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Stability of desiccated rhizosphere soil aggregates of mycorrhizal *Juniperus oxycedrus* grown in a desertified soil amended with a composted organic residue

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Abstract

Adequate soil structural stability favours the establishment and viability of a stable plant cover, protecting the soil against water erosion in desertified Mediterranean environments. We studied the effect of soil drying–rewetting, inoculation with a mixture of three exotic arbuscular mycorrhizal (AM) fungi (*Glomus intraradices* Schenck & Smith, *Glomus deserticola* (Trappe, Bloss. & Menge) and *Glomus mosseae* (Nicol & Gerd.) Gerd. & Trappe) and addition of a composted organic residue on aggregate stabilisation of the rhizosphere soil of *Juniperus oxycedrus*. The AM fungi and composted residue produced similar increases in plant growth, independently of the water conditions. Under well-watered conditions, the highest percentages of stable aggregates were recorded in the amended soil, followed by the soil inoculated with AM fungi. Excepting microbial biomass C, the soil drying increased labile C fractions (water soluble C, water soluble and total carbohydrates), whereas the rewetting decreased significantly such C fractions. Desiccation caused a significant increase in aggregate stability of the rhizosphere soil of all plants, particularly in the amended and inoculated plants. In all treatments, the aggregates formed after soil drying were unstable, since, in the rewetting, they disappear, reaching the initial levels before soil drying. Our results suggest that the aggregation mechanisms developed by rhizosphere microbial community of the amended and inoculated plants under water stress can be particularly relevant in desertified soils exposed to long desiccation periods.

Keywords: Aggregate stability; Arbuscular mycorrhizal fungi; Enzyme activity; Mediterranean shrubs; Microbial biomass; Organic amendment; Soil drying and rewetting cycles

1. Introduction

Soil structure has a prevailing role in soil infiltration and biogeochemistry processes (Hamblin, 1985). Improved soil structure means increased water retention, nutrient uptake, drainage, aeration and root growth. Soil aggregate stability is one of the most important properties controlling plant growth in semi-arid Mediterranean environments which, in turn, protects the soil against water erosion.

The agents responsible for aggregate stability are mainly organic, and hence biological in origin. The importance of microbial populations, either as free-living organisms or associated with plant roots, has been stressed by Roldán et al. (1994). In addition to the evidence of mechanical entanglement by hyphae, there is speculation that extracellular polysaccharides of fungi or bacteria provide a cementing agent to large, transiently stable aggregates (Chenu, 1993). Arbuscular mycorrhizal (AM) fungi are thought to make their most important contributions to the stabilisation of macroaggregates (>250 μm), in which they are hypothesised to help stabilise aggregates via hyphal enmeshment (Miller and Jastrow, 2000) and by deposition of organic substances. In particular, AM fungal hyphae produce a glycoprotein, glomalin, that stabilises soil aggregates (Wright and Anderson, 2000). Rillig et al. (2002) described significant indirect effects of AM fungal hyphal length on water-stable aggregate stabilisation via the production of glomalin-related soil protein in a natural grassland system.

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The Mediterranean climate exposes soil to frequent and severe dessication interspersed with relatively rapid rewetting events. Soil drying and rewetting represents a common physiological stress for the microbial communities residing in surface soil. A drying–rewetting cycle may induce lysis in a significant proportion of the microbial biomass (Fierer et al., 2003). This decrease in soil microbiota is largely dependent on its physical stabilisation in the soil matrix or on its ability to survive desiccation in soil. The effects of drying–rewetting on microbial biomass and activity have been well studied (Rosacker and Kieft, 1990). Soil microbial activity has been assessed frequently through biological and biochemical parameters such as biomass C and enzyme activities. Enzymes have been used as indicators of perturbations caused by microbial inoculation and other soil treatments, including application of drying–rewetting stress on the functioning of the soil-plant ecosystem (Naseby and Lynch, 1997). However, no studies have examined the effects of soil drying–rewetting on the interaction between natural microbial population and introduced mycorrhizal species in relation to soil aggregate stability.

The addition of organic matter in the form of composted urban residue has been reported to enhance proliferation of AM 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 (Caravaca et al., 2002). To our knowledge, there are no reports on the effect of organic amendment and AM fungi on aggregate stability during a soil drying and rewetting cycle.

The purpose of this study was to determine the influence of soil drying–rewetting, inoculation with a mixture of AM fungi and addition of a composted residue on the aggregation of the rhizosphere soil of *Juniperus oxycedrus*, and its consequences for the growth of the plant. We hypothesised that native microbial community, which is well-adapted to the whole environment of the desertification-threatened Mediterranean ecosystems in Southeast Spain, would be partly responsible for the development of aggregation processes under water stress.

