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Geoderma 108 (2002) 133–144

GEODERMA

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Improvement of rhizosphere aggregate stability of afforested semiarid plant species subjected to mycorrhizal inoculation and compost addition

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Received 10 October 2001; received in revised form 8 February 2002; accepted 28 February 2002

Abstract

Soil aggregate stability is one of the most important properties controlling plant growth in semiarid Mediterranean environments. A field study was carried out to evaluate the effect of the rhizosphere of *Olea europaea* subsp. *sylvestris* and *Rhamnus lycioides*, the addition of a composted residue and mycorrhizal inoculation with *Glomus intraradices* on rhizosphere aggregate stability and on the viability of both plant species in a semiarid structureless soil. For both plant species, water-soluble carbon (WSC) content and enzyme activities (urease, acid phosphatase and β -glucosidase) measured in the rhizosphere aggregate were higher than in the non-rhizosphere soil. Rhizosphere aggregate stability of both plant species was on average 1.8-fold higher than that of non-rhizosphere aggregate. The addition of composted residue was the most effective treatment for increasing rhizosphere aggregate stability. The water-soluble carbon content was correlated positively with aggregate stability of the *O. europaea* rhizosphere. The mycorrhizal component was increasingly important for improving the growth of both seedlings following the addition of composted residue to soil under the severe climatological conditions of the area. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: *Olea europaea* subsp. *sylvestris*; *Rhamnus lycioides*; *Glomus intraradices*; Organic residue addition; Enzyme activities

1. Introduction

Adequate soil structural stability favours the establishment and viability of a stable plant cover which, in turn, protects the soil against water erosion in semiarid Mediterra-

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nean environments (Albaladejo et al., 1996). The viability of semiarid plant species can be improved also by the inoculation of symbiotic microorganisms, especially with vesicular-arbuscular mycorrhiza (VAM) fungi. An enhanced mycorrhizal infection may account for enhanced plant growth through improved uptake of nutrients and water by plants (Lansac et al., 1995). Mycorrhizal colonisation also increases soil aggregation in eroded soil (Bearden and Petersen, 2000), primarily influencing the stability of macroaggregates (Jastrow et al., 1998).

Plant roots increase the stability of surrounding aggregates (Lynch and Bragg, 1985) through several interacting mechanisms. Roots and associated mycorrhizal hyphae may form a three-dimensional network that enmeshes fine particles of soil into aggregates. In addition, the organic C released by roots promotes a dense microbial community in the immediate environment of the root which, in turn, produces exocellular mucilaginous polysaccharide material that has the capacity to stabilise soil aggregates (Jastrow et al., 1998). Nonetheless, changes in aggregate stability under different management practices have been shown to occur rapidly before any changes in total organic C or total acid-hydrolysable carbohydrate content are detected (Haynes et al., 1991). Thus, Sparling and Cheshire (1985) have reported that polysaccharides may be less important for rhizosphere soil than for non-rhizosphere soil.

The addition of organic matter in the form of urban residue has been reported to enhance proliferation of VAM fungal hyphae in soil (Douds et al., 1997), but negative effects have also been reported (Roldán and Albaladejo, 1993). The beneficial role of organic matter may be related to an improvement of physical properties, such as increased soil aggregate stability, and/or to an increase in microbial activity (García et al., 1998). Improvement of aggregate stability in degraded soil by amendment with urban residue has been frequently measured in aggregates from non-rhizosphere soil (Díaz et al., 1994; Roldán et al., 1996). However, there are no reports on the effect of organic amendment and VAM fungi on stability of rhizosphere aggregates. We hypothesised that since rhizosphere microbial biomass and its activities are extremely involved in soil aggregation, early and sensitive changes in aggregate stability would mainly occur in rhizosphere aggregates. The objective of this study was to determine and compare the short-term influences of the rhizosphere of *Olea europaea* and *Rhamnus lycioides*, mycorrhizal inoculation with *Glomus intraradices* and the addition of composted residue on the stabilisation of rhizosphere aggregates and to evaluate whether such treatments can be considered as appropriate techniques in revegetation programmes in semiarid environments.

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) (coordinates: 1°10' W and 38°23' N). The climate is semiarid Mediterranean with an average annual rainfall of 196 mm and a mean annual temperature of 20 °C during the experiment. The soil used was a Petrocalcic Xerosol (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 organic residue was mechanically produced by fast fermentation (60 days), with the waste heap mixed daily under aerobic conditions. The analytical characteristics of the composted organic residue, determined by standard methods (Page et al., 1982), are shown in Table 1.

