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Formation of stable aggregates in rhizosphere soil of *Juniperus oxycedrus*: Effect of AM fungi and organic amendments

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Abstract

The effects of mycorrhizal inoculation, with an arbuscular mycorrhizal (AM) fungus (*Glomus intraradices* Schenck and Smith) or with a mixture of three AM fungi (*G. intraradices*, *G. deserticola* (Trappe, Bloss. and Menge) and *G. mosseae* (Nicol and Gerd.) Gerd. and Trappe), and addition of composted sewage sludge or *Aspergillus niger*-treated dry olive cake (DOC) residue on aggregate stabilisation of the rhizosphere soil of *Juniperus oxycedrus* were studied. The influence of such structural improvements on the establishment of *J. oxycedrus* was evaluated. Six months after planting, the inoculation with the mixture of three AM fungi and the combination of *G. intraradices* with both organic amendments had significantly enhanced the structural stability. Water soluble C and carbohydrates values were increased only with the addition of composted sewage sludge or fermented DOC. The addition of both organic amendments, particularly fermented DOC, decreased significantly the dehydrogenase, urease, protease and β-glucosidase activities. Rhizosphere soil from the inoculation treatments had higher dehydrogenase and β-glucosidase activities than control soil. Both the organic amendments addition and the mycorrhizal inoculation treatments increased significantly shoot biomass in *J. oxycedrus*. AM inoculation treatments were more effective with respect to increasing shoot biomass than the addition of organic amendments alone, and there were no significant differences between the two mycorrhization treatments (on average, about 53% higher with respect to control plants and about 18% higher with respect to plants grown in the amended soil).

Keywords: Aggregate stability; Arbuscular mycorrhizal fungi; Dry olive cake; Enzyme activity; Sewage sludge

1. Introduction

Revegetation programmes based on planting shrubs would assist in the conservation of biodiversity in degraded Mediterranean areas and help to prevent the processes of erosion and desertification of arid and semiarid landscapes (Caravaca et al., 2003). The vegetation cover helps avoid soil losses, together with improving soil physical properties. The improvement of soil quality should be an essential step in establishing vegetation. One method to reverse the degradation in soil quality is the addition of carbon-rich materials (Roldán et al., 1996). The agronomic utilisation of agrowastes of lignocellulosic nature, such as dry olive cake (DOC), and of urban organic waste, such as sewage sludge, has increased steadily in recent years as an alternative nutrient and organic matter source, and as

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an acceptable method for their disposal. However, the use of DOC has been seen to have a detrimental effect on seed germination, plant growth and microbial activity. In fact, several studies have reported the phytotoxic and antimicrobial effects of olive-mill residues, due to their phenol, organic acid and fatty acid contents (Linares et al., 2003). Efforts to decrease the environmental impact of olive-mill wastes include biological fermentation with filamentous fungi, such as *Aspergillus niger* (Vassilev et al., 1995) or white-rot fungi (Linares et al., 2003). Such bio-systems, involving agrowastes and microorganisms, have been used for rock-phosphate (RP) solubilisation and improvement of crop plant growth and nutrition in agricultural soils (Vassilev et al., 1996).

There is growing evidence that soil biological and biochemical parameters may have a potential role as early and sensitive indicators of soil ecological stress and restoration (García et al., 2000). In particular, enzyme activities are especially significant because of their major contribution to the ability of the soil to degrade organic matter. Several investigations have been performed regarding the effects of sewage sludge on soil microorganisms. Undesirable constituents potentially associated with sewage sludge application include elevated levels of heavy metals and xenobiotica, which severely affect the soil microbial biomass. In response to sewage sludge amendments, both increases (Fliessbach et al., 1994) and decreases (Chander and Brookes, 1993) in microbiological activity have been shown, as well as a lack of effect (Johansson et al., 1999).