2. Materials and methods

2.1. Materials

The soil was collected from Los Cuadros in the Province of Murcia (SE Spain) (coordinates: 1°05′W and 38°10′N). 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 y^{-1}. The soil used was a Typic Haplocalcid (Soil Survey Staff, 1999) developed from Quaternary sediments with a loam texture. The main characteristics of the soil used were: pH (1:5) 8.50; electrical conductivity 0.225 dS m^{-1}; total organic C 1.03%; total N 0.95 g kg^{-1}; available P, 7 μg g^{-1}; extractable K, 222 μg g^{-1}.

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 d), mixing the waste heap daily under aerobic conditions. The main characteristics of the composted residue used were: pH (1:10) 6.7; total organic C 276.0 g kg^{-1}; water soluble C 1950 μg g^{-1}; water soluble carbohydrates 76 μg g^{-1}; total N 14.5 g kg^{-1}; total P 3.8 g kg^{-1}.

2.2. Plant 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 is widely distributed in the Mediterranean area and well-adapted to drought conditions. Seedlings were grown for 6 months in peat substrate under nursery conditions, without any fertilization treatment.

The mycorrhizal inoculum used in the experiment was a mixture of *Glomus intraradices* Schenck and Smith (EEZ 1), *G. deserticola* (Trappe, Bloss. & Menge) (EEZ 45) and *G. mosseae* (Nicol & Gerd.) Gerd. & Trappe (EEZ 43), 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.

2.3. Drying and rewetting treatment

In early February 2004, the experimental soil was placed in 1500-ml (13-cm diameter, 11.3-cm height) capacity pots. Composted residue was mixed manually with the soil at a rate of 5% (v/v) into half of the pots. The AM inoculum was applied at a rate of 5% (v/v). The same amount of the autoclaved inoculum was added to control plants, supplemented with a filtrate (Whatman no. 1 paper) of the culture to provide the microbial populations accompanying the mycorrhizal fungi. *J. oxycedrus* seedlings were transplanted to pots (one per pot). Sixteen replicates per treatment were set up, making a total of 64 seedlings. Plants were grown and watered regularly with decalcified water during 14 months maintaining soil moisture adjusted to 70% of water-holding capacity (corresponding with a soil matric potential of −0.2 MPa). Then, the irrigation was interrupted and the soil were allowed to dry during 12-day until gravimetric water content reached approximately 5% (corresponding with a soil matric potential of −1.2 MPa). After the soil drying period, plants were rewatered (corresponding with a soil matric potential of −0.2 MPa) and kept at this potential for a week. The soil water content of each pot was adjusted daily with decalcified water, which was added by spraying onto the soil surface and by

capillary action from the bottom. The experiment was conducted in a greenhouse, located in the Campus of Espinardo (Murcia, Spain). During the experiment, the temperature ranged from 11 °C to 34 °C, and the relative humidity was between 40% and 80%. Midday photosynthetically active radiation (PAR) averaged 260 $\mu E\,m^{-2}\,s^{-1}$.

2.4. Soil water potential

Soil water potential was determined by a pressure plate apparatus and soil water content was measured by weighing the soil before and after drying at 110 °C for 24 h (Richards, 1941). A characteristic soil moisture curve was constructed and used to correlate soil water content and soil water potential (Ψ) by gravimetric measurement of soil water content in the pots.

2.5. Plant analyses

In the beginning of drying period, and at times corresponding to the middle (corresponding with a soil matric potential of −0.6 MPa), and end of drying–rewetting cycle, five plants per treatment were harvested. To collect the rhizosphere soil the roots with adhering soil was introduced into a plastic bag, shaken and separated the rhizosphere soil from the root system. Fresh and dry (105 °C, 5 h) weights of shoots were recorded. The foliar contents of phosphorus were determined, after digestion in nitric–perchloric acid (5:3) for 6 h, by colorimetry (Murphy and Riley, 1962). The percentage of root length colonised by AM fungi was calculated by the gridline intersect method (Giovannetti and Mosse, 1980) after staining with trypan blue (Phillips and Hayman, 1970).

2.6. Soil biological, biochemical and physical analyses

In a 1:5 (w/v) aqueous solution of soil sieved between 0.2 and 4 mm, water soluble carbon was determined with an automatic carbon analyser for liquid samples (Shimadzu TOC-5050A) and total and water soluble carbohydrates were determined by the method of Brink et al. (1960).

Microbial biomass C was determined using a fumigation–extraction method (Vance et al., 1987). Ten grams of soil at 60% of its field capacity are fumigated in a 125-ml Erlenmeyer flask with purified $CHCl_3$ for 48 h. After removal of residual $CHCl_3$, 40 ml of 0.5 M K_2SO_4 solution is added and the sample is shaken for 1 h before filtration of the mixture. The K_2SO_4-extracted C was measured as indicated for water soluble carbon and microbial biomass C is calculated as the difference between fumigated and non-fumigated samples.