The plants used for the re-afforestation experiments were *O. europaea* subsp. *sylvestris* and *R. lycioides*, which are low-growing shrubs reaching heights of 1.5 and 3 m, respectively, and are widely distributed in the Mediterranean region. They are also well adapted to water stress and, therefore, potentially could be used in the re-afforestation 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 experimental field station of Zaidin, 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. Control seedlings were added with the same amount of the autoclaved mixture of the inocula, supplemented with a filtrate (< 20 μm) of them to provide the microbial populations accompanying the mycorrhizal fungi. Inoculated and noninoculated seedlings were grown for 8 months under nursery conditions without any fertilisation. Nursery procedures were conducted at Paisajes del Sur (Granada, Spain). At the end of the nursery period, inoculated seedlings (32.4 ± 8.5 and 15.4 ± 5.5 cm for *O. europaea* and *R. lycioides* seedlings, respectively) were slightly larger than were noninoculated ones (24.8 ± 2.9 and 10.0 ± 1.1 cm for *O. europaea* and *R.*

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

Ash (g kg^{-1})	448
pH (1:10, H_2O)	6.7
Electrical conductivity EC (1:5, $\mu\text{S cm}^{-1}$)	4700
Total organic C (g kg^{-1})	276.0
Water-soluble C ($\mu\text{g g}^{-1}$)	1950
Water-soluble carbohydrates ($\mu\text{g g}^{-1}$)	76
Total N (g kg^{-1})	14.5
N- NH_3 ($\mu\text{g g}^{-1}$)	3350
Total P (g kg^{-1})	3.8
Total K (g kg^{-1})	12.0
Cu ($\mu\text{g g}^{-1}$)	146
Zn ($\mu\text{g g}^{-1}$)	261
Ni ($\mu\text{g g}^{-1}$)	25
Cr ($\mu\text{g g}^{-1}$)	62.9
Cd ($\mu\text{g g}^{-1}$)	5
Pb ($\mu\text{g g}^{-1}$)	98

lycioides seedlings, respectively), although differences in size were not statistically significant.

2.4. Experimental design and layout

The experiments had 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* and *R. lycioides* plants with *G. intraradices* in the nursery. In addition, four treatments in each experiment were established: seedlings without mycorrhizal treatment and soil without composted residue addition (control soil, C2), seedlings without mycorrhizal treatment and soil with composted residue addition (R), seedlings inoculated with *G. intraradices* and soil without composted residue addition (M), and seedlings inoculated with *G. intraradices* and soil with composted residue addition (RM). In September 1999, two adjacent plots of 1200 m² were mechanically prepared with a subsoiler to carry out each planting experiment. In both plots, eight rows (1 m wide, 25 m long, 3 m apart) were established. In early December 1999, to half of the rows added composted residue (0–20 cm depth) at a rate of 6.7 kg m⁻² following the randomised design, which is sufficient to raise the soil total organic carbon content by 1%. Three weeks after the addition of the compost, *O. europaea* and *R. lycioides* seedlings (inoculated and noninoculated) 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). At the time of planting, the *G. intraradices*-inoculated seedlings of *O. europaea* and *R. lycioides* exhibited average percentages of root colonisation of 62% and 38%, respectively. The noninoculated seedlings of both plant species showed a low mycorrhizal colonisation level, which was in both cases less than 2%.

2.5. Sampling and laboratory procedures

One year after planting, four randomly selected seedlings of each treatment were carefully dug from the field (16 seedlings in total), and the root systems were gently shaken to remove aggregates loosely adhered to roots. Soil strongly adhering to roots and collected at 0–4 mm from the root surface was defined as rhizosphere aggregates. Soil samples were also collected from areas between plant rows and were defined as non-rhizosphere soil (control soil, C1). Each sample (six soil samples in total) consisted of five bulked subsamples (200 cm³ soil cores) randomly taken from the top 20 cm. Samples of rhizosphere and non-rhizosphere soil were sieved between 0.2 and 4 mm before being stored at 5 °C until required for analysis. The sampling was carried out in early December after the autumn rainy season, when the highest microbial activity would be expected (Lax et al., 1997).

In soil aqueous extracts, water-soluble carbon (WSC) was determined by wet oxidation with K₂Cr₂O₇ and measurement of the absorbance at 590 nm (Sims and Haby, 1971).

