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Aggregate stability changes after organic amendment and mycorrhizal inoculation in the afforestation of a semiarid site with *Pinus halepensis*

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Abstract

The recovery of soil structural stability is a precondition for successful afforestation programmes in semiarid environments. A multifactorial field experiment was carried out in a semiarid rangeland in south-eastern Spain to evaluate the influence of a fresh organic residue addition (first factor), mycorrhizal inoculation with *Pisolithus arhizus* (second factor), and the rhizosphere of *Pinus halepensis* (third factor) on soil aggregate stability. A total of 6 years after planting, the addition of residue was seen to increase the levels of stable aggregates to a greater extent than the mycorrhizal inoculation. Both reafforestation methods increased C-fractions and enzyme activities measured (dehydrogenase and phosphatase). The rhizosphere also affected aggregate stability, particularly when *P. halepensis* was inoculated with *P. arhizus*. Aggregate stability in the rhizosphere of *P. halepensis* was strongly correlated ($P < 0.01$) with the C-biomass and soluble C-fractions (WSC and WSCH) as well as with dehydrogenase ($r = 0.901$, $P < 0.05$) and phosphatase ($r = 0.903$, $P < 0.05$) activities. It was concluded that the combination of residue amendment and inoculation of *P. halepensis* with *P. arhizus* significantly improves soil aggregate stability, this beneficial effect appearing to be mainly due to a reactivation of microbiological activity.

Keywords: Aggregate stability; Municipal waste; Organic residue; *Pisolithus arhizus*; Semiarid soils

1. Introduction

In large areas of arid and semiarid environments, the natural vegetation is so degraded that it hardly protects the soil surface against rain splash (Lopez Bermudez and Albaladejo, 1990). Under such conditions, physical soil degradation is common, particularly during periods of rainfall. Aggregate stability is one of the main factors controlling topsoil hydrology, crustability and erodibility (De Ploey and Poesen, 1985). When the different factors that affect aggregate stability are taken into account, it is clear that any improvement in the structure of semiarid soils will depend on increased organic matter levels and reactivated microbial activity (Diaz et al., 1994).

The agents responsible for aggregate stability are mainly organic and hence biological in origin and are usually developed in the rhizosphere. The importance of microbial populations, either as free-living organisms or associated with plant roots, has been stressed by Jastrow and Miller (1991). Any increase in stability produced by microorganisms may be of a physical nature (Tisdall and Oades, 1982) or arise from the formation and excretion of microbial polysaccharides,

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which act as binding agents (Cheshire et al., 1983). Such binding agents are associated with large transiently stable aggregates.

The addition of organic amendment rich in readily decomposable carbon compounds promotes microbial activity, which, in turn, increases the microbial polysaccharide content and, as a consequence, may improve soil stability (Lax and Garcia-Orenes, 1993; Diaz et al., 1994; Roldan et al., 1996a). *Pinus halepensis* is a mycorrhizal forest-tree species, which has successfully adapted to severe drought conditions and which offers effective soil protection against hydric erosion processes. The viability of *P. halepensis* seedlings in revegetation programmes can be improved by inoculation with symbiotic microorganisms (Torres and Honrubia, 1993; Roldan and Albaladejo, 1994). Roldan et al. (1996b) demonstrated that the combination of soil terracing, refuse amendment and *P. arhizus* inoculation considerably improved the performance of *P. halepensis* seedlings. However, these reforestation techniques using *P. halepensis* have not been assessed in relation to changes in soil structure.

Thus, the general objective of our study is to determine the influence of the rhizosphere of *P. halepensis*, mycorrhizal inoculation with *P. arhizus* and the addition of fresh municipal solid waste on aggregate stabilisation in a semiarid rangeland in south-eastern Spain, as well as to ascertain the relationships between any physical changes and chemical, biochemical and microbiological variations induced by the reafforestation practices.

