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Organic amendment and mycorrhizal inoculation as a practice in afforestation of soils with *Pinus halepensis* Miller: effect on their microbial activity

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Abstract

Soil amendment with organic materials prior to afforestation as well as the use of mycorrhizal inoculation, are advisable practices in afforestation of semiarid areas. In this work, the effect of both organic amendment and mycorrhizal treatment on the microbial activity of a soil afforested with Aleppo pine (*Pinus halepensis* Miller) was studied. Labile carbon fractions (water soluble carbon and water soluble carbohydrates), microbiological parameters (microbial biomass carbon, basal respiration and metabolic quotient), and enzyme activities such as oxydoreductases (deshydrogenase and catalase activities) and hydrolases (urease, protease and phosphatase) were determined. All these parameters were found positively influenced by organic amendment and mycorrhizal treatment of plant roots by inoculation of fungi or forest soil addition. The best result was obtained when mycorrhizal inoculation with fungi was supplemented by organic amendment.

Keywords: Organic amendment; Mycorrhizal inoculation; Afforestation; Microbial activity

1. Introduction

The climate conditions existing in southeast Spain, with an average temperature of 18°C and annual rainfall lower than 300 mm, together with inadequate agricultural soil management have led to a decrease in soil productivity and fertility, favouring soil degradation processes (Albaladejo et al., 1994). It is necessary to afforest these areas but it is a difficult task due to both, poor quality of the soil and water shortage. *Pinus halepensis* Miller is perhaps the best adapted forest tree species to these hostile conditions (Elena-Rosello et al., 1990).

One of the main problems of these soils is their low organic matter content (Albaladejo and Diaz, 1990), and, therefore, the use of organic amendments is very important in afforestation programs (Roldan et al., 1994; Garcia et al., 1994). The use of mycorrhizae in afforestation has been studied widely and its effectiveness has been demonstrated (Valdes, 1986; Roldan et al., 1996a, 1996b). Mycorrhizae increase nutrient uptake and promote the transport of water to plant roots (Harley and Smith, 1983). Since *P. halepensis* is obligatory mycorrhizal (Roldan and Albaladejo, 1994), inoculation at the nursery or alternative mycorrizhal methods (addition of forest soil to the planting holes) can favour afforestation.

In the afforestation of soils under semiarid conditions it is important to determine the soil microbial activity due to the fundamental role that microorganisms play in the establishment of the biogeochemical cycles of nutrients which has a direct influence on ecosystems' stability and fertility (Smith et al., 1993). Sev-

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eral microbiological and biochemical parameters have been used as biomarkers for the microbial activity in soils (Nannipieri et al., 1990; Dick, 1994): microbial biomass C, soil respiration, metabolic quotient, and enzyme activities such as dehydrogenase, urease and phosphatase.

In this work, we have evaluated the influence of soil amendment with urban waste and two different mycorrhizal methods (mycorrhizal inoculation with fungi or addition of forest soil to the planting holes) on the microbial activity of a soil revegetated with *P. halepensis*.

2. Materials and methods

2.1. Study sites

The study sites were located in El Aguilucho experimental area (UTM: 30S XG5395) on the Carrascoy range in Murcia Province (southeast Spain). The climate is semiarid Mediterranean with an average annual rainfall of 300 mm, which occurs mostly in autumn and spring, and a mean annual temperature of 18°C; the potential evapo-transpiration reaches 900–1000 mm y^{-1}. The predominant soil is Haplocalcid type developed from limestone (Soil Survey Staff, 1996).

2.2. Materials

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

The forest soil was taken from an established *P. halepensis* spot located 300 m away from the experimental plot. The soil was collected 3 h before planting from the feeder-root zone (top 20 cm of mineral soil) of randomly selected pine trees.

The mycorrizhal fungus used in the experiment was *Pisolithus 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 and Albaladejo (1994).

