a single row and 3 m between blocks. At least, 32 seedlings per replication block were planted (eight plants × four treatments in each block).

2.5. Sampling procedures

One year after planting, four soil samples of each treatment were collected (16 soil samples in total). Each sample consisting of five bulked subsamples ($200\,cm^3$ soil cores) randomly collected at 0–20 cm in the rhizospheres of five individual plants. The sampling was carried out in early December after the autumn rainy season, when the highest microbial activity could be expected (Lax et al., 1997).

2.6. Physical-chemical, biological and biochemical analyses

The pH and electrical conductivity (EC) were measured in a 1:5 (w:v) aqueous solution. Total nitrogen (TN) was determined by the Kjeldhal method, and the total organic C by Yeomans and Bremner's method (Yeomans and Bremner, 1989). Available P (with sodium bicarbonate (Olsen et al., 1954)) was determined by colorimetry, according to Murphy and Riley (1962). Extractable (with ammonium acetate) K was determined by flame photometry.

In soil aqueous extracts, water-soluble carbon (WSC) was determined by wet oxidation with $K_2Cr_2O_7$ and measurement of the absorbance at 590 nm (Sims and Haby, 1971). Water-soluble carbohydrates (WSCH) and total carbohydrates (TCH) were determined by the method of Brink et al. (1960), and polyphenol soluble compounds by the method of Kuwatsuka and Shindo (1973).

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

Dehydrogenase activity was determined following Skujins' (1976) method modified by García et al. (1997a). For this, 1 g of soil at 60% of its field capacity was exposed to 0.2 ml of 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium

chloride) in distilled water for 20 h at 22 °C in darkness. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with 10 ml of methanol by shaking vigorously for 1 min and filtration through a Whatman No. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

Urease and *N*-α-benzoyl-L-argininamide (BAA) 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 millilitres 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 millilitres of 0.5 M sodium acetate buffer at pH 5.5 using acetic acid (Naseby and Lynch, 1997) and 0.5 ml of substrate were added to 0.5 g of soil and incubated at 37 °C for 90 min. The reaction was stopped by cooling at 2 °C for 15 min. Then, 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were added, and the mixture was centrifuged at 4000 rpm for 5 min. The *p*-nitrophenol (PNP) formed was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969). Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

β-Glucosidase was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M; Hayano and Tubaki, 1985; modified by Masciandaro et al., 1994) as substrate. This assay is based on the release and detection of PNP. Two millilitres of 0.1 M maleate buffer pH 6.5 and 0.5 ml of substrate was added to 0.5 g of sample and incubated at 37 °C for 90 min. The reaction was stopped with Tris–hydroxymethyl aminomethano (THAM) according to Tabatabai (1982). The amount of PNP was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969).

2.7. Physical analysis

The percentage of stable aggregates was determined by the method described by Lax et al. (1994). A 4 g aliquot of sieved (0.2–4 mm) soil was placed on a small 0.250 mm sieve and wetted by spray. After 15 min the soil was subjected to an artificial rainfall of 150 ml with an energy of 270 J m^{-2}. The remaining soil on the sieve was put in a previously weighed capsule (T), dried at 105 °C and weighed (P_1). Then, the soil was soaked in distilled water and, after 2 h, passed through the same 0.250 mm sieve with the assistance of a small stick to break the remaining aggregates. The residue remaining on the sieve, which was made up of plant debris and sand particles, was dried at 105 °C and weighed (P_2). The percentage of stable aggregates with regard to the total aggregates was calculated by $(P_1 - P_2) \times 100/(4 - P_2 + T)$. The four soil samples of each treatment were analysed in triplicate for percentage of stable aggregates.

BD was determined by the paraffin method described by Barahona and Santos (1981) after maintaining soil moisture at 60% of field capacity for 1 month.

One year after planting, basal stem diameters and heights of the seedlings were measured with calipers and rule.

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

2.8. Statistical analysis

Residue addition, mycorrhizal inoculation and their interactions effects on measured variables were tested by a two-way analysis of variance and comparisons among means were made using least significant difference (LSD) multiple range 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 packages Statgraphics for Windows 7.0.