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 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.

Phosphatase activity was determined using *p*-nitrophenyl phosphate disodium as substrate (Tabatabai and Bremner, 1969). Two millilitres of 0.1 M maleate buffer at pH 6.5 and 0.5 ml of substrate were added to 0.5 g soil sample and incubated at 37 °C for 90 min. The reaction was stopped by cooling at 2 °C for 15 min; 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were then added and the mixture centrifuged at 2287 × *g* for 5 min. The amount of PNP was determined in a spectrophotometer at 398 nm.

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 Jm^{-2}. The remaining soil on the sieve was placed in a previously weighed capsule (T), dried at 105 °C and weighed (P1). Then, the soil was soaked in distilled water and, after 2 h, passed through the same 0.250 mm sieve with the assistance of a small stick to break the remaining aggregates. The residue remaining on the sieve, which was made up of plant debris and sand particles, was dried at 105 °C and weighed (P2). The percentage of stable aggregates with regard to the total aggregates was calculated by (P1–P2) × 100/(4–P2 + T).

Easily, extractable glomalin was extracted from soil samples sieved between 0.2 and 4 mm with 20 mM sodium citrate (pH 7.0) at a rate of 250 mg of aggregates in 2 ml of buffer and autoclaving at 121 °C for 30 min (Wright and Anderson, 2000). The supernatant was removed and two additional sequential 1-h extractions were performed. All supernatants from a sample were combined, the volume was measured, an aliquot was centrifuged at 10,000 × *g* for 15 min to remove soil particles and Bradford-reactive total protein was measured.

2.7. Statistical analysis

Data were log-transformed to achieve normality. The effects of composted residue addition, inoculation with mycorrhizal fungi and soil drying, and their interactions, on measured variables were tested by a three-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. Growth, nutrient assimilation and mycorrhizal colonisation

Both the inoculation with exotic mycorrhizal fungi and addition of composted residue increased significantly shoot

dry biomass of seedlings with respect to the control plants, although the combination of both treatments produced even higher values (Tables 1 and 2). Before soil drying, the mixture of AM fungi and composted residue produced similar increases in plant growth (about 150% greater than control plants). Twelve days of soil desiccation did not have significant effects on the shoot dry biomass (Table 1). The increases produced by the mycorrhizal inoculation and composted residue at the end of the soil drying period were similar to those observed for plants cultivated under well-watered conditions. This effect of the assayed treatments on plant growth was also observed after 1 week of rewatering. The highest increases in the shoot dry biomass of *J. oxycedrus* plants were recorded in the combined treatment of composted residue addition and mycorrhizal inoculation (about 3.4-fold greater than control plants), whatever the soil water potential.

As observed for shoot biomass, the highest contents of foliar phosphorus were seen in the plants inoculated with the mixture of three exotic AM fungi and grown in the soil

Table 1
Shoot dry biomass, shoot P content, mycorrhizal colonisation and easily extractable glomalin concentration of *J. oxycedrus* in response to mycorrhizal inoculation and composted residue addition during drying–rewetting cycle ($n = 5$)

	Well-watered (−0.2 Mpa)	Stressed (−0.6 MPa)	Stressed (−1.2 MPa)	Rewatered (−0.2 MPa)
Shoot (g dw)				
C	5.7±0.8	6.0±0.4	6.4±0.6	5.9±0.3
M	13.5±0.5	13.5±0.5	13.0±0.4	14.0±0.5
R	15.0±0.6	16.6±1.4	15.6±0.7	16.0±0.8
M+R	19.3±1.8	22.0±0.3	20.1±1.3	19.9±2.2
Phosphorus (mg plant^{-1})				
C	1.9±0.4	2.3±0.2	2.3±0.2	4.6±0.4
M	15.9±2.1	13.3±0.7	11.9±0.6	14.4±0.4
R	12.4±0.8	15.5±1.2	16.4±0.5	25.7±0.3
M+R	19.3±2.1	22.7±0.5	20.9±1.6	35.7±0.1
Colonisation (%)				
C	14.0±2.0	13.5±0.7	12.5±1.2	15.3±0.6
M	67.3±4.7	72.0±1.6	70.3±1.3	74.5±1.8
R	19.3±2.3	14.3±1.7	13.8±1.1	15.8±2.1
M+R	72.0±1.8	80.3±2.2	79.5±1.2	81.8±1.5
Easily extractable glomalin (μg g^{-1} soil)				
C	717±16	801±27	843±11	835±30
M	788±31	824±48	1048±15	782±18
R	1600±34	1579±48	1775±89	1487±46
M+R	1713±54	1735±57	1673±49	1432±26

C: control; M: inoculation with a mixture of three AM fungi; R: addition of a composted residue; M + R: addition of a composted residue and inoculation with a mixture of three AM fungi.