Urease activity was determined in 0.1 M phosphate buffer at pH 7; 1 M urea was used as substrate. 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 90 min. Activity was determined as the NH₄⁺ released in the hydrolysis reaction (Nannipieri et al., 1980).

Acid phosphatase activity was determined using *p*-nitrophenyl phosphate, disodium salt (PNPP, 0.115 M) as substrate. Two millilitres of 0.5 M sodium acetate buffer, adjusted to pH 5.5 using acetic acid (Naseby and Lynch, 1997), and 0.5 ml of substrate were added to 0.5 g of sample and incubated at 37 °C for 90 min. The reaction was stopped by cooling at 2 °C for 15 min. Then, 0.5 ml of 0.5 M CaCl₂ 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 CaCl₂ 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 were added to 0.5 g of sample and incubated at 37 °C for 90 min. The reaction was stopped with tris-hydroxymethyl aminomethane (THAM), according to Tabatabai (1982). The amount of PNP was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969).

The percentage of stable aggregates was determined by the method described by Lax et al. (1994). Four grams of sieved (0.2–4 mm) soil was placed on a 0.250-mm sieve and wetted by spraying with water. 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 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 respect to the total aggregates was calculated by $(P1 - P2) \times 100 / (4 - P2 + T)$.

Seedling height was measured, with a ruler, at the time of planting and 1 year later.

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

2.6. Statistical analysis

Treatment effects, and differences between non-rhizosphere soil and rhizosphere aggregates, with respect to measured variables were tested by analysis of variance, and comparisons among means were made using the least significant difference (LSD) multiple range test, calculated at $P < 0.05$. Statistical procedures were carried out with the software package Statgraphics for Windows 7.0.

3. Results and discussion

The labile C fraction, including water-soluble carbon (WSC), is made up of biodegradable substrates and this C fraction has been frequently considered as an indicator of soil microbiological activity (De Luca and Keeney, 1993). Also, WSC can be used as an

indicator of early changes in soil organic matter (Bolinder et al., 1999). Significant differences in WSC between non-rhizosphere soil and rhizosphere aggregates were found for *O. europaea* and *R. lycioides* (Figs. 1 and 2). This indicated that the rhizosphere aggregates had an important source of energy and nutrients available for the micro-organisms associated with them.