Table 1
Characteristics of the Organic Waste (ORG)

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

2.3. Field procedures

In October 1992 an area of 1800 m^2 on an east-facing hillslope ranging from 25–30% grade was set up for a reforestation experiment by two methods. The main method was the addition of organic waste (ORG) to the soil. The other method was the direct mycorrhizal inoculation of pine plants or the indirect miycorrhizal inoculation by addition of the forest soil (M or F respectively, refer to Table 2). *P. halepensis* seedlings were planted following a randomised design. More detailed information on the experimental area can be found in Roldan et al. (1996a, b). Eight manual terraces (0.8 m wide, 30 m long) were stabilised in the previously terraced hillslope. *P. halepensis* seedlings were planted in holes, using a shovel, at least 1m apart, one in each hole, in a single row per terrace. In four terraces, ORG was added (0–20 cm depth) into the planting holes at the rate of 10 kg m^{-2} soil. In each terrace, and regardless of the addition of ORG, one third of the pine seedlings were inoculated with *P. arhizus*; another third of the pine seedlings was planted, adding 200 g forest soil into the planting holes (indirect mycorrhizal method), and the another third of plants were used as control. A distance of 3 m or more separated the pines inoculated with *P. arhizus* and the non inoculated plants. At least, 25 seedlings per treatment were planted.

After three years, four samples of each treatment Control (C), pines mycorrhized with *P. arhizas* (M), and pines planted with forest soil (F) and the same treatment with ORG (C + ORG, M + ORG and F + ORG) were collected. Each sample consisted of six bulked subsamples (150 cm^3 cores) randomly collected at 0–15 cm depth into the planting holes. The sampling was carried out in April, when the highest microbial activity could be expected (Garcia et al., 1998).

2.4. Chemical analysis of soil samples

pH and electrical conductivity were measured in a 1/5 (w/v) aqueous solution. Total N was determined by Kjeldhal method, and the total organic C by Yeoman and Bremner method (Yeomans and Bremner, 1989).

Total P and K were determined in the HNO_3–$HClO_4$ digestion extract. Available P (extracted with sodium bicarbonate (Olsen et al., 1954)) and total P were determined by colorimetry according to Murphy and Riley's method (1962). Extractable K (with ammonium acetate) and total K, were determined by flame photometry.

In the aqueous extract of soils, water soluble carbon (WSC) was determined by wet oxidation with $K_2Cr_2O_7$ and measurement of absorbance at 590 nm (Sims and Haby, 1971), and water soluble carbohydrates by the method of Brink et al. (1960). Humic C fraction and non-humic fraction C were determined in the $Na_4P_2O_7$ extract (1/10, solid–liquid), at pH 2 with HCl; C was determined by wet oxidation following the method of Sims and Haby (1971).

2.5. Microbiological analysis

Microbial biomass C was determined using a fumigation-extraction method (Vance et al., 1987). The basal respiration was determined in 50g of dry soil placed in sealed flasks, moistened to 60% of their water holding capacity, and incubated in the dark; the CO_2 released was measured by an IR CO_2 detector. The microbial biomass C to total organic C ratio (Hart et al., 1989) and the metabolic quotient (qCO_2) which was calculated by dividing the C of CO_2 released from sample in 1 h by the microbial biomass C content (Santruckova and Straskraba, 1991) were also obtained.

2.6. Enzyme activities

2.6.1. Dehydrogenase activity

Dehydrogenase activity was determined following the Skujins' method (1976) modified by García et al., (1997a). Dehydrogenase activity was assessed in 1 g of soil at 60% of its field capacity, 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 a mixture of 1:1.5 methanol by shaking vigorously for 1 min and filtration through a Whatman No 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

2.6.2. Catalase activity

Catalase activity was determined by measuring the O_2 consumed by $KMnO_4$ after addition of H_2O_2 to the samples (Rodriguez-Kavana and Truelove, 1982).