Table 2
Three factor ANOVA (mycorrhizal inoculation, composted residue addition and drying) for all parameters studied

Source of variation	Mycorrhiza (M)	Composted residue (R)	Drying (D)	Interactions			
				M × R	M × D	R × D	M × R × D
Shoot biomass	<0.001	<0.001	0.776	<0.001	0.939	0.896	0.900
Foliar P	<0.001	<0.001	0.629	<0.001	0.200	0.628	0.693
Colonisation	<0.001	0.064	0.334	0.569	0.087	0.245	0.182
Easily extractable glomalin	0.105	<0.001	0.057	0.551	0.898	0.227	0.325
pH	0.620	<0.001	0.055	0.349	0.833	0.305	0.364
EC	0.495	0.733	0.695	0.684	0.832	0.887	0.879
Aggregate stability	0.001	<0.001	<0.001	<0.001	0.277	<0.001	0.047
Water soluble C	0.256	<0.001	0.044	0.538	0.048	0.133	0.172
Total carbohydrates	0.030	<0.001	<0.001	0.098	0.073	0.001	0.120
Water soluble CH	<0.001	<0.001	<0.001	0.048	0.742	<0.001	<0.001
Microbial biomass C	<0.001	<0.001	0.282	0.505	0.732	0.352	0.664
Dehydrogenase	<0.001	<0.001	0.071	0.150	0.045	0.023	0.044
Phosphatase	<0.001	<0.001	<0.001	0.434	0.021	0.047	0.247

P significance values.

with composted residue, under either well-watered or drought-stressed conditions (Table 1). Likewise, the soil drying did not have an effect on foliar P in shoots of *J. oxycedrus* seedlings (Table 2). However, the contents of foliar P in rewatered plants were higher than those recorded in the plants before and after soil drying, particularly for plants inoculated and soil amended with composted residue.

Only the mycorrhizal inoculation treatment had significant effect on the level of colonisation in roots of *J. oxycedrus* ($P<0.001$, Table 2). Before and at the end of the drying period, the plants inoculated with the mixture of exotic *Glomus* species presented a similar level of root colonisation. Naturally-colonised seedlings grown in the soil with or without composted residue addition showed about 15% colonisation of the root length. The rewatering did not affect significantly mycorrhizal colonisation of the inoculated and non-inoculated plants.

3.2. Easily extractable glomalin and aggregate stability

Mycorrhizal inoculation had no significant effect on the easily extractable glomalin concentration of the rhizosphere soil of *J. oxycedrus* (Table 2). The addition of composted residue affected easily extractable glomalin to a very significant degree ($P<0.001$), under either well-watered or drought-stressed conditions. Concentrations of easily extractable glomalin did not vary with the soil drying. At the end of the rewetting period, the easily extractable glomalin concentration in the rhizosphere soil of recovered plants was similar to that recorded after drying (Table 1).

Both the inoculation with the mixture of exotic AM fungi and the addition of composted residue significantly enhanced the structural stability of the rhizosphere soil of *J. oxycedrus* (Fig. 1). The highest increase was recorded with the addition of composted residue (about 85% greater, compared to the control soil under well-watered conditions). Desiccation caused a significant increase in the aggregate stability of the rhizosphere soil of all plants, particularly in the amended soil. During the soil drying, the effect of desiccation on aggregate stability firstly appeared in the inoculated and amended plants, corresponding with a soil matric potential of −0.6 MPa. There was a positive interaction, with respect to increasing the percentage of stable aggregates, between the soil drying and the addition of composted residue (Table 2). At the end of the drying period, the percentage of stable aggregates of the rhizosphere soil of amended plants was about 40% higher than that under well-watered conditions. After 1 week of rewatering, the aggregate stability of the rhizosphere soil of all plants sharply decreased, reaching values similar to those observed with plants cultivated under well-watered conditions. The decrease in stable aggregates due to rewatering was less pronounced in the amended soil.