Inoculation with symbiotic microorganisms, especially arbuscular mycorrhizal (AM) fungi, is an effective method of enhancing the ability of the host plants to become established and to cope with stress situations such as nutrient deficiency, drought and soil disturbance. In fact, several authors have indicated that mycorrhizal fungi may improve the performance of seedlings, by stimulating water uptake (Augé, 2001) or increasing nutrient uptake by the plant, particularly N and P (Jeffries et al., 2003), or by improving soil aggregation in eroded soils (Caravaca et al., 2002). Recent studies have indicated that AM fungi produce glomalin, that stabilises soil aggregates (Wright and Anderson, 2000). Thus, AM fungi are essential components of ecosystems, for both revegetation of degraded lands and maintenance of soil structure, thereby reducing the risks of erosion and desertification. However, there are no data on the effects and interactions of DOC or sewage sludge on mycorrhizal symbiosis or on soil properties in revegetation programmes.

The objectives of this study were: (1) to determine the influence of mycorrhizal inoculation, with an AM fungus or with a mixture of three AM fungi, and addition of composted sewage sludge or *A. niger*-treated DOC residue on aggregate stabilisation of rhizosphere soil of *Juniperus oxycedrus* L., as well as to ascertain the relationships between any physical changes and biochemical and microbiological variations induced by these treatments and (2) to evaluate the influence of such improvements on the establishment of *J. oxycedrus* seedlings.

2. Materials and methods

2.1. Plants and mycorrhizal treatments

The plant used for the experiment, *J. oxycedrus*, is a low-growing tree reaching a height of 3–4 m, although often it grows as a shrub. This shrub has a typical Mediterranean distribution and is well-adapted to drought conditions because it can thrive with mean annual rainfall of less than 230 mm and a summer drought period which can extend for 4 months (Amaral Franco, 1964). However, knowledge of revegetation strategies involving *J. oxycedrus* is still very limited.

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

AM fungal inoculum consisted of a mixture of rhizospheric soil from trap cultures (*Sorghum* sp.) containing spores, hyphae and mycorrhizal root fragments (an average of 30 spores g^{-1} and roots with 75% of AM colonisation). Once germinated, the *J. oxycedrus* seedlings were transplanted into the growth substrate, consisting of peat and cocopeat (1:1, v/v). The corresponding arbuscular mycorrhizal inoculum was applied at a rate of 5% (v/v) in 120-mL containers. The same amount of an autoclaved mixture of the inocula was added to control plants, supplemented with a filtrate ($<$ 20 μm) of the 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.

2.2. Soil

An agricultural soil, used to cultivate citrus fruits was collected near Murcia (SE Spain). The climate is semi-

arid Mediterranean with an average annual rainfall of 300 mm and a mean annual temperature of 19.2 °C; the potential evapo-transpiration reaches 1000 mm year^{-1}. The main characteristics of the agricultural soil used were: pH (1:5) 8.89; electrical conductivity 0.18 dS m^{-1}; COT 1.80%; total N 2.01 g kg^{-1}; available P, 70 μg g^{-1}; extractable K, 440 μg g^{-1}; cationic exchange capacity, 15 cmol kg^{-1}.

2.3. Materials

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

Dry olive cake (DOC), a lignocellulosic material obtained from an olive-mill located in Granada (Spain), was dried at 60 °C and then ground to pass a 2-mm-pore screen. Portions of 5 g of DOC were mixed with 50 mL of Czapek solution (agar 15.0 g L^{-1}; dipotassium hydrogen phosphate 1.0 g L^{-1}; iron(II) sulfate heptahydrate 0.01 g L^{-1}; potassium chloride 0.5 g L^{-1}; magnesium sulfate heptahydrate 0.5 g L^{-1}; sodium nitrate 3.0 g L^{-1}; sucrose 30.0 g L^{-1}; pH 7.3) for static fermentation in 250 mL Erlenmeyer flasks. Rock phosphate (Morocco fluorapatite, 12.8% P, 1 mm mesh), was added at a rate of 0.75 g per flask. This medium was sterilized by autoclaving at 120 °C for 30 min. A spore suspension of *A. niger* NB2 (1.2×10^7) was spread carefully over the surface of the media. The mixture was allowed to ferment at 30 °C for 20 days without shaking. The characteristics of the DOC after fermentation were: pH, 4.0; electrical conductivity (1:10), 1231 μS cm^{-1}; total P, 0.38%; total N, 0.62%; total organic C, 22.2% and water soluble C, 1146 μg g^{-1}.