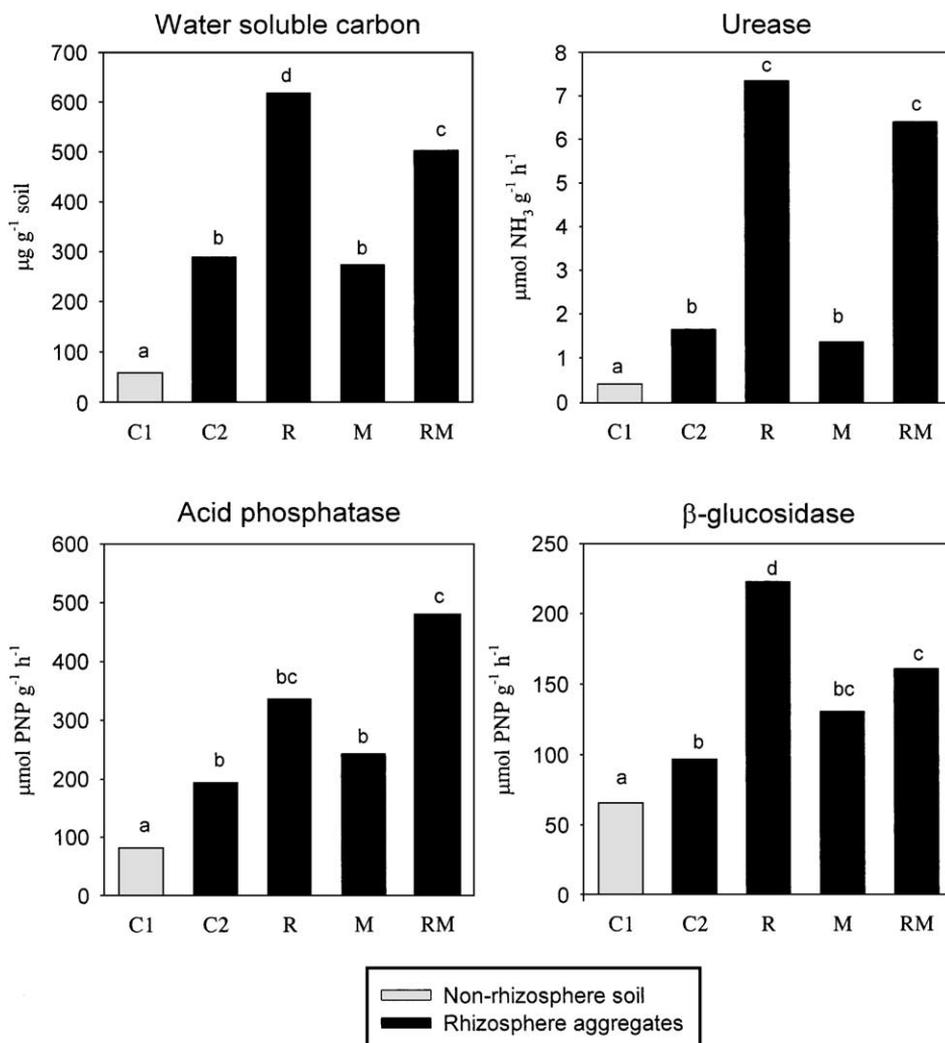


Fig. 1. Water-soluble carbon content and urease, acid phosphatase and β -glucosidase activities changes in the *O. europaea* subsp. *sylvestris* plantation following the different treatments (C1=control non-rhizosphere soil; C2=control in rhizosphere soil; R=composted residue addition; M=inoculation; RM=composted residue addition+inoculation) ($n=4$). Values with the same letter are not significantly different values at $P < 0.05$, according to LSD test.

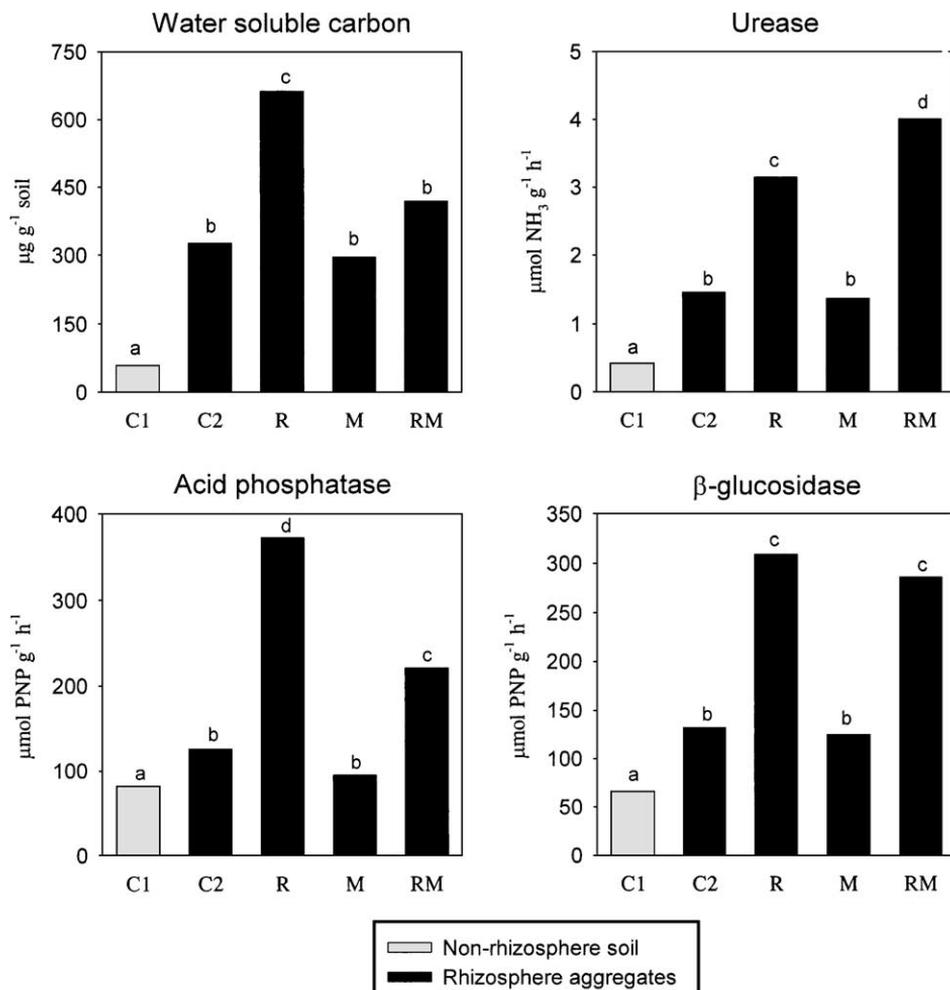


Fig. 2. Water-soluble carbon content and urease, acid phosphatase and β -glucosidase activities changes in the *R. lycioides* plantation following the different treatments (C1=control non-rhizosphere soil; C2=control in rhizosphere soil; R=composted residue addition; M=inoculation; RM=composted residue addition+inoculation) ($n=4$). Values with the same letter are not significantly different values at $P<0.05$, according to LSD test.