3.3. Biological and biochemical parameters

Water soluble C and water soluble carbohydrates values were increased only with the addition of composted residue and drying (Tables 2 and 3), particularly when the soil water potential was −0.6 MPa. The inoculated plants presented lower values of water soluble carbohydrates than control plants under well-watered conditions and at a water potential of −0.6 MPa. There was a positive interaction, with respect to increasing water soluble carbohydrates, between the mycorrhizal inoculation and the addition of composted residue, coinciding with a soil

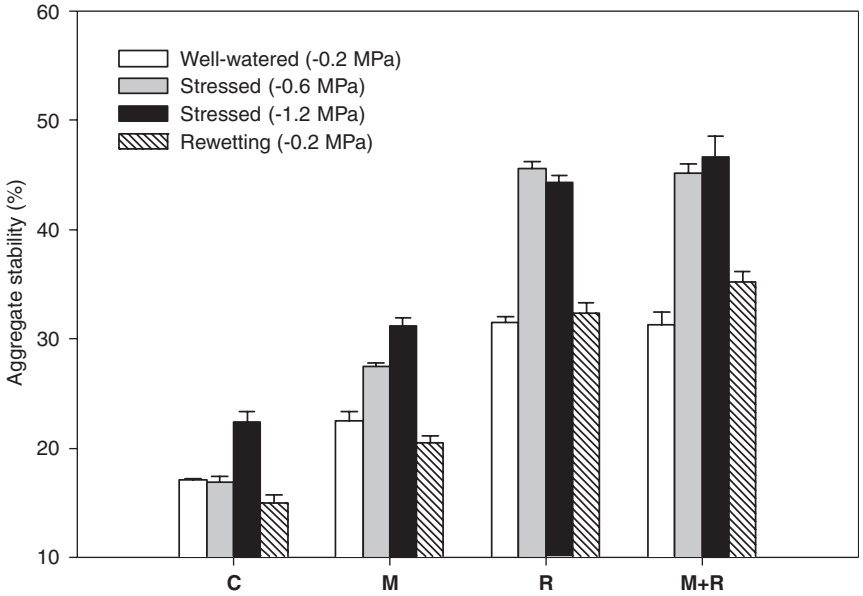

Fig. 1. Percentage of stable aggregates of rhizosphere soil of *J. oxycedrus* in response to composted residue addition and mycorrhizal inoculation during drying–rewetting cycle ($n = 5$). C: control; M: inoculation with a mixture of three AM fungi; R: addition of a composted residue; M + R: addition of a composted residue and inoculation with a mixture of three AM fungi. Bars represent standard errors.

Table 3
Carbon fractions of rhizosphere soil of *J. oxycedrus* in response to mycorrhizal inoculation and composted residue addition during drying–rewetting cycle ($n = 5$)

	Well-watered (−0.2 MPa)	Stressed (−0.6 MPa)	Stressed (−1.2 MPa)	Rewatered (−0.2 MPa)
Water soluble C ($\mu g\,g^{-1}$)				
C	151±1	161±1	169±5	154±1
M	158±2	158±4	171±2	128±2
R	197±10	231±8	230±6	177±2
M+R	231±2	237±3	234±5	148±4
Total carbohydrates ($\mu g\,g^{-1}$)				
C	1458±15	1924±57	2580±106	2397±78
M	1772±45	2205±85	2981±50	2247±102
R	2233±28	3205±30	3223±56	2677±115
M+R	2011±60	3740±26	3399±138	2327±39
Water soluble CH ($\mu g\,g^{-1}$)				
C	33±1	35±1	39±2	23±0
M	24±1	26±0	41±1	22±1
R	40±1	54±2	44±0	21±2
M+R	42±0	61±3	44±1	15±0
Microbial biomass C ($\mu g\ g^{-1}$)				
C	291±29	290±7	245±54	287±22
M	402±18	420±29	354±22	432±12
R	788±22	853±47	824±40	811±23
M+R	1145±4	1179±57	1127±33	1167±8

C: control; M: inoculation with a mixture of three AM fungi; R: addition of a composted residue; M + R: addition of a composted residue and inoculation with a mixture of three AM fungi.

Table 4
Enzyme activities of rhizosphere soil of *J. oxycedrus* in response to mycorrhizal inoculation and composted residue addition during drying–rewetting cycle ($n = 5$)

	Well-watered (−0.2 MPa)	Stressed (−0.6 MPa)	Stressed (−1.2 MPa)	Rewatered (−0.2 MPa)
Dehydrogenase (μg INTF g^{-1} soil $20\,h^{-1}$)				
C	55±3	59±1	57±1	57±1
M	64±1	66±4	67±4	67±1
R	91±2	108±2	93±6	109±2
M+R	153±7	122±2	98±2	94±3
Phosphatase (μmol PNP $g^{-1}\,h^{-1}$)				
C	0.36±0.02	0.61±0.04	0.51±0.03	0.32±0.02
M	0.32±0.01	0.93±0.04	0.84±0.05	0.64±0.04
R	0.68±0.02	0.95±0.02	1.07±0.03	1.03±0.03
M+R	0.75±0.07	1.28±0.07	1.35±0.06	1.05±0.03

C: control; M: inoculation with a mixture of three AM fungi; R: addition of a composted residue; M + R: addition of a composted residue and inoculation with a mixture of three AM fungi.

water potential of −0.6 MPa (Table 3). Both the mycorrhizal inoculation with exotic AM fungi and the addition of composted residue increased significantly total carbohydrates and biomass C (Table 3), the greatest increases being observed in the biomass C fraction (about 38% and 171% greater than for control soil, respectively). The addition of composted residue was more effective for increasing both C fractions. Except for biomass C, the drying had a significant effect on the labile C fractions, producing an increase. In contrast, the rewatering of plants decreased significantly such C fractions (except biomass C), reaching values even lower than those recorded under well-watered conditions.