Table 1
Analytical characteristics of the composted sewage sludge used in the experiment

Ash (%)	18.6 ± 0.1[a]
pH (1:5)	6.1 ± 0.0
Electrical conductivity EC (1:5, μS cm^{-1})	3095 ± 48
Total organic C (g kg^{-1})	380 ± 4
Water soluble C (μg g^{-1})	7245 ± 22
Water soluble carbohydrates (μg g^{-1})	590 ± 53
Total N (g kg^{-1})	14.5 ± 0.1
N–NH_3 (μg g^{-1})	312 ± 13
N–NO_3 (μg g^{-1})	1967 ± 49
Total P (g kg^{-1})	4.5 ± 0.1
Total K (g kg^{-1})	2.3 ± 0.1
Fe (μg g^{-1})	6562 ± 165
Cu (μg g^{-1})	212 ± 8
Zn (μg g^{-1})	588 ± 30
Ni (μg g^{-1})	44 ± 3
B (μg g^{-1})	85 ± 2
Cd (μg g^{-1})	9 ± 1
Pb (μg g^{-1})	180 ± 28
Porosity (%)	78 ± 1

[a] Mean ± standard error ($N = 6$).

2.4. Experimental design and layout

The experiment was a mesocosm assay, conducted as a completely randomised factorial design with two factors. The first factor had three levels: addition or not of either composted sewage sludge or fermented DOC residue to the soil, and the second had three levels: non-inoculation, inoculation of *J. oxycedrus* plants with either *G. intraradices* or with an mixture of *G. intraradices*, *G. deserticola* and *G. mosseae* in the nursery. Five replicates per treatment were carried out, making a total of 45 pots.

Four hundred grams of soil were placed in 600 mL pots. In early February 2004, the amendments (composted sewage sludge or fermented DOC residue) were mixed manually with the experimental soil at a rate of 5% (w/w). *J. oxycedrus* seedlings (inoculated and non-inoculated) were transplanted to 600-mL capacity pots (one per pot). The experiment was carried out in the nursery of the University of Murcia, in Murcia, without any fertiliser treatment. The plants were well watered and kept outdoors under ambient irradiance, temperature and air humidity. Six months after planting, plants were harvested and soil samples of the pots were taken. Soil samples, air-dried and sieved to <2 mm, were divided into two subsamples. One soil subsample was stored at 2 °C for microbiological analysis and another subsample was allowed to dry at room temperature for physical–chemical analysis.

2.5. Plant analyses

Fresh and dry (105 °C, 5 h) mass of shoots and roots were recorded.

The percentage of root length colonised by arbuscular mycorrhizal fungi was calculated by the

gridline intersect method (Giovannetti and Mosse, 1980) after staining with trypan blue (Phillips and Hayman, 1970).

2.6. Soil physical–chemical, chemical and biochemical analyses

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous solution. In soil aqueous extracts, water soluble carbon was determined in a Shimadzu TOC-5050A analyser of C for liquid samples. Water soluble carbohydrates were determined by the method of Brink et al. (1960).

Dehydrogenase activity was determined according to García et al. (1997). For this, 1 g of soil at 60% of its field capacity was exposed to 0.2 mL of 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h at 22 °C in the dark. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with 10 mL of methanol by shaking vigorously for 1 min and filtering through Whatman no. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

Urease and *N*-(-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).

β-Glucosidase was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) as substrate. This assay is based on the release and detection of PNP. Two 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 by spectrophotometry 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 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 (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 $(P_1 - P_2) \times 100/(4 - P_2 + T)$.

2.8. Statistical analysis

Data were log transformed to achieve for normality. Composted sewage sludge or fermented DOC residue additions, 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 the Least Significant Difference (LSD) test, calculated at $p < 0.05$. Statistical procedures were carried out with the software package SPSS 10.0 for Windows.