2.6.3. Urease activity

Two ml of pH 7 phosphate buffer and 0.5 ml of 6.4% urea were added to 0.5 g of soil which was incubated at 30°C for 90 min and the volume then made up to 10 ml with distilled water. The ammonium released after addition of 0.1 ml 10M NaOH was measured using an ammonium selective electrode (CRISON micro pH 2002). A control without urea was carried out for each sample.

Table 2
Chemical parameters in the plots studied[a]

	Without ORG	With ORG	Without ORG	With ORG
Treatments	pH		Electrical Conductivity (dS m^{-1})	
C	8.75 (0.31)	8.42(0.21)	92.1(3.9)	117.5 (4.2)
M	8.63 (0.39)	8.34 (0.14)	92.7 (5.2)	128.8 (2.5)
F	8.47 (0.22)	8.45 (0.19)	99.5 (4.5)	131.1 (4.5)
Treatments	Total N (g kg^{-1})		NO_3^-–N (mg kg^{-1})	
C	0.162 (0.005)	0.211(0.004)	2.53 (0.15)	4.46 (0.13)
M	0.171 (0.008)	0.242 (0.011)	3.66 (0.18)	4.78 (0.08)
F	.168 (0.017)	0.246 (0.004)	4.23 (0.22)	4.36 (0.12)
Treatments	NH_4^+–N (mg kg^{-1})		Total P (g kg^{-1})	
C	0.74 (0.12)	2.82 (0.24)	17.4 (0.71)	22.1 (0.78)
M	0.54 (0.06)	1.78 (0.18)	20.4 (0.59)	26.8 (0.89)
F	0.52 (0.10)	1.40 (0.16)	22.7 (1.81)	25.3 (0.22)
Treatments	Extractable P (mg kg^{-1})		Total K (mg kg^{-1})	
C	2700 (97)	5102 (194)	9.8 (0.12)	10.1 (0.33)
M	3491 (165)	5436 (267)	8.6 (0.26)	8.70 (0.69)
F	3060 (243)	4527 (196)	9.8 (0.43)	12.1 (1.09)
Treatments	Extractable K (mg kg^{-1})			
C	950 (50)	1550 (55)		
M	1200 (140)	2080 (216)		
F	1100 (58)	2130 (182)		

[a] C: Pines without mycorrhizal treatment. M: pines mycorrhized with *P. arhizus*. F: pines planted with forest soil (indirect mycorrhizal treatment). In parenthesis, standard deviation for each measure ($n = 4$).

2.6.4. Protease activity on N-α Benzoyl-L-argininamide (protease-BAA)

Two ml of phosphate buffer (pH 7) and 0.5 ml of 0.03M Nα-benzoyl-L-argininamide (BAA) substrate were added to 0.5 g of soil. The mixture was incubated at 37°C for 90 min and then diluted to 10 ml with distilled water. The ammonium released was measured in the same way as for urease (Nannipieri et al., 1980).

2.6.5. Phosphatase activity

Two ml of 0.1M maleate buffer (pH 6.5) and 0.5 ml of 0.115M *p*-nitrophenyl phosphate (PNPP) were added to 0.5 g of soil and incubated at 37°C for 90 min. The reaction was stopped by cooling to 2°C for 15 min. Then, 0.5 ml of 0.5M $CaCl_2$ and 2 ml of 0.5M 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 addition.

3. Results and discussion

3.1. Chemical parameters

3.1.1. Electrical conductivity and pH

Direct mycorrhizal inoculation did not influence soil pH, however, ORG amendment and F treatment decreased soil pH (Table 2). This can be due to the formation of organic acids of low molecular weight (acetic acid, propionic acid, butyric acid, etc.) during in situ mineralization of the added organic matter (Pascual et al., 1997).

In agreement with other authors (Guidi et al., 1982) the organic amendment raised soil electrical conductivity. This fact is important because Garcia and Hernandez (1996) showed that soil salinity (particularly salinity due to chloride) negatively affected microbial activity by decreasing soil basal respiration, dehydrogenase and hydrolase activities.