2. Materials and methods

2.1. Study sites

The study sites were located in the El Aguilucho experimental area (UTM: 30S XG5395) in the Carrascoy range in Murcia Province (south-east Spain). The climate is semiarid Mediterranean with an average annual rainfall of 300 mm, occurring mostly in autumn and spring, and a mean annual temperature of 18 °C; the potential evapo-transpiration reaches 900–1000 mm per year. The predominant soil is Haplocalcid type developed from limestone (Soil Survey Staff, 1996).

Table 1
Characteristics of the organic residue (R)

Ash (%)	40.6
pH (1:10)	6.8
Electrical conductivity EC (1:5, dS m^{-1})	4.4
Organic C (g kg^{-1})	253
Total N (g kg^{-1})	11.9
Total P (g kg^{-1})	5.5
Extractable C (g kg^{-1})	48.1
Humic C (g kg^{-1})	16.4
Carbohydrates (g kg^{-1})	49.5
Zn (mg kg^{-1})	281
Cu (mg kg^{-1})	77
Cd (mg kg^{-1})	2
Pb (mg kg^{-1})	75
Ni (mg kg^{-1})	168
Cr (mg kg^{-1})	252

2.2. Materials

The organic residue (R) used was the organic fraction of a municipal solid waste from a municipal waste treatment plant in Murcia, which had been stored for 15 days without treatment (precomposting stage). The inert materials (plastic, glass, paper, etc.) were removed from the organic material. Analytical characteristics of the R, determined by standard methods (Page et al., 1982), are shown in Table 1.

The mycorrhizal fungus used in the experiment was *P. arhizus* (Pers. I) Rauschert (=*P. tinctorius* (Pers.) Coker and Couch). To prepare the spore inoculum, fresh mature fruiting bodies were cut into pieces (3–5 cm) and blended with tap water at high speed for 2–3 min. Spore concentration of the resulting suspension was determined with a heamocytometer and it was stored in the dark at 2 °C until used as inoculum (Castellano and Molina, 1990). More information about the mycorrhizal inoculation of the seedlings can be found in Roldan et al. (1996b).

2.3. Field procedures

In October 1992, an area of 1800 m^2 on an east-facing hillslope with a 25−30% gradient was prepared for the reforestation experiment by two methods: the addition of organic residue to the soil, and the direct mycorrhizal inoculation of pine plants with *P. arhizus*. *P. halepensis* seedlings were planted following a randomised design. More detailed information

on the experimental area can be found in Roldan et al. (1996b). Twelve manual terraces (0.8 m wide, 30 m long) were established on the hillslope. *P. halepensis* seedlings were planted in individual holes, at least 1 m apart, in a single row per terrace. In six terraces (replication blocks), R was added in the first 20 cm top layer with a rotovator at a rate of 10 kg m^{-2} soil. In each terrace, and regardless of the addition of R, half of the pine seedlings were inoculated with *P. arhizus*, while the other half were used as control. A distance of 3 m or more separated the inoculated and the non-inoculated pines. At least 25 seedlings per treatment and replication block were planted.

After 6 years, six samples of each treatment (one per block) were collected. Each sample consisted of six bulked subsamples (150 cm^3 cores) randomly collected at 0–15 cm depth from six planting holes (considered as rhizosphere soil). The sampling was carried out in early December after the autumn rainy season, when the highest microbial activity could be expected (Lax et al., 1997). At the same time, the same number of soil samples was taken from outside the canopy of the pines for aggregate stability determination.

2.4. Chemical, microbiological and biochemical analyses

Chemical, biochemical and microbiological analyses were made in the soil samples taken from the planting holes (four treatments × six blocks).

Electrical conductivity was potentiometrically evaluated from the 1:5 water extract. The pH values were determined in H_2O saturated paste. Cation exchange capacity was determined by Ba^{2+} retention after percolation with a solution of 0.2 N $BaCl_2$-triethanolamine at pH 8.1, according to Carpena et al. (1972). Total organic carbon and total nitrogen were assessed by pretreatment with HCl to eliminate carbonates (Navarro et al., 1991) followed by combustion at 1020 °C and determination in an Automatic Nitrogen and Carbon Analyzer. Extractable (with sodium bicarbonate (Olsen et al., 1954)) P was determined by colorimetry, according to Murphy and Riley (1962). Extractable (with ammonium acetate) K was determined by flame photometry.