3. Results

3.1. Physical–chemical properties and labile C fractions

Neither mycorrhizal inoculation treatments nor organic amendments, nor the interaction of amendment × mycorrhizal inoculation, had any significant effect on soil electrical conductivity (Table 3).

Water soluble C and water soluble carbohydrates values were increased only with the addition of composted sewage sludge or fermented DOC (Tables 2 and 3), the greatest increases being observed in the water soluble carbohydrates fraction (about 61 and 97% greater than for non-amended soil, respectively). The addition of fermented DOC was more effective for increasing both labile C fractions. The inoculation with the mixture of three AM fungi and the combination of *G. intraradices* with both organic amendments significantly enhanced the structural stability of the rhizosphere soil of *J. oxycedrus* (Table 2). The highest increase was recorded in the combined treatment of mycorrhizal inoculation with *G. intraradices* and addition of composted sewage sludge (about 35% greater, compared to the control soil).

3.2. Biochemical parameters

Rhizosphere soil from the mycorrhizal inoculation treatments had significantly higher dehydrogenase and

Table 2
Physical–chemical properties and labile C fractions of rhizosphere soil of *J. oxycedrus* in response to mycorrhizal inoculation treatments and composted sewage sludge and fermented DOC addition 6 months after planting ($n = 5$)

	C	S	D	G	M	SG	SM	DG	DM
EC (1:5, μS cm^{-1})	845[a] (10)	829 (2)	783 (28)	825 (26)	761 (9)	907 (29)	780 (6)	770 (1)	729 (14)
Water soluble C (μg g^{-1})	281 (1)	385 (2)	446 (5)	278 (6)	300 (1)	335 (7)	367 (1)	411 (3)	405 (3)
Water soluble CH (μg g^{-1})	33 (1)	53 (1)	65 (1)	34 (2)	38 (0)	45 (2)	57 (1)	70 (1)	58 (0)
Aggregate stability (%)	28.0 (0.1)	26.0 (0.2)	26.9 (0.3)	27.9 (0.4)	33.7 (0.1)	37.9 (0.3)	25.2 (0.4)	34.8 (0.4)	33.5 (0.6)

C, control; S, composted sewage sludge addition; D, fermented DOC addition; G, inoculation with *G. intraradices*; M, inoculation with a mixture of three AM fungi; SG, composted sewage sludge addition and inoculation with *G. intraradices*; SM, composted sewage sludge addition and inoculation with a mixture of three AM fungi; DG, fermented DOC addition and inoculation with *G. intraradices*; DM, fermented DOC addition and inoculation with a mixture of three AM fungi.
[a] Mean (standard error).

Table 3
Two factor ANOVA (mycorrhizal inoculation treatments and composted sewage sludge and fermented DOC addition) for all parameters studied in the rhizosphere soil of *J. oxycedrus* seedlings (*P* significance values)

	Amendment (A)	Mycorrhiza (M)	Interaction (A × M)
Dehydrogenase	<0.001	<0.001	<0.001
Urease	0.015	0.286	<0.001
Protease	<0.001	0.083	0.010
β-Glucosidase	<0.001	0.042	<0.001
Water soluble C	<0.001	0.256	0.062
Water soluble carbohydrates	<0.001	0.868	0.420
Electrical conductivity	0.546	0.303	0.480
Aggregate stability	0.828	0.001	0.187
Shoot	0.001	<0.001	0.029
Colonisation	0.010	<0.001	0.846

β-glucosidase activities and lower urease activity than the control soil, but there were no significant differences between mycorrhization treatments (Table 4). The addition of composted sewage sludge or fermented DOC, particularly fermented DOC decreased significantly the dehydrogenase, urease, protease and β-glucosidase activities. The negative interaction between the amendments and the mycorrhizal inoculation

Table 4
Enzyme activities of rhizosphere soil of *J. oxycedrus* in response to mycorrhizal inoculation treatments and composted sewage sludge and fermented DOC addition 6 months after planting ($n = 5$)