The highest values of WSC measured in the rhizosphere of both plant species were observed in soils with addition of composted residue, particularly in R soils (Figs. 1 and 2), thus indicating an improvement of rhizosphere microbial activity due to organic amendment.

Measurement of soil hydrolases provides an early indication of changes in soil fertility, since they are related to the mineralisation of biological origin of important nutrient elements such as N, P and C (Ceccanti and García, 1994). Since the WSC fraction was much greater in the rhizosphere aggregates than that in non-rhizosphere soils (C1) for both

plant species, so too were the hydrolase activities (urease, acid phosphatase and β -glucosidase) (Figs. 1 and 2).

Many researchers have found that soil hydrolases are enhanced by the addition of organic materials (Dick, 1992; García et al., 1998). We also found that the hydrolase activities involved in the N (urease activity), P (acid phosphatase activity) and C (β -glucosidase) cycles were higher in the rhizosphere aggregates of soils with addition of composted residue (R and RM) for *O. europaea* and *R. lycioides*. For both plant species, the highest increases were observed for the urease activity, due to the organic materials increasing the N substrates in the soil (García et al., 2000).

Inoculation with VAM fungi did not increase enzyme activities in rhizosphere aggregates of either plant species compared with control C2. However, Vázquez et al. (2000) found significant increases in phosphatase activity in rhizosphere soil as a result of mycorrhizal colonisation by natural VAM fungi.

Rhizosphere aggregates (C2, R, M and RM) of *O. europaea* and *R. lycioides* were significantly more stable than non-rhizosphere soil aggregates (C1) (Fig. 3). Thus, aggregate stability measured in the rhizosphere (soil C2) of both plant species was on average 1.8-fold higher than that of non-rhizosphere soil aggregates (soil C1). Similar results were obtained by Haynes and Francis (1993), who indicated that the positive effect of rhizosphere soil on the stability of aggregates was due to a higher microbial biomass C and water-soluble carbohydrate content in the rhizosphere soil. In our experiment, the differences in stability between aggregates from within and outside the rhizosphere soil might be attributed to notably increased soluble carbon fraction content and enzyme activities compared with those in the non-rhizosphere soil, which points to a greater degree of biological activity in the rhizosphere aggregates. Likewise, the roots themselves and the presence of fungi, possibly vesicular-arbuscular mycorrhiza, could mechanically bind soil particles together, with stabilisation being enhanced by polymers produced either directly by the fungus or by bacteria associated with the hyphae. In both plant species, the percentage colonised root length in plants inoculated with *G. intraradices* (M, RM) was significantly higher than for noninoculated plants (C2, R) (Table 2).

It is generally recognised that the benefits of organic amendment are not only due to the supply of nutrient elements, but also to the improvement of the soil's physical characteristics (Roldán et al., 1996; McCoy, 1998). The percentages of stable aggregates measured in the rhizosphere of both plant species were significantly increased by the addition of composted residue (R, RM) (Fig. 3) compared with those without composted residue (C2). Several authors have pointed out that the water-soluble organic C also has a structural function because it contains polysaccharides, mainly of fungal origin, that are involved in aggregating and stabilizing soil aggregates (Metzger et al., 1987; Haynes and Swift, 1990). In this regard, a close relationship between WSC content and the percentage of stable aggregates was observed for *O. europaea* ($r=0.992$, $P<0.01$).