3. Results and discussion

3.1. Physical-chemical parameters

Only the composted residue addition significantly decreased soil pH and increased soil EC (Table 2). However, neither mycorrhizal inoculation nor the interaction of compost × mycorrhizal inoculation had any significant effect on soil physical-chemical parameters (Table 4). Similar soil EC results after

Table 2
Changes in physical-chemical properties of the soil in response to mycorrhizal inoculation (M) and composted residue (CR) addition ($n = 4$) (C: control soil, without mycorrhizal inoculation and without composted residue addition and CRM: composted residue addition + mycorrhizal inoculation)

	C	CR	M	CRM
pH (H_2O)	8.56 (0.02)	8.25 (0.05)	8.62 (0.04)	8.22 (0.05)
EC (1:5, $\mu S\,cm^{-1}$)	251 (6)	427 (39)	245 (10)	460 (5)
TOC ($g\,kg^{-1}$)	19.9 (1.6)	29.6 (1.0)	17.4 (1.4)	26.8 (1.7)
TCH ($\mu g\,g^{-1}$)	2273 (68)	3889 (270)	2194 (476)	3733 (182)
WSC ($\mu g\,g^{-1}$)	242 (17)	419 (69)	232 (14)	591 (115)
WSCH ($\mu g\,g^{-1}$)	11 (0)	17 (2)	14 (1)	18 (0)
WSPP ($\mu g\,g^{-1}$)	5 (0)	31 (1)	4 (1)	25 (3)
TN ($g\,kg^{-1}$)	1.4 (0.0)	1.7 (0.1)	1.5 (0.1)	2.4 (0.1)
Available P ($\mu g\,g^{-1}$)	3.4 (0.9)	19.7 (4.2)	3.5 (0.8)	20.0 (4.9)
Extractable K ($\mu g\,g^{-1}$)	438 (17)	845 (46)	436 (54)	655 (45)

The values shown in the parenthesis represent S.D. for each measure. TOC: total organic carbon; TCH: total carbohydrates; WSC: water-soluble carbon; WSCH: water-soluble carbohydrates; WSPP: water-soluble polyphenols; TN: total nitrogen.

uncomposted organic residue amendment were recorded by Roldán et al. (1996a) and García et al. (2000). The increases observed in soil EC (on average $193\,\mu S\,cm^{-1}$) were not sufficient to limit plant growth.

Organic amendment was more effective than mycorrhizal (M) treatment in increasing TOC and all the C-fractions of revegetated soils, as shown in Tables 2 and 4. After 1 year of planting, the TOC content had increased by about 1% with respect to the control soil (C), which corresponded with the dose of TOC added from the composted residue.

Polysaccharides, which are mostly by-products of the microbial activity developed in the C-containing substrate, are considered as the main chemical aggregate-stabilising agents (Chesire et al., 1983; Lax and García-Orenes, 1993; Roldán et al., 1994). The fraction of polysaccharides involved in soil structure would be contained in the total polysaccharides measured (TCH). The increased TCH levels found after adding compost were of a lower magnitude (about 68% with respect to non-amended soil) than those generally found in soils amended with non-composted residues (Roldán et al., 1994).

The water-soluble organic matter fraction consists of a heterogeneous mixture of components of varying molecular weight, such as mono- and polysaccharides, polyphenols, proteins and low molecular weight organic acids (Kuiters and Dennenman, 1987). This fraction can be used as carbon and energy sources for soil microflora (Roldán et al., 1994) and may also have a structural function (Metzger and Yaron, 1987). The increased soluble C-fraction values (WSC, WSCH and WSPP) were mainly due to the composted residue added to the soil. Among the WSC-fractions, the greatest increase in response to the addition of compost was observed in the soil phenolic compounds, which may be phytotoxic (Kuwatsuka and Shindo, 1973).

Both reafforestation methods assayed significantly increased the TN and extractable K contents of the soil (Tables 2 and 4), while the combination of both reafforestation methods produced even higher TN values possibly due to the increase in soil microbial biomass that is able to fix N_2. However, the soil available phosphorus content was only increased by the addition of compost. Thus, the available phosphorus content in compost-amended soils (CR and CRM) was about six-fold higher than in non-amended soils (C and M). This is in accordance with the finding of Roldán et al. (1996a), who found that the positive effect of a composted residue on chemical parameters was primarily due to phosphorus.