Rhizosphere soil from plants inoculated with AM fungi and plants grown in the amended soil had significantly higher dehydrogenase activity than the control soil (Table 4), but there were no significant effects of the drying on such enzyme (Table 2). The addition of composted residue and drying increased significantly the phosphatase activity of rhizosphere soil (Table 4). At the end of the drying period, the plants had higher phosphatase activity than under well-watered conditions. The significant interaction

between the amendment and the mycorrhizal inoculation, with the drying, promoted the positive effect of these treatments on phosphatase activity. The rewatering had hardly any effect on dehydrogenase activity. Excepting the plants grown in the amended soil, phosphatase activity was decreased due to rewatering.

4. Discussion

4.1. Effect of the mycorrhizal fungi and composted residue on soil structure

The present study confirms the influence of the inoculation with a mixture of exotic AM fungi on the aggregate 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). The concentrations of total carbohydrates were higher in the rhizosphere soil of the plants inoculated with AM fungi than in that of the control plants. 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 AM 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 AM fungi produce a glycoprotein, glomalin, that acts as an insoluble glue to stabilise aggregates (Wright and Anderson, 2000). In this study, there was no evidence for the major participation of glomalin produced by exotic AM fungi with respect to that produced by local indigenous AM fungi from the soil, in the improvement of soil structural stability. The reactivation of rhizosphere microbial activity is clearly demonstrated in our case, since the mycorrhizal inoculation treatment increased the dehydrogenase activity, which is strongly related to microbial activity (Nannipieri, 1994) and biomass C in the rhizosphere soil. Oxidoreductases, such as dehydrogenase, are involved in oxidative processes in soils and their activity mainly depends on the metabolic state of the soil biota; thus, they are considered as good indicators of the soil microbial activity in semiarid areas (García et al., 1997).

The addition of composted organic residue to soil increased the abundance of stable aggregates to a greater extent than the inoculation with exotic AM fungi. It is important to emphasise that the combined treatment of mycorrhizal inoculation of seedlings and addition of amendment to soil produced effects on aggregate stability similar to residue alone. Such material has a cementing effect, due to the polysaccharides present (Lax and García-Orenes, 1993), and reactivates microbial populations as consequence of the input of biodegradable organic matter and other major nutrients such as N and P. Indeed, the levels of soluble C fractions (water soluble C, total and water soluble carbohydrates) were higher in the soil of plants amended with composted residue than in the soil of inoculated plants. A positive correlation between the soluble C fractions and microbial activity exists in soil (Ghani et al., 2003). The soluble C fractions can be used as C and energy sources for soil microflora and may also participate in soil aggregation (Caravaca et al., 2002). As observed for the soluble C fractions, dehydrogenase activity and biomass C also increased with the addition of composted residue, to a greater degree than with the mycorrhizal inoculation of the plants. Increased microbiological activity was also revealed by the increases in phosphatase activity. Phosphatases are enzymes with a relatively broad specificity, capable of hydrolyzing various organic and inorganic phosphate esters, and are involved in the P cycle. The highest increase in phosphatase activity was recorded in the rhizosphere soil amended with composted residue, which could indicate that the composted residue contained organic phosphorus compounds capable of activating the synthesis of this enzyme. On the other hand, the high easily extractable glomalin concentration in the amended soil of non-inoculated plants could indicate that a significant portion of proteins from composted residue was not eliminated during the glomalin extraction process, interfering in its determination. Recently, Rosier et al. (2006) proved that the use of the Bradford method to assess glomalin pools may not be useful when organic matter additions occur.