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

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

Dehydrogenase activity was determined following Skujins' method (1976) modified by Garcia et al. (1997a). For this, 1 g of soil at 60% of its field capacity was exposed to 0.2 ml of 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h at 22 °C in darkness. The iodo-nitrotetrazolium formazan (INTF) 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 (PNPP, 0.115 M) as substrate. Two millilitres of 0.1 M maleate buffer at pH 6.5 and 0.5 ml of substrate were added to 0.5 g of soil and incubated at 37 °C for 90 min. The reaction was stopped by cooling at 2 °C for 15 min. Then, 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were added, and the mixture was centrifuged at 4000 rpm for 5 min. The *p*-nitrophenol (PNP) formed was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969). Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

2.5. Physical analysis

Aggregate stability was determined in the soil samples taken from inside the planting holes and from outside the canopy of the pines (four treatments × six blocks × two positions).

The percentage of stable aggregates was determined by the method described by Lax et al. (1994). A 4 g aliquot of sieved (0.2–4 mm) soil was placed on a small 0.250 mm sieve and wetted by spray. After 15 min the soil was subjected to an artificial rainfall of 150 ml with an energy of 270 Jm^{-2}. The remaining soil on the sieve was put in a previously weighed capsule (T), dried at 105 °C and weighed (P1). Then, the soil was soaked in distilled water and, after 2 h, passed through the same 0.250 mm sieve with the assistance of a small stick to break the remaining aggregates. The

residue remaining on the sieve, which was made up of plant debris and sand particles, was dried at 105 °C and weighed (P2). The percentage of stable aggregates with regard to the total aggregates was calculated by $(P1 - P2) \times 100/(4 - P2 + T)$.

2.6. Statistical analysis

Residue addition, mycorrhizal inoculation and their interactions effects on measured variables were tested by a two-way analysis of variance and comparisons among means were made using least significant difference (LSD) multiple range test calculated at $P < 0.05$. Pearson's rank correlation coefficients between all the soil parameters measured were assessed. Statistical procedures were carried out with the software packages Statgraphics for Windows7.0.

3. Results

3.1. Chemical parameters

Soil electrical conductivity and pH values were not affected by either of the reafforestation methods assayed (Table 2). However, the application of organic residue significantly increased the other chemical parameters considered (Tables 2 and 3). Particularly of note was the increase in assimilable nutrients (P, K) and the total N values. Neither mycorrhizal inoculation nor the interaction residue × mycorrhizal (R × M) inoculation had any significant effect on the chemical properties (Table 3).

3.2. Microbiological and biochemical parameters

The total organic carbon content of the soil was significantly increased by the addition of residue but not by mycorrhizal inoculation (Fig. 1, Table 4). Both mycorrhizal inoculation and residue significantly increased the water-soluble carbon, water-soluble carbohydrates, C-biomass values (Fig. 1) and enzyme activities (dehydrogenase and phosphatase, Fig. 2). Application of the residue increased all the microbiological and biochemical parameters to a greater extent than mycorrhizal inoculation, although the interaction of both reafforestation methods produced even higher values, the differences being statistically significant in all cases except for dehydrogenase activity.