3.2. Biological and biochemical parameters

In amended soils the C-biomass values were higher than in non-amended soils (Table 3). This is in agreement with the results found by García et al. (2000) in a soil treated with an non-composted organic residue, although the C-biomass increase they reported was greater. De Luca and Keeney (1993) defined the WSC content as a reflection of soil microbial

Table 3
Changes in biochemical properties in response to mycorrhizal inoculation (M) and composted residue (CR) addition ($n = 4$)[a]

	C	CR	M	CRM
C-biomass ($\mu g\, g^{-1}$)	237 (10)	534 (34)	300 (39)	552 (66)
C-biomass/TOC (%)	1.19 (0.05)	1.80 (0.16)	1.72 (0.23)	2.06 (0.26)
C-biomass/WSC	0.98 (0.13)	1.27 (0.20)	1.29 (0.18)	0.93 (0.07)
Dehydrogenase (μg INTF g^{-1} soil)	102 (4)	167 (28)	97 (7)	164 (16)
Urease ($\mu mol\, NH_3\, g^{-1}\, h^{-1}$)	0.42 (0.03)	0.88 (0.22)	0.29 (0.05)	1.58 (0.01)
Protease–BAA ($\mu mol\, NH_3\, g^{-1}\, h^{-1}$)	0.80 (0.02)	1.59 (0.06)	0.63 (0.07)	1.23 (0.06)
Acid phosphatase (μmol PNP $g^{-1}\, h^{-1}$)	45.92 (2.38)	78.87 (6.42)	46.82 (3.09)	91.52 (6.61)
β-Glucosidase (μmol PNP $g^{-1}\, h^{-1}$)	85.36 (3.28)	159.97 (11.00)	59.90 (6.14)	153.03 (17.68)

The values shown in the parenthesis represent S.D. for each measure. TOC: total organic carbon; WSC: water-soluble carbon.
[a] C: control soil, without mycorrhizal inoculation and without composted residue addition and CRM: composted residue addition + mycorrhizal inoculation).

activity, and in our experiment there was a significant correlation ($P < 0.05$) between C-biomass and WSC fractions. In compost-amended soil the quantity of these easily biodegradable compounds was low in comparison with uncomposted residue amended soils (Pascual et al., 1999) and, hence, the microbial activity was less marked (García et al., 1997b). Organic amendment was more efficient than mycorrhizal treatment for enhancing soil microbial biomass (Table 4). Both assayed methods significantly increased the C-biomass/TOC ratio (Table 3), indicating that both residue addition and mycorrhizal inoculation favoured the soil organic matter turnover. According to Biederbeck et al. (1994), fluctuations in organic matter occur primarily in the readily decomposable labile fractions. This can be deduced from the high values of the C-biomass/WSC ratio in both the organic amendment and mycorrhizal inoculation.

The application of the composted residue increased all the enzyme activities to a greater extent than

Table 4
Two factor ANOVA (mycorrhizal inoculation and composted residue addition) for all parameters studied[a]

	CR	M	CRM
pH (H_2O)	73.27 (<0.01)	0.14 (0.72)	1.35 (0.27)
EC	89.72 (<0.01)	0.43 (0.53)	0.92 (0.37)
TOC	36.26 (<0.01)	2.83 (0.12)	0.01 (0.93)
TCH	29.60 (<0.01)	0.16 (0.70)	0.02 (0.91)
WSC	15.67 (<0.01)	1.43 (0.26)	1.80 (0.21)
WSCH	14.46 (<0.01)	2.10 (0.17)	0.30 (0.6)
WSPP	139.13 (<0.01)	3.22 (0.10)	2.00 (0.18)
TN	40.77 (<0.01)	12.75 (<0.01)	9.20 (0.01)
Available P	98.93 (<0.01)	0.01 (0.91)	0.01 (0.98)
Extractable K	211.41 (<0.01)	19.79 (<0.01)	19.02 (<0.01)
C-biomass	42.02 (<0.01)	0.91 (0.37)	0.29 (0.61)
Dehydrogenase	15.96 (<0.01)	0.05 (0.84)	0.01 (0.98)
Urease	58.96 (<0.01)	6.44 (0.03)	13.06 (<0.01)
Protease–BAA	135.60 (<0.01)	19.07 (<0.01)	2.63 (0.13)
Acid phosphatase	59.99 (<0.01)	1.69 (0.22)	1.32 (0.27)
β-Glucosidase	58.41 (<0.01)	2.23 (0.16)	0.73 (0.42)
AS	0.23 (0.65)	6.40 (0.03)	6.37 (0.03)
BD	18.00 (<0.01)	1.80 (0.21)	0.74 (0.42)
Basal diameter	15.23 (0.01)	9.29 (0.03)	5.16 (0.07)
Height	61.44 (<0.01)	12.98 (0.02)	14.58 (0.01)
Mycorrhizal roots	0.01 (0.96)	195.40 (<0.01)	0.08 (0.80)