3.1.2. Nutrients

Although the organic wastes added for soil amendment cannot be considered as a typical fertiliser, it has been demonstrated that organic wastes act as slow release fertiliser with respect to some macro (N and P) and micronutrients (Ayuso et al., 1996). All factors (mycorrhizal treatments and organic wastes) increased the content of total N, NO_3^-–N, total P, and available P and K in the soil (Table 2). It should be noted that mycorrhizal treatments (M and F) increased the NO_3^-–N content while decreased the NH_4^+–N content (Table 2). This fact suggests that nitrification processes were favoured by these treatments. Plant mycorrhizal inoculation in nursery highly raised the content of available P (extracted with $NaHCO_3$) in soil. This could be expected taken into account that mycorrhizal fungi can actively solubilize P from non available substrates (Querejeta et al., 1998).

The ANOVA (Analysis of Variance) showed that organic amendment was more effective than direct or indirect mycorrhizal treatments in improving nutrient content (N, P) of revegetated soils (Table 3).

3.1.3. Carbon fractions

As it was expected, ORG addition increased all C fractions in soil (Tables 3 and 4). These C fractions found in the soils three years after the organic amendment, correspond both to the initial organic waste added to the soil which has not yet been mineralized and to the vegetal remains added to the soil during this period of three years. This vegetal contribution was higher in amended soils due to enhanced plant growth induced by this treatment. The addition of organic waste improved pine growth with respect to the same treatments without ORG addition by 30% and more (Roldan and Albaladejo, 1994; Querejeta et al., 1998).

Both mycorrhizal treatments (M and F), particularly direct mycorrhizal inoculation (M), increased the content of total organic C (Cmic) and humic substances (C fraction extracted with $Na_4P_2O_7$) in soil (Tables 3 and 4). This may be attributed to increased plant growth obtained with these treatments compared to the growth with the control (medium height in control tree: 350 cm; medium height in mycorrhized tree: 510 cm), suggesting also an enhanced C allocation to the soil (Roldan et al., 1996a).

The water soluble C fractions (WSC and carbohydrates) were the most favoured by the different treatments (Table 4). The WSC content represents the most labile fraction of soil organic matter because it is susceptible to microbial attack (Cook and Allan, 1992). These labile C fractions are energetic sources for soil microorganisms being closely related with soil microbial activity (Pascual et al., 1998). In addition, root exudates and the below ground plant mass contribute to the increase in these parameters (Campbell and Zentner, 1993). Mycorrhizal inoculation and organic amendment increased the content of water soluble carbohydrates, reflecting the soil microbial activity (de Luca and Keeney, 1993).

3.2. Microbial parameters

3.2.1. Microbial biomass C

In recent years, interest in knowing the soil microbial biomass C (Cmic) has increased due to the role played by microorganisms in the fertility of soil (Paul and Voroney, 1989). This parameter has been used as

Table 3
Two factorials ANOVA (organic fertilization and mycorrizhal treatment) for all parameters studied