	C	S	D	G	M	SG	SM	DG	DM
Dehydrogenase (μg INTF g^{-1} soil)	52.4[a] (1.2)	52.8 (0.1)	50.8 (0.6)	68.1 (1.0)	64.8 (0.1)	54.4 (0.4)	51.0 (0.4)	47.8 (0.8)	47.1 (0.3)
Urease (μmol NH_3 g^{-1} h^{-1})	1.34 (0.02)	0.90 (0.01)	0.75 (0.03)	1.05 (0.04)	1.10 (0.01)	0.93 (0.06)	1.12 (0.02)	1.66 (0.04)	1.26 (0.02)
Protease-BAA (μmol NH_3 g^{-1} h^{-1})	2.23 (0.03)	1.91 (0.01)	1.20 (0.03)	2.36 (0.06)	2.38 (0.01)	1.03 (0.03)	1.12 (0.01)	1.13 (0.09)	1.32 (0.02)
β-Glucosidase (μmol PNP g^{-1} h^{-1})	1.21 (0.01)	0.59 (0.01)	0.73 (0.01)	1.42 (0.01)	1.36 (0.01)	0.41 (0.01)	0.51 (0.01)	0.50 (0.01)	0.56 (0.01)

C, control; S, composted sewage sludge addition; D, fermented DOC addition; G, inoculation with *G. intraradices*; M, inoculation with a mixture of three AM fungi; SG, composted sewage sludge addition and inoculation with *G. intraradices*; SM, composted sewage sludge addition and inoculation with a mixture of three AM fungi; DG, fermented DOC addition and inoculation with *G. intraradices*; DM, fermented DOC addition and inoculation with a mixture of three AM fungi.
[a] Mean (standard error).

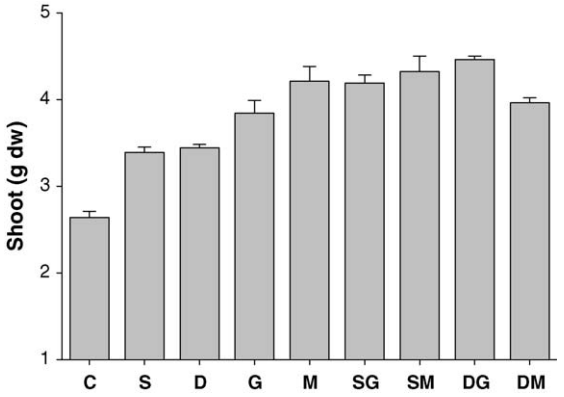

Fig. 1. Shoot dry weight of *J. oxycedrus* in response to mycorrhizal inoculation treatments and composted sewage sludge and fermented DOC addition 6 months after planting (C, control; S, composted sewage sludge addition; D, fermented DOC addition; G, inoculation with *G. intraradices*; M, inoculation with a mixture of three AM fungi; SG, composted sewage sludge addition and inoculation with *G. intraradices*; SM, composted sewage sludge addition and inoculation with a mixture of three AM fungi; DG, fermented DOC addition and inoculation with *G. intraradices*; DM, fermented DOC addition and inoculation with a mixture of three AM fungi). Bars represent standard error for each measure ($n = 5$).