[a] The values shown in the parenthesis represent *F*-values (*P*-values).

mycorrhizal inoculation. The increases observed in C-biomass and dehydrogenase point to the greater microbiological activity (García et al., 1997b) as a consequence of the organic amendment.

3.3. Physical parameters

The percentage of water-stable soil aggregates was significantly increased by mycorrhizal inoculation (M), which resulted in a 35% increase with respect to non-amended soil and non-inoculated seedlings (C), as shown in Fig. 1. As suggested by Bearden and Petersen (2000), the symbiosis between arbuscular-mycorrhizal fungi and plants would have increased the stability of the soil aggregates. Mycorrhiza primarily influence the stability of macroaggregates (>250 μm) (Tisdall and Oades, 1982; Bearden and Petersen, 2000). According to Roldán et al. (1994), the binding effect of polysaccharides is short-lived and the maintenance and increase in AS is attributable to the increases in microbial populations, and particularly to the proliferation of fungal mycelium. The mechanism is thought to function through the enmeshment of soil particles by hyphae and roots and the exudation of polysaccharides (Miller and Jastrow, 1990, 1992; Bearden and Petersen, 2000). Recent studies have also indicated that arbuscular-mycorrhizal fungi produce a glycoprotein, glomalin, that acts as an insoluble glue to stabilise aggregates (Wright and Anderson, 2000).

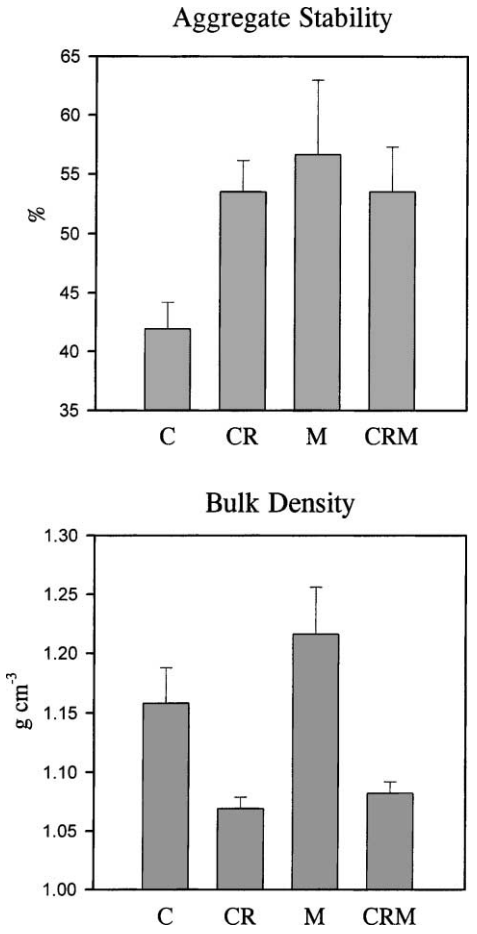

Fig. 1. Percentage of stable aggregates and BD in response to mycorrhizal inoculation (M) and composted residue (CR) addition ($n = 4$) (C: control soil, without mycorrhizal inoculation and without composted residue addition and CRM: composted residue addition + mycorrhizal inoculation). Bars represent S.D. for each measure ($n = 4$).