Parameters	Organic wastes		Mycorrhizal treatment		Interaction	
	F values	Significance level	F values	Significance level	F values	Significance values
pH	18.41	< 0.01	4.08	0.06	2.35	0.14
Elec. conduct.	86.23	< 0.01	6.15	0.02	1.55	0.26
Total N	74.84	< 0.01	7.19	0.01	5.09	0.03
$N–NO_3^-$	63.58	< 0.01	22.74	< 0.01	16.65	< 0.01
$N–NH_4^+$	414.97	< 0.01	86.52	< 0.01	43.23	< 0.01
Total P	27.56	< 0.01	21.63	< 0.01	0.01	0.91
Extractable P	100.78	< 0.01	1.20	0.28	2.92	0.10
Total K	1.39	0.25	0.11	0.76	0.77	0.34
Extractable K	42.44	< 0.01	8.56	0.08	1.25	0.27
Total organic carbon	166.18	< 0.01	2.91	0.09	4.46	0.04
Extractable C	281.03	< 0.01	25.91	< 0.01	1.85	0.18
Non-Humic C	176.69	< 0.01	29.14	< 0.01	4.13	0.05
Humic C	10.71	0.04	0.03	0.8745	0.089	0.77
Water soluble carbon (WSC)	73.85	< 0.01	12.26	< 0.01	8.17	0.01
WSCarbohy	146.81	< 0.01	43.90	< 0.01	4.58	0.04
Biomass C	850.37	< 0.01	70.93	< 0.01	7.94	0.02
Basal Resp.	508.65	< 0.01	114.81	< 0.01	27.51	< 0.01
Dehy. Activity	27.77	< 0.01	3.53	0.07	0.15	0.70
Catalase Activity	45.34	< 0.01	9.73	< 0.01	0.27	0.61
Urease Activity	127.73	< 0.01	13.22	< 0.01	1.25	0.27
Protease Activity	65.38	< 0.01	21.17	< 0.01	1.09	0.31
Phosphatase Act.	156.52	< 0.01	59.65	< 0.01	17.24	< 0.01

an index for comparing natural and degraded systems (Ross et al., 1982). As shown in Tables 3 and 5, ORG was more efficient than mycorrhizal treatments (M or F) for enhancing soil microbial biomass. However, the highest values of Cmic were obtained with the combination of both treatments, direct mycorrhizal inoculation and organic amendment. The positive effect on microbial biomass observed in the amended soils is due to a direct (microbial growth in ORG, Pascual et al., 1997) and indirect effect (improvement of plant growth).

The Cmic-to-Corg ratio is considered by some authors as a good index of the changes in soil organic matter (Insam and Merschak, 1997). The higher values of this ratio were found in the organic amended soils (Table 5). This indicates that in spite of the time

Table 4
Carbon fractions in the studied plots[a,b]

	Without ORG	With ORG	Without ORG	With ORG
Treatments	TOC ($\mu g\ g^{-1}$)[c]		Extractable C ($\mu g\ g^{-1}$)	
C	10.8 (0.3)	20.1 (0.3)	1488 (76)	2703 (68)
M	12.5 (0.6)	22.0 (1.0)	1952 (48)	3274 (115)
F	13.0 (0.4)	19.0 (0.8)	1613 (38)	3149 (79)
Treatments	Non-humic acid C ($\mu g\ g^{-1}$)		Humic acid C ($\mu g\ g^{-1}$)	
C	898 (23)	1728 (95)	589 (91)	950 (88)
M	1145 (43)	2116 (89)	806 (71)	1153 (98)
F	1147 (26)	2434 (105)	465 (36)	715 (81)
Treatments	WSC ($\mu g\ g^{-1}$[d])		WSCarbohy (μg Glucose g^{-1}[e])	
C	45 (5.7)	104 (9.8)	25 (1.1)	43 (1.5)
M	76 (4.9)	112 (3.2)	35 (1.6)	55 (2.0)
F	69 (7.1)	124 (8.4)	37 (1.9)	51 (1.6)

[a] C: pines without mycorrhizal treatment. M: pines mycorrhized with *P. arhizus*. F: pines planted with forest soil (indirect mycorrhizal treatment).
[b] In parenthesis, standard deviation for each measure ($n = 4$).
[c] TOC: Total organic carbon.
[d] WSC: water soluble carbon.
[e] WSCarbohy: Water soluble carbohydrates.

elapsed since the ORG addition (three years), soil organic matter was still active. This organic matter is composed of some fractions derived from the organic matter added with the ORG and from vegetal remains derived from the growing plants. Both, direct mycorrhizal inoculation with *P arhizus* and the incorporation of forest soil in the planting hole also increased the Cmic-to-Corg ratio values (Table 5), indicating that mycorrhizal treatments favour soil organic matter dynamics. The most