The percentage of plant survival was about 95% in each treatment and there were no significant differences between treatments. Table 2 shows the *O. europaea* and *R. lycioides* plant heights. Inoculation with *G. intraradices* significantly enhanced height of both plant species. The highest increment of plant height was reached for *O. europaea* grown in the combined treatment of composted residue addition and mycorrhizal inoculation, RM soil (107%), due possibly to the highest percentage of mycorrhizal colonisation being recorded

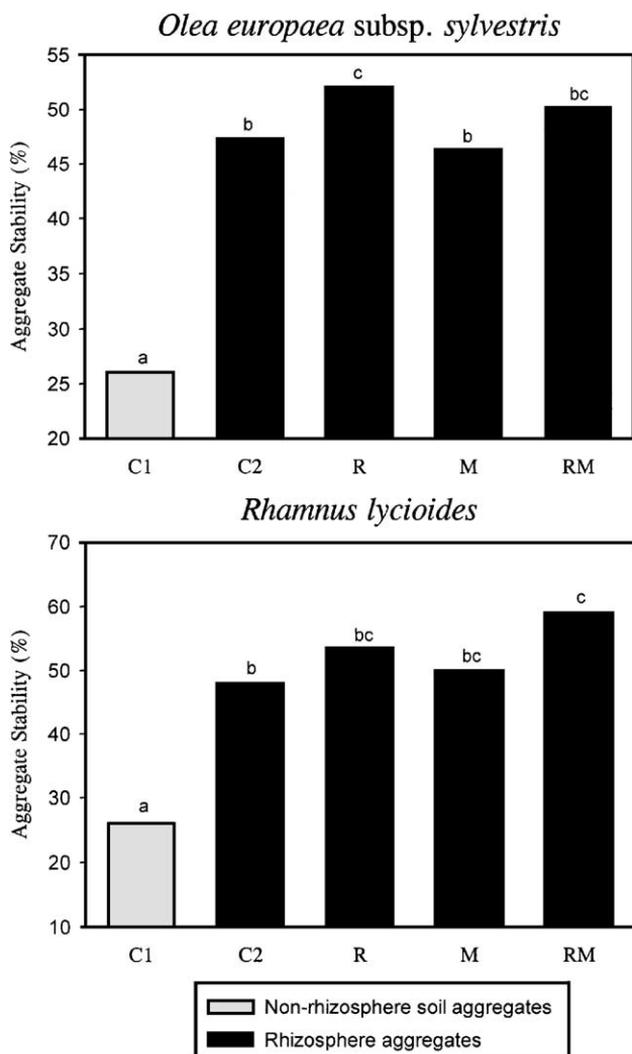


Fig. 3. Percentage of stable aggregates in rhizosphere soil following the different treatments in the *O. europaea* and *R. lycioides* plantations (C1 = control non-rhizosphere soil; C2 = control in rhizosphere soil; R = composted residue addition; M = mycorrhization; RM = composted residue addition + inoculation) ($n=4$). Values with the same letter are not significantly different values at $P < 0.05$, according to LSD test.

in the roots of this plant species, as shown in Table 2 (65%). The rapid growth of *O. europaea* seedlings inoculated with *G. intraradices*, as compared with the noninoculated seedlings in the soil with addition of composted residue, may have been due to the capacity of the fungus to promote the transport of water to plant roots, to increase nutrient uptake from composted residue (Hodge et al., 2001) or to produce growth promoting substances.

Table 2

Effect of composted residue (R) addition and mycorrhizal inoculation (M) on growth and root infection in *O. europaea* subsp. *sylvestris* and *R. lycioides* ($n=4$)

	<i>O. europaea</i>		<i>R. lycioides</i>	
	Height (cm)	Mycorrhization (%)	Height (cm)	Mycorrhization (%)
C2	29a*	15a	23a	0a
R	36ab	14a	24a	2a
M	45b	65b	37ab	48c
RM	60c	65b	45b	28b

C2=control soil, without mycorrhization and without composted residue addition; RM=composted residue addition + inoculation.

* Values in columns that followed the same letter do not differ significantly ($P<0.05$) as determined by the LSD test.

Soil structure is one of the most important properties controlling plant growth (De Freitas et al., 1996). In *R. lycioides*, the addition of composted residue (RM) decreased the colonisation level of inoculated seedlings compared with that in the soil without composted residue addition (M) (Table 2). This suggests that the observed increase in *R. lycioides* seedling growth may be related mainly to the increase in rhizosphere aggregate stability following the combined treatment of mycorrhizal inoculation and compost addition (Fig. 3).