4.2. Effect of the mycorrhizal fungi and composted residue on the growth of J. oxycedrus

The composted residue and the exotic AM fungi produced similar increases in the shoot biomass of *J. oxycedrus*. The improvement in aggregate stability may have contributed positively to the establishment of the *J. oxycedrus* plants. However, under our experimental conditions is not possible to demonstrate the effect that soil structure had on plant growth. Mycorrhizae increase nutrient uptake, especially of P, by providing a larger absorbing surface, favour root system development and produce substances that promote seedling growth (Jeffries et al., 2003). The extent of mycorrhizal infection is of importance when studying the influence of AM fungi on the host plant. Large differences in AM percentage colonisation between non-inoculated seedlings and those inoculated with exotic AM fungi seedlings persisted throughout the drying–rewetting experiment, as the local indigenous AM fungi from the soil and/or composted residue showed little capacity to colonise shrub roots. The local AM fungi community from the experimental soil was much less effective than the added *Glomus* inoculum with regard to stimulating host plant growth. Remarkably, *Juniperus* shrubs inoculated with the exotic AM fungi species were of comparable size to those treated with composted residue. This could be due to an improvement

in the available nutrient supply in the soil, arising from the composted residue. Cox et al. (2001) showed that the use of soil amendments can improve soil productivity, increasing the soil nutrient status for some limiting nutrients such as N and P. Thus, we report here that plants grown in the amended soil had higher P contents in their tissues than plants grown in the non-amended soil. Likewise, differential improvement of host plant P nutrient status by the exotic AM fungi inoculum, compared with native AM fungi from the soil, was also recorded before and after drying.

4.3. Effect of drying–rewetting on soil structure

The positive effect of the exotic AM fungi and composted residue on soil structural stability was more pronounced after soil drying, particularly in the amended soil. Likewise, the soil desiccation produced an improvement of the aggregate stability of the rhizosphere soil of control plants. Some studies have shown that soil drying may represent a significant stress for the soil microbiota, provoking a substantial loss of its biomass and activity (Rosacker and Kieft, 1990). Decreasing the water content of soil restricts the diffusion of nutrients to microorganisms (Harris, 1981). In the present experiment, neither the biomass C nor the dehydrogenase activity of soil were affected by the soil drying. One possible explanation for this observation is that the native soil community is physiologically and genetically adapted to water-limited environments. Soil microorganisms have developed various mechanisms to survive desiccation in soil. For example, bacteria have been reported to change the structure of their membrane or to synthesise exopolysaccharide in order to increase their survival during periods of low external water potential. Polysaccharides are hygroscopic and therefore may maintain a higher water content in the colony microenvironment than in the bulk soil as water potential declines. This increase in water content could increase nutrient availability within the bacterial colony. Thus, it has been shown that a *Pseudomonas* sp. strain isolated from soil increased its exopolysaccharide production during desiccation (Roberson and Firestone, 1992). The extracellular polysaccharides of bacteria can form, with the surrounding mineral particles, an organo-mineral sheath around the cells (Chenu, 1993), which leads to an increase in macroaggregates as an indirect additional effect. In this study, large differences in total and water soluble carbohydrates between well-watered and stressed plants accompanied by an increase in aggregate stability after soil drying could support this idea. It has been demonstrated that inoculation with an exopolysaccharide-producing rhizobacterium increased the mass of sunflower root-adhering soil under water stress conditions, although the values reached were lower than under well-watered conditions (Alami et al., 2000). It is worth noting that the existence of this type of mechanism, developed by the native microbial community to increase structural stability during soil drying, has a great ecological importance in desertified soils highly susceptible to water erosion and exposed to long desiccation periods. Differential enhancement of stable aggregates during drying of both the amended and inoculated soils versus the control soil could indicate that the added microbial biomass was more effective than native ones at developing this protective effect against drought. These results also support the fact that the native microbial biomass was too degraded to be effective and so may be necessary to reactivate it by appropriate inoculation with AM fungi or additions of organic amendments. The contribution of AM fungal hyphae in the formation of stable aggregates during soil drying was not demonstrated in this study due to the lack of changes in root colonisation and easily extractable glomalin concentration with the water potential of soil. On the other hand, the aggregates formed during the soil drying were relatively unstable, since the percentages of aggregates of the rhizosphere soil of rewatered plants was lower than those recorded after the drying period. This could be due to the organic matter involved in the formation of stable aggregates during the soil drying being a labile fraction of soil organic matter, which can be used as carbon and energy sources by soil microflora when the aggregates are slaked in water. In fact, after the rewetting period, the levels of water soluble C, total and water soluble carbohydrates decreased markedly with respect to those observed after the drying period. The aggregates of the amended soil were more resistant to disaggregation produced by the rewatering than those of the control and inoculated soils.

It can be concluded that the desiccation improved the aggregate stability in a degraded soil, producing the greatest increases in the soil with composted residue and with mycorrhizal plants. The mechanisms involved in aggregate formation during soil drying were of a biological type, mainly involving secretion of exopolysaccharides. These aggregates formed after soil drying were unstable, since in the rewetting, they disappear, reaching the initial levels before soil drying. The importance of this physical process is evident since it takes place in desertified soils highly susceptible to water erosion. More research is needed to elucidate the ecological implication of this process in desertified environments.