In the determination of aggregate stability, the effects of both reafforestation methods used and the effect of the rhizosphere of *P. halepensis* were considered by sampling soil from both the centre and from outside the pine canopy. Residue addition had the strongest effect on the percentage of water-stable

Table 2
Variations in chemical parameters in response to mycorrhizal inoculation (M) and residue (R) addition ($n = 6$)[a]

Treatments	pH (H_2O)	EC ($dS\,m^{-1}$)[b]	CEC ($cmol\,kg^{-1}$)	TN ($g\,kg^{-1}$)	P ($mg\,kg^{-1}$)	K ($mg\,kg^{-1}$)
M^-R^-	7.64 (0.17)	0.226 (0.109)	5.7 (0.7)	0.5 (0.1)	4 (0)	39 (6)
M^-R^+	7.53 (0.10)	0.321 (0.099)	7.5 (2.3)	1.2 (0.4)	35 (11)	209 (48)
M^+R^-	7.78 (0.19)	0.267 (0.122)	4.9 (0.9)	0.4 (0.1)	2 (1)	50 (9)
M^+R^+	7.55 (0.08)	0.496 (0.147)	8.5 (1.5)	1.1 (0.3)	30 (10)	221 (86)

[a] In parenthesis, standard deviation for each measure.
[b] EC: electrical conductivity; CEC: cation exchange capacity; TN: total nitrogen.

Table 3
Results of two factors ANOVA (mycorrhizal inoculation and residue addition) for the chemical parameters studied[a]

Source of variation	pH	EC	CEC	TN	P_{ext}	K_{ext}
Residue (R)	0.08	0.16	0.02	<0.01	<0.01	<0.01
Mycorrhiza (M)	0.38	0.33	0.92	0.54	0.58	0.55
R × M	0.51	0.55	0.38	1.00	0.79	0.98

[a] *P* values.