In semiarid environments, the water and nutrient contents of soil are the major limiting factors for plant growth (Albaladejo et al., 1994). The addition of compost alone to soil did not significantly promote *O. europaea* and *R. lycioides* seedling growth, although it has been demonstrated elsewhere that this type of composted residue increases the nutrient content of soil, in particular, the level of available P (Roldán et al., 1996). This fact may be due to the lack of mycorrhizal activity, particularly in *R. lycioides*, and the severe climatological conditions of the area, characterised by low and irregular precipitation and frequent drought periods. The increase in water uptake that a high level of mycorrhizal colonisation provides may be critical under these conditions, and can increase the seedling growth rate. Thus, mycorrhizae may become increasingly important for improving the performance of seedlings grown in soils with addition of composted residue under severe drought.

It can be concluded that the high proportion of stable aggregates was attributable to a higher microbial activity of root biomass and particularly to the presence of vesicular arbuscular mycorrhiza in the rhizosphere aggregates. At the same time, the re-forestation techniques based on the addition of composted residue and mycorrhizal inoculation in the nursery could be used as a tool for improving soil structure, with a subsequently improved plant growth in a semiarid soil.

Acknowledgements

This research was supported by the EC+CICYT co-financed FEDER programme (1FD97-0507 FOREST). We acknowledge the technical support of Paisajes del Sur and TRAGSA. F. Caravaca acknowledges a grant from European Commission (HPMF-CT-2000-00822).

References

- Albaladejo, J., Martínez-Mena, M., Castillo, V., 1994. Changes in soil physical properties induced by soil degradation. Transaction of the 15th World Congress of Soil Science, Acapulco, Mexico, pp. 250–252.
- Albaladejo, J., Castillo, V., Roldán, A., 1996. Rehabilitation of degraded soils by water erosion in semiarid environment. In: Rubio, J.L., Calvo, A. (Eds.), *Soil Degradation and Desertification in Mediterranean Environments*. Geoforma Logroño, Spain, pp. 265–278.
- Bearden, B.N., Petersen, L., 2000. Influence of arbuscular mycorrhizal fungi on soil structure and aggregate stability of vertisols. *Plant Soil* 218, 173–183.
- Bolinder, M.A., Angers, D.A., Gregorich, E.G., Carter, M.R., 1999. The response of soil quality indicators to conservation management. *Can. J. Soil Sci.* 79, 37–45.
- Ceccanti, B., García, C., 1994. Coupled chemical and biochemical methodologies to characterize a composting process and the humic substances. In: Senesi, N., Miano, T. (Eds.), *Humic Substances in the Global Environment and Its Implication on Human Health*. Elsevier, New York, pp. 1279–1285.
- De Freitas, P.L., Zobel, R.W., Snyder, V.A., 1996. A method for studying the effects of soil aggregate size and density. *Soil Sci. Soc. Am. J.* 60, 288–290.
- De Luca, T.H., Keeney, D.R., 1993. Soluble anthrone-reactive carbon in soils: effect of carbon and nitrogen amendments. *Soil Sci. Soc. Am. J.* 57, 1296–1300.
- Díaz, E., Roldán, A., Lax, A., Albaladejo, J., 1994. Formation of stable aggregates in degraded soil by amendment with urban refuse and peat. *Geoderma* 63, 277–288.
- Dick, R.P., 1992. A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. *Agric. Ecosyst. Environ.* 40, 25–36.
- Douds, D.D., Galvez, L., Franke-Snyder, M., Reider, C., Drinkwater, L.E., 1997. Effect of compost addition and crop rotation point upon VAM fungi. *Agric. Ecosyst. Environ.* 65, 257–266.
- FAO, 1988. Soil map of the world: revised legend, final draft. Food Agriculture Organization of the United Nations.
- García, C., Hernández, T., Albaladejo, J., Castillo, V., Roldán, A., 1998. Revegetation in semiarid zones: influence of terracing and organic refuse on microbial activity. *Soil Sci. Soc. Am. J.* 62, 670–676.
- García, C., Hernández, T., Roldán, A., Albaladejo, J., Castillo, V., 2000. Organic amendment and mycorrhizal inoculation as a practice in afforestation of soils with *Pinus halepensis* Miller: effect on their microbial activity. *Soil Biol. Biochem.* 32, 1173–1181.
- Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* 84, 489–500.
- Hayano, B., Tubaki, K., 1985. Origin and properties of β -glucosidase activity of tomato field soil. *Soil Biol. Biochem.* 17, 553–557.
- Haynes, R.J., Francis, G.S., 1993. Changes in microbial biomass C, soil carbohydrate composition and aggregates stability induced by growth of selected crop and forage species under field conditions. *J. Soil Sci.* 44, 665–675.
- Haynes, R.J., Swift, R.S., 1990. Stability of soil aggregates in relation to organic constituents and soil water content. *J. Soil Sci.* 41, 73–83.
- Haynes, R.J., Swift, R.S., Stephen, R.C., 1991. Influence of mixed cropping rotations (pasture-arable) on organic matter content, water stable aggregation and clod porosity in a group of soils. *Soil Tillage Res.* 19, 77–87.
- Hodge, A., Campbell, C.D., Fitter, A.H., 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* 413, 297–299.
- Jastrow, J.D., Miller, R.M., Lussenhop, J., 1998. Contributions of interacting biological mechanisms to soil aggregates stabilization in restored prairie. *Soil Biol. Biochem.* 30, 905–916.
- Lansac, A.R., Martín, A., Roldán, A., 1995. Mycorrhizal colonization and drought interactions of Mediterranean shrubs under greenhouse conditions. *Arid Soil Res. Rehabil.* 9, 167–175.
- Lax, A., Díaz, E., Castillo, V., Albaladejo, J., 1994. Reclamation of physical and chemical properties of a salinized soil by organic amendment. *Arid Soil Res. Rehabil.* 8, 9–17.
- Lax, A., Roldán, A., Caravaca, F., García-Orenes, F., 1997. Relationships between aggregate improvement, microbiological activity and organo-mineral complex formation in soils from semiarid areas. In: Pandalai, S.G. (Ed.), *Recent Research Developments in Soil Biology and Biochemistry*. Research Signpost, Trivandrum, India, ISBN: 81-86481-51-6, pp. 77–92.