The soil structural stability did not significantly improve with the organic addition (Table 4). Dinel et al. (1992) found that the restoration of soil structure may depend on the amount and nature of the organic matter added. Thus, the biological transformations that a compost undergoes in the waste treatment plant reduce the quantity of chemical aggregate-stabilizing agents such as polysaccharide or water-soluble organic matter and increase the number of carbon fractions most resistant to rapid decomposition (García, 1990). Presumably, the dose applied was not high enough to result in significant differences with respect to the control soil (C), which coincides with the results of other authors (Roldán et al., 1996a). Hence, this type of composted residue is less effective for improving soil structure although, on the positive side, its use leads to fewer problems related to toxic substances, which are eliminated during the composting process (Pascual et al., 1999). AS did not differ significantly between the two reafforestation methods assayed. Furthermore, no significant correlations were found between soil stable aggregates and soil chemical and biochemical parameters (Table 5).

The organic materials are less dense than the mineral fraction of soils and play an important role in improving soil structure. Thus, their application reduces the soil BD and leads to an increase in soil porosity. In addition, the organic carbon of compost may affect the BD of a soil by improving its structural stability. The addition of compost increases the percentage of transmission and storage pores (Pagliai et al., 1981).

Table 5
Pearson rank correlation matrix between physical, chemical and biochemical parameters ($n = 4$)[a]

	AS	BD	Dehydrogenase (Dhase)	Urease	BAA	Acid phosphatase (PHPase)	β-Glucosidase (βglucos)	C-biomass (CBiom)	TOC	TCH	WSC	WSCH	WSPP
AS	1.000	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
BD		1.000	−0.955**	NS	−0.949*	NS	−0.990***	NS	−0.979**	−0.950**	NS	NS	−0.944*
Dehydrogenase (Dhase)			1.000	NS	0.945*	0.964**	0.987**	0.974**	0.977**	0.999***	NS	NS	0.989***
Urease				1.000	NS	0.953**	NS	NS	NS	NS	0.994***	NS	NS
Protease–BAA					1.000	NS	0.958**	NS	0.989***	0.953**	NS	NS	0.977**
Acid phosphatase (PHPase)						1.000	NS	0.974**	NS	0.955**	0.979**	NS	NS
β-Glucosidase (βglucos)							1.000	NS	0.989***	0.985**	NS	NS	0.977**
C-biomass (CBiom)								1.000	NS	0.974**	0.945*	0.968**	0.957**
TOC									1.000	0.980**	NS	NS	0.988**
TCH										1.000	NS	NS	0.994***
WSC											1.000	NS	NS
WSCH												1.000	NS
WSPP													1.000

[a] Correlation coefficient (significance level). NS: not significant.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.

This would explain the reduced BD measured in compost-amended soil (Fig. 1). Furthermore, the BD of the amended soil was negatively correlated with the TOC, TCH and WSPP contents and some enzyme activities such as dehydrogenase, protease–BAA and β-glucosidase (Table 5), pointing to the involvement of microbiological agents in the changes observed in soil structure and, in particular, in the soil BD.

Unlike AS, mycorrhizal treatment did not affect soil BD (Fig. 1). Thus, the inoculation of *O. europaea* seedlings with *G. intraradices* did not decrease the soil BD with respect to the others treatments, which reached similar values to those measured in the control soil (C).

3.4. Mycorrhizal infection and growth of Olea europaea subsp. sylvestris

At the time of planting, the *G. intraradices*-inoculated seedlings had significantly higher percentages of root colonisation (on average 62%) than the uninoculated plants (on average 0.4%). Fig. 2 shows data for the percentages of root mycorrhizal colonisation corresponding to the plants randomly sampled 1 year after planting. The degree of mycorrhizal colonisation of the non-inoculated seedlings increased to an average of 14% as a result of natural infection. The inoculate seedlings grown in amended and non-amended soils show the highest percentages of root colonisation and there were no significant differences between the values.

One year after planting, both the addition of the composted organic residue and mycorrhizal inoculation improved *O. europaea* basal diameter growth with respect to the growth measured in the control soil (C) by at least 38% (Fig. 2). A similar trend was observed with plant height. There were no significant differences between the assayed treatments (CR, M and CRM) as regards *O. europaea* seedling growth.