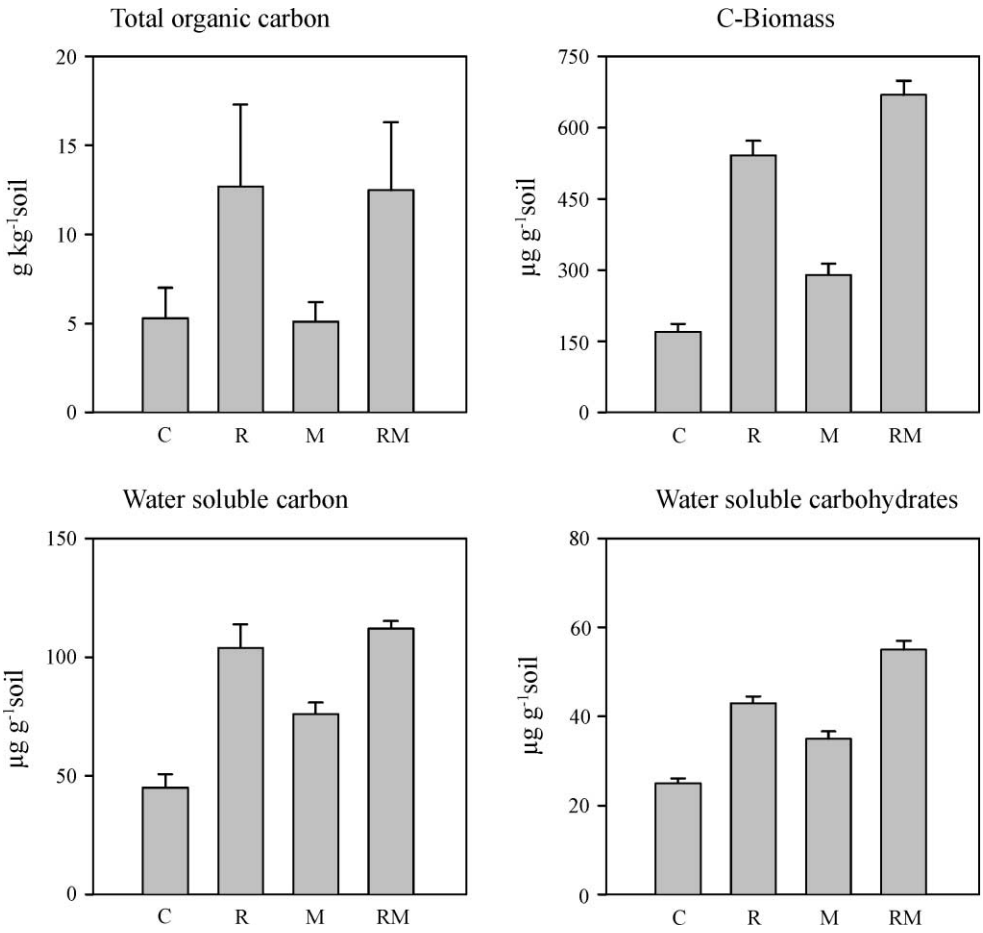

Fig. 1. Changes in total organic carbon and carbon-fractions in a *P. halepensis* plantation following the different treatments (C: control; R: residue addition; M: mycorrhizal inoculation; RM: residue addition + mycorrhizal inoculation). Bars represent standard deviation for each measure ($n = 6$).

Table 4
Results of two factors ANOVA (mycorrhizal inoculation and residue addition) for total organic carbon, C-fractions and enzymatic activities studied[a]

Source of variation	Total organic carbon	Water-soluble carbon	Water-soluble carbohydrates	C-biomass	Dehydrogenase	Phosphatase
Residue (R)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Mycorrhiza (M)	0.93	<0.01	<0.01	<0.01	0.07	<0.01
R × M	0.99	0.01	0.04	0.02	0.70	0.01

[a] *P* values

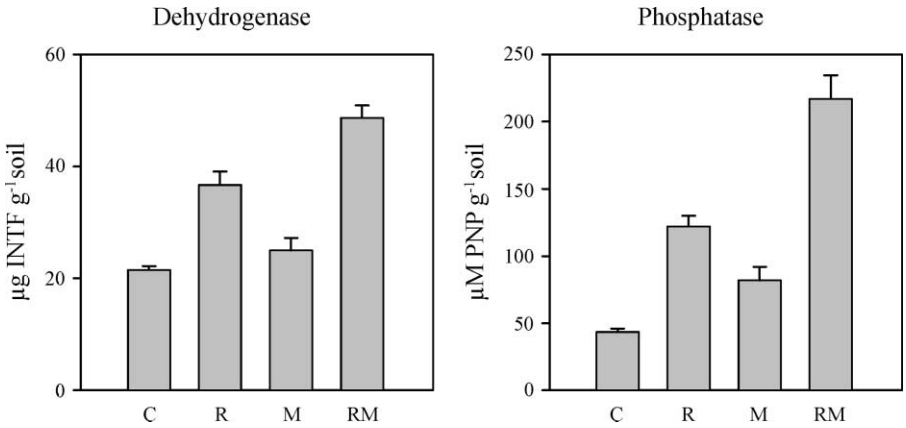

Fig. 2. Changes in dehydrogenase and phosphatase activities in a *P. halepensis* plantation following the different treatments (C: control; R: residue addition; M: mycorrhizal inoculation; RM: residue addition + mycorrhizal inoculation). Bars represent standard deviation for each measure ($n = 6$).

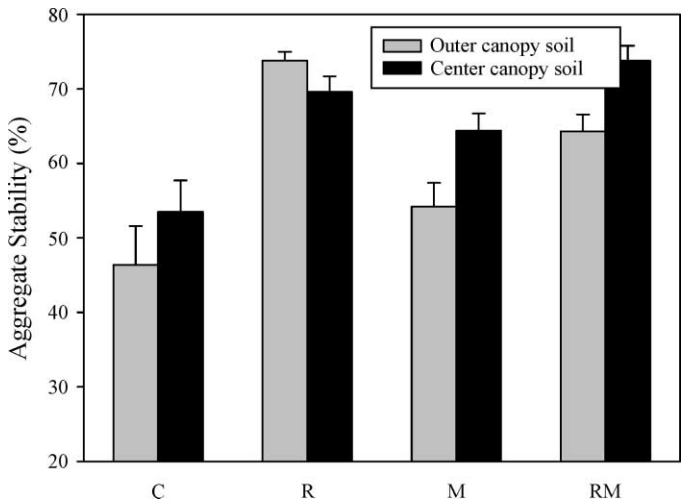

Fig. 3. Percentage of stable aggregates in soil following the different treatments in the *P. halepensis* plantation (C: control; R: residue addition; M: mycorrhizal inoculation; RM: residue addition + mycorrhizal inoculation). Bars represent standard deviation for each measure ($n = 6$).