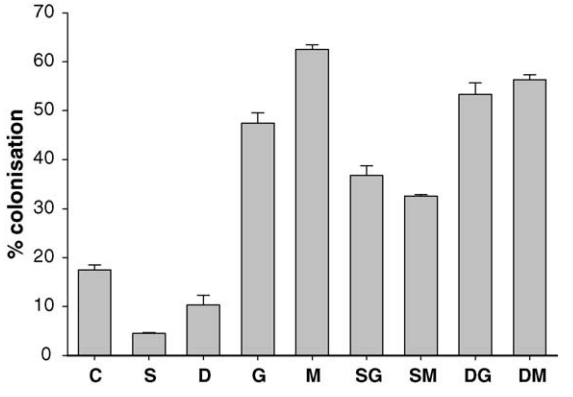

Fig. 2. Root infection of *J. oxycedrus* in response to mycorrhizal inoculation treatments and composted sewage sludge and fermented DOC addition 6 months after planting (C, control; S, composted sewage sludge addition; D, fermented DOC addition; G, inoculation with *G. intraradices*; M, inoculation with a mixture of three AM fungi; SG, composted sewage sludge addition and inoculation with *G. intraradices*; SM, composted sewage sludge addition and inoculation with a mixture of three AM fungi; DG, fermented DOC addition and inoculation with *G. intraradices*; DM, fermented DOC addition and inoculation with a mixture of three AM fungi). Bars represent standard error for each measure ($n = 5$).

treatments affected dehydrogenase, protease and β-glucosidase activities to a very significant degree ($P \leq 0.01$). However, there was a positive interaction, with respect to increasing urease activity, between the mycorrhizal inoculation treatments and the addition of fermented DOC.

3.3. Growth and mycorrhizal infection of *J. oxycedrus*

Six months after planting, both the addition of composted sewage sludge or fermented DOC and the mycorrhizal inoculation treatments had increased significantly shoot dry weight in *J. oxycedrus* seedlings with respect to the control plants (Fig. 1). Arbuscular mycorrhizal inoculation treatments were more effective with respect to increasing shoot biomass than the addition of organic amendments alone, and there were no significant differences between the two mycorrhization treatments (on average, about 53% higher with respect to control plants and about 18% higher with respect to plants grown in the amended soil). The combined treatments, involving mycorrhizal inoculation of seedlings with *G. intraradices* and the addition of composted sewage sludge or fermented DOC to soil, increased the growth of seedlings to a higher extent than each treatment applied separately.

Inoculated *J. oxycedrus* seedlings had significantly higher percentages of root colonisation than the non-inoculated plants, particularly those inoculated with the mixture of three AM fungi (Fig. 2). The percentages of root colonisation decreased with the addition of composted sewage sludge or fermented DOC to soil, reaching values of 3.8 and 1.7 times lower, respectively, than for control plants.

4. Discussion

4.1. Effectiveness of the mycorrhizal inoculation treatments with respect to soil structure

Soil structure largely determines soil quality and fertility, which, in turn, favour the establishment and viability of a stable plant cover. The present study confirms the influence of mycorrhizal inoculation treatments on soil aggregate stability. The mycorrhizal inoculation treatments produced increases in aggregate stability, alone or in combination with both organic amendments. In particular, the inoculation with the mixture of three AM fungi and the combination of *G. intraradices* with both organic amendments significantly enhanced the structural stability of the rhizosphere soil of *J. oxycedrus*. The mechanisms involved in aggregate stabilisation are based on the enmeshment of soil particles by hyphae and roots, and on the exudation