As suggested by several authors, mycorrhizal fungi may improve the performance of seedlings either by stimulating water uptake (Roldán et al., 1996b), by producing growth promoting substances (Álvarez, 1991) or by increasing nutrient uptake (Roldán and Albaladejo, 1994; Roldán et al., 1996b). In the case of the amended soil, the nutrient

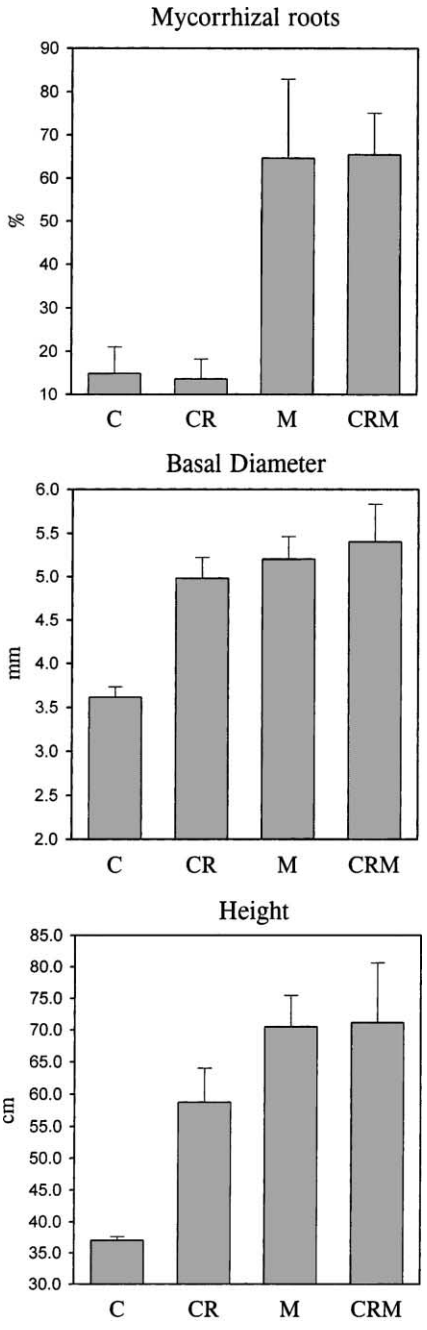

Fig. 2. Effect of composted residue addition and mycorrhizal inoculation on root mycorrhizal colonisation and growth of *O. europaea* subsp. *sylvestris* under field conditions ($n = 4$) (C: control soil, without mycorrhizal inoculation and without composted residue addition and CRM: composted residue addition + mycorrhizal inoculation). Bars represent S.D. for each measure ($n = 4$).

concentration was sufficiently high so as not to limit seedling growth. Therefore, the positive interaction observed between both reafforestation methods may be related to the capacity of the fungus to increase the uptake of nutrients from the composted residue added.

It is well documented that root growth in plants is restricted by increasing soil compaction (Bengough and Mullins, 1990; Materechera et al., 1991). Nadian et al. (1996) found that the total root length colonised was lower in highly compacted soil than in slightly compacted soil. Thus, the combination of high fertility levels and low BD may also favour the significantly enhanced growth of *O. europaea* seedlings in the soil receiving compost and the mycorrhizal inoculation treatment.

Soil structure is one of the most important properties controlling plant growth (De Freitas et al., 1996). Moreover, the positive effect of the symbiosis between arbuscular-mycorrhizal fungi and plants on soil structural characteristics has been widely demonstrated (Tisdall and Oades, 1980; Miller and Jastrow, 1990; Bearden and Petersen, 2000). This suggests that the observed increase in *O. europaea* seedling growth may also be related to the improvement in soil AS following mycorrhizal inoculation. In this regard, there was a positive correlation between soil AS and seedling basal diameter with a significance level of $P = 0.08$.

In conclusion, both mycorrhizal inoculation and the addition of composted residue were very effective in improving soil quality and the performance of *O. europaea* seedlings under our experimental conditions. It would be of interest to carry out further long-term surveys to ascertain the effects of both methods and the validity of the selected bioindicators for monitoring physical and biological properties of revegetated soils in semiarid environments.

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