- Lynch, J.M., Bragg, E., 1985. Microorganisms and soil aggregate stability. *Adv. Soil Sci.* 2, 133–171.
- Masciandaro, G., Ceccanti, B., García, C., 1994. Anaerobic digestion of straw and piggery wastewater: II. Optimization of the process. *Agrochimica* 3, 195–203.
- McCoy, E.L., 1998. Sand and organic amendment influences on soil physical properties related to turf establishment. *Agron. J.* 90, 411–419.
- Metzger, L., Levanon, D., Mingelgrin, U., 1987. The effect of sewage sludge on soil structural stability: microbiological aspects. *Soil Sci. Soc. Am. J.* 51, 346–351.
- Nannipieri, P., Ceccanti, B., Cervelli, S., Matarese, E., 1980. Extraction of phosphatase, urease, protease, organic carbon and nitrogen from soil. *Soil Sci. Soc. Am. J.* 44, 1011–1016.
- Naseby, D.C., Lynch, J.M., 1997. Rhizosphere soil enzymes as indicators of perturbation caused by a genetically modified strain of *Pseudomonas fluorescens* on wheat seed. *Soil Biol. Biochem.* 29, 1353–1362.
- Page, A.L., Miller, R.H., Keeney, O.R., 1982. *Methods of Soil Analysis*. American Society of Agronomy, Madison, USA, 1159 pp.
- Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhiza fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55, 159–161.
- Roldán, A., Albaladejo, J., 1993. Vesicular-arbuscular mycorrhiza (VAM) fungal populations in a Xeric Torriorthent receiving urban refuse. *Soil Biol. Biochem.* 25, 451–456.
- Roldán, A., Albaladejo, J., Thornes, J.B., 1996. Aggregate stability changes in a semiarid soil after treatment with different organic amendments. *Arid Soil Res. Rehabil.* 10, 139–148.
- Sims, J.R., Haby, V.A., 1971. Simplified colorimetric determination of soil organic matter. *Soil Sci.* 112, 137–141.
- Sparling, G.P., Cheshire, M.V., 1985. Effect of periodate oxidation on the polysaccharide content and micro-aggregate stability of rhizosphere and non-rhizosphere soils. *Plant Soil* 88, 113–122.
- Tabatabai, M.A., 1982. Soil enzymes. In: Page, A.L., Miller, E.M., Keeney, D.R. (Eds.), *Methods of Soil Analysis: Part 2*, 2nd edn. *Agron Monogr.*, vol. 9. ASA and SSSA, Madison, WI, pp. 501–538.
- Tabatabai, M.A., Bremner, J.M., 1969. Use of *p*-nitrophenol phosphate in assay of soil phosphatase activity. *Soil Biol. Biochem.* 1, 301–307.
- Vázquez, M.M., César, S., Azcón, R., Barea, J.M., 2000. Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. *App. Soil Ecol.* 15, 261–272.