of polysaccharides (Bearden and Petersen, 2000). According to Roldán et al. (1994), the binding effect of roots and hyphae is long-lived, while that of polysaccharides is transient because they are decomposed rapidly by microbes. As suggested by Bearden and Petersen (2000), the symbiosis between arbuscular mycorrhizal fungi and plants would have increased the stability of the soil aggregates. The percentage of colonised root length in plants inoculated with AM fungi was significantly higher than for non-inoculated plants. Recent studies have indicated also that arbuscular mycorrhizal fungi produce a glycoprotein, glomalin, that acts as an insoluble glue to stabilise aggregates (Wright and Anderson, 2000). The water soluble C fraction is also regarded as one of the key labile components of organic matter responsible for soil aggregation (Puget et al., 1999). In fact, the concentrations of water soluble carbohydrates and water soluble C were higher in the rhizosphere soil of the plants inoculated with AM fungi and grown in the amended soils. The increased levels of stable aggregates resulting from the mycorrhizal inoculation treatments can be attributed also to the greater stimulation of the rhizosphere microbial population and, particularly, to the proliferation of fungal hyphae (Roldán et al., 1994; Jeffries and Barea, 2000). This is clearly demonstrated in our case, since only the mycorrhizal inoculation treatments increased the levels of dehydrogenase activity, which is strongly related to microbial activity (Nannipieri, 1994). Oxidoreductases, such as dehydrogenase, are involved in oxidative processes in soils and their activity mainly depends on the metabolic state of soil biota; thus, they are considered as good indicators of the soil microbial activity in semiarid areas (García et al., 1997). The reactivation of microbial populations depended on the assayed mycorrhizal inoculation treatment. *G. intraradices*-inoculated *J. oxycedrus* was the most effective at increasing dehydrogenase activity (by about 30% with respect to the control). Increased biological activity was also revealed by the variations in activities of hydrolases such as protease-BAA and β-glucosidase. The measurement of these hydrolases provides an early indication of changes in soil fertility, since they are related to the mineralisation of important nutrient elements such as N and C (Alguacil et al., 2005). Enzyme activities also are sufficiently sensitive to indicate perturbations caused by microbial inoculation (Naseby and Lynch, 1997). They give an indication of ecosystem function rather than just a measurement of perturbation. The increases observed in protease-BAA and β-glucosidase activities may be related mainly to reactivation of the rhizosphere microbial population as a consequence of the inoculation treatments. To our knowledge, there is no evidence regarding the secretion of these enzymes by AM fungi.

4.2. Effectiveness of the organic amendments with respect to soil structure

The results of this study demonstrate the limited effectiveness of the addition of composted sewage sludge or fermented DOC to soil with respect to improving soil structural stability. There was a significant increase in the labile C fractions, water soluble C and carbohydrates, after addition of the organic amendments to soil, but only those derived from a microbial origin seem to play a principal role in soil stabilisation (Albiach et al., 2001). Thus, this lack of change in soil aggregate stability may be attributed to the addition of organic amendments to soil producing very significant decreases in the soil microbial activity. Some authors have suggested that bacterial communities, which restrict their action to the stabilisation of aggregates measuring <500 μm, are inhibited by the addition of DOC due to its content of phenols (Martínez et al., 1998). The presence of heavy metals in sewage sludge may adversely affect the soil microbial population or activity (Chander and Brookes, 1993). In our study, there was a clear negative effect of the addition of composted sewage sludge on urease, protease-BAA and β-glucosidase activities. The heavy metal contents of the composted sewage sludge used did not exceed the maximum levels authorised by EU law (Directive of European Communities Council, 86/278/CEE). In contrast, García-Gil et al. (2004) described an increase in phosphatase, urease, protease and β-glucosidase activities 9 months after a single sewage sludge application of 40 t ha^{-1} in a semiarid Mediterranean soil.

4.3. Effectiveness of the treatments with respect to the performance of J. oxycedrus

Inoculation with an AM fungus or a mixture of three AM fungi stimulated significantly the production of shoot biomass by *J. oxycedrus*. Mycorrhizae increase nutrient uptake, especially of P and N, by providing a larger absorbing surface, favour root system development and produce substances that promote seedling growth (Jeffries et al., 2003). Both mycorrhizal inoculation treatments showed the same effectiveness with respect to improving the performance of *J. oxycedrus*. The improvement of the soil structure by the mycorrhizal inoculation treatments could have

contributed positively to the establishment of the *J. oxycedrus* plants. It is worth noting that mycorrhizas played a key role in the first stage of the establishment of *J. oxycedrus* seedlings (6 months after planting), which is the most critical period for revegetation, particularly in Mediterranean semiarid areas. Furthermore, arbuscular mycorrhizal inoculation treatments were more effective with respect to increasing shoot biomass than the addition of organic amendments alone.

It can be concluded that, in the short-term, the mycorrhizal inoculation with an AM fungus or a mixture of three AM fungi was the most effective treatment for improving soil structural stability, possibly due to a reactivation of microbial populations, leading to enhanced plant growth. The addition of the organic amendments alone was not sufficient to restore soil structure but was effective for improving the performance of *J. oxycedrus* plants.

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