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References

Alami, Y., Achouak, W., Marol, C., Heulin, T., 2000. Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing *Rhizobium* sp. strain isolated from sunflower roots. Applied and Environmental Microbiology 66, 3393–3398.

Bearden, B.N., Petersen, L., 2000. Influence of arbuscular mycorrhizal fungi on soil structure and aggregate stability of vertisols. Plant and Soil 218, 173–183.

Brink, R.H., Dubach, P., Lynch, D.L., 1960. Measurements of carbohydrates in soil hydrolyzates with anthrone. Soil Science 89, 157–166.

Caravaca, F., Hernández, M.T., García, C., Roldán, A., 2002. Improvement of rhizosphere aggregates stability of afforested semi-arid plant species subjected to mycorrhizal inoculation and compost addition. Geoderma 108, 133–144.

Chenu, C., 1993. Clay-or sand-polysaccharide associations as models for the interface between microorganisms and soil: water related properties and microstructure. Geoderma 56, 143–156.

Cox, D., Bezdicek, D., Fauci, M., 2001. Effects of compost, coal ash, and straw amendments on restoring the quality of eroded Palouse soil. Biology and Fertility of Soils 33, 365–372.

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. Agriculture, Ecosystems and Environment 65, 257–266.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Influence of drying–rewetting frequency on soil bacterial community structure. Microbial Ecology 45, 63–71.

García, C., Hernández, M.T., Costa, F., 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Communications in Soil Science and Plant Analysis 28, 123–134.

Ghani, A., Dexter, M., Perrott, K.W., 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biology & Biochemistry 35, 1231–1243.

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

Hamblin, A.P., 1985. The influence of soil structure on water movement, crop root growth, and water uptake. Advances in Agronomy 38, 95–158.

Harris, R., 1981. Effect of water potential on microbial growth and activity. In: Parr, J., Gardener, W. (Eds.), Water Potential Relations in Soil Microbiology. Soil Science Society of America, Madison, WI, pp. 23–95.

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

Lax, A., García-Orenes, F., 1993. Carbohydrates from municipal solid wastes as aggregation factor of soils. Soil Technology 6, 157–162.

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

Miller, R.M., Jastrow, J.D., 2000. Mycorrhizal fungi influence soil structure. In: Kapulnik, Y., Douds, D.D. (Eds.), Arbuscular Mycorrhizas: Molecular Biology and Physiology. Kluwer Academic, Dordrecht, The Netherlands, pp. 3–18.

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

Nannipieri, P., 1994. The potential use of soil enzymes as indicators of productivity, sustainability and pollution. In: Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R., Grace, P.R. (Eds.), Soil Biota: Management in Sustainable Farming Systems. CSIRO, Australia, pp. 238–244.

Naseby, D.C., Lynch, J.M., 1997. Rhizosphere soil enzymes as indicators of perturbations caused by enzyme substrate addition and inoculation of a genetically modified strain of *Pseudomonas fluorescens* on wheat seed. Soil Biology & Biochemistry 29, 1353–1362.

Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55, 158–161.

Richards, L.A., 1941. A pressure-membrane extraction apparatus for soil solution. Soil Science 51, 377–386.

Rillig, M.C., Wright, S.F., Eviner, V.T., 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant and Soil 238, 325–333.

Roberson, E.B., Firestone, M.K., 1992. Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. Applied and Environmental Microbiology 58, 1284–1291.

Roldán, A., Albaladejo, J., 1993. Vesicular–arbuscular mycorrhiza (VAM) fungal populations in a Xeric Torriorthent receiving urban refuse. Soil Biology & Biochemistry 25, 451–456.

Roldán, A., García-Orenes, F., Lax, A., 1994. An incubation experiment to determine factors involving aggregation changes in an arid soil receiving urban refuse. Soil Biology & Biochemistry 26, 1699–1707.

Rosacker, L.L., Kieft, T.L., 1990. Biomass and adenylate energy charge of a grassland soil during drying. Soil Biology & Biochemistry 22, 1121–1127.

Rosier, C.L., Hoye, A.T., Rillig, M.C., 2006. Glomalin-related soil protein: assessment of current detection and quantification tools. Soil Biology & Biochemistry (in press). DOI 10.1016/j.soilbio.2006.01.021.

Soil Survey Staff, 1999. Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys. USDA Natural Resources Conservation Service, Agricultural Handbook, vol. 436. U.S. Government Printing Office, Washington, DC.

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

Vance, E.D., Brookes, P.C., Jenkinson, D., 1987. An extraction method for measuring microbial biomass carbon. Soil Biology & Biochemistry 19, 703–707.

Wright, S.F., Anderson, R.L., 2000. Aggregate stability and glomalin in alternative crop rotations for the central Great Plains. Biology and Fertility of Soils 31, 249–253.