aggregates (Fig. 3). Mycorrhisation on its own seems to have had a moderate effect (on average 19% over control) but conferred no additional benefit when combined with residue addition. The sampling position (rhizosphere effect) also affected aggregate stability values to a very significant degree (Table 5), with the levels determined in samples from outside the canopy being significantly lower than in those within the canopy. This effect was independent of residue application but strongly dependent on mycorrhizal inoculation.

Table 5
Results of three factors ANOVA (mycorrhizal inoculation, residue addition and position) for aggregate stability

Source of variation	*P* values
Residue (R)	<0.001
Mycorrhiza (M)	0.083
R × M	0.001
Position (P)	0.002
R × P	0.119
M × P	0.010
R × P × M	0.178

4. Discussion

Reafforestation with *P. halepensis* effectively increased the structural stability of the semiarid soil studied. Besides the protection against hydric erosion afforded by the plant cover itself, the increased resistance of aggregates to the action of rainwater considerably reduced the risks of erosion (Albaladejo et al., 1996). The experimental factors had different effects on the increased stability of soil aggregates and deserve further discussion.

The addition of an organic residue provides to soil an immature organic matter rich in easily assimilable carbon compounds. Such material has a cementing effect through the polysaccharides present (Lax and Garcia-Orenes, 1993) and reactivates microbial populations (Roldan et al., 1994), to such an extent that it has been used as amendment in the recovery of soils in semiarid areas (Diaz et al., 1994; Roldan et al., 1996b). In our particular case, there was a very significant increase in the levels of total organic carbon and of the soluble C-fractions, pointing to a greater degree of biological activity. Other authors too, such as De Luca and Keeney (1993) have observed positive correlations between the soluble C-fractions and microbial activity. A more direct measurement of an increased microbial population is the C-biomass, which was used by Ross et al. (1982) as an index to compare natural and degraded systems. In our experiment, the application of residue, the mycorrhizal inoculation and the combined treatment of both reafforestation methods increased the C-biomass by 219, 71 and 294%, respectively with respect to the control. Increased biological activity was also revealed by the variations in dehydrogenase and phosphatase activities. Application of organic amendment to soil can increase dehydrogenase activity which has been frequently used as an indicator of soil microbial activity (Garcia et al., 1997b), while the processes related to the degradation of organic matter by microbial activity may be followed measuring hydrolases such as phosphatase (Ceccanti and Garcia, 1994). In the present experiment, there is a good correlation between enzyme activities, the C-biomass and the levels of stable aggregates (Table 6), which suggests that the reason for the increased aggregate stability observed after the addition of residue is fundamentally microbiological. Likewise, the highest increases in dehydrogenase and phosphatase activities in response to the combined treatment of both reafforestation methods might be due to high levels of stable aggregates, which protect the organic fraction on which enzymes are immobilised from microbial degradation (Nannipieri, 1994).

Reactivation of the microbial population leads to increased levels of bacteria, and particularly of fungal populations, which are principally responsible for the formation of aggregates larger than 0.2 mm (Lax et al., 1997; Andrade et al., 1998). The mycelium of mycorrhizal fungi is also an important aggregating factor, as many authors have stated (Barea and Jeffries, 1995). In the experiment we describe, the increased levels of stable aggregates resulting from mycorrhizal inoculation can be attributed to the greater proliferation of fungal hyphae in the rhizosphere. It should also be mentioned that mycorrhizal inoculation also has a beneficial effect on root and above ground growth in pine (Querejeta et al., 1998), and this greater growth is translated into a greater presence of root exudates and increased microbial activity in the rhizosphere. Furthermore, the hyphae of ectomycorrhizal fungi may release enzymes involved in mineralisation of organic matter. A positive correlation has been reported between phosphatase activity and the length of fungal hyphae associated with ectomycorrhizal mantles (Haussling and Marschner, 1989). This is clearly demonstrated in our case since the mycorrhizal inoculation factor increased the levels of C-biomass, WSC, WSCH and enzyme activities. Other authors (Andrade et al., 1998) have also found that the fungal components of mycorrhiza enhanced soil aggregate stability, and suggested that they affected the numbers of microorganisms indirectly by providing a favourable and protective habitat through the creation of habitable pore space in the water-stable aggregates. We also registered an increase in the total populations of bacteria and fungi (Roldan et al., 1996b), an increase which was directly related to the levels of stable aggregates, as already mentioned. The addition of residue leads to strong growth in the mycelium of saprophytic fungi (Roldan et al., 1994; Roldan et al., 1996a) and has also been shown to favour the development of some mycorrhizal fungi, as is the case of *P. arhizus* in symbiosis with *P. halepensis* (Roldan and Albaladejo, 1994). This would explain the substantial increase in the aggregate stability produced by the residue × mycorrhizal inoculation interaction.

Table 6
Pearson's rank correlation matrix between all the parameters studied ($n = 6$)

	A. S.	Dhase	PHPase	C-Biom	WSCH	WSC	pH	EC	CEC	TOC	TN	P_{ext}	K_{ext}
Aggregate stability (AS)	1.000	0.901*	0.903*	0.951**	0.964**	0.990**	NS[a]	NS	NS	NS	NS	NS	NS
Dehydrogenase (Dhase)		1.000	0.984**	0.980**	0.978**	0.913*	NS	0.971**	0.939*	0.900*	NS	NS	0.933*
Phosphatase (PHPase)			1.000	0.949*	0.984**	NS	NS	0.993***	NS	NS	NS	NS	NS
C-Biomass (C-Biom)				1.000	0.978**	0.971**	NS	0.914*	0.914**	0.941*	NS	0.901*	0.968**
Water-soluble carbohydrates (WSCH)					1.000	0.956**	NS	0.958**	NS	NS	NS	NS	NS
Water-soluble carbon (WSC)						1.000	NS	NS	NS	NS	NS	NS	0.925*
pH							1.000	NS	NS	NS	−0.911*	NS	NS
Electrical conductivity (EC)								1.000	NS	NS	NS	NS	NS
Cation exchange capacity (CEC)									1.000	0.947*	0.934*	0.913*	0.949*
Total organic carbon (TOC)										1.000	0.993***	0.993***	0.996***
Total nitrogen (TN)											1.000	0.998***	0.979**
Extractable P (P_{ext})												1.000	0.980**
Extractable K (K_{ext})													1.000

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.
[a] NS: not significant.

Finally, some explanation should be offered for the positive effect which position within canopy had on the aggregates, which showed significantly ($P < 0.01$) higher levels when recorded within the "area of influence" of the canopy, i.e. in the rhizosphere, except in the soil treated with the residue. In this case, the residue factor still had a significant effect, which however did not change with position, hence the residue × position interaction had no statistically significant effect. This can be explained by the fact that the positive effect that the residue had on fertility and microbial populations in the soil is also, to a large extent, produced outside the canopy since the same organic matter was added at the same rate inside and outside the planting holes. On the other hand, there was a very significant interaction between mycorrhizal inoculation and position ($P < 0.01$), meaning that the beneficial effect of mycorrhizal fungus was confined to the canopy of the pine.

In conclusion, it can safely be said that reafforestation is a proven tool for improving soil structure and mitigating the risks of erosion in semiarid areas. At the same time, the use of new techniques such as, for example organic amendment and mycorrhizal inoculation in the nursery, increases this beneficial effect basically through the reactivation of microbial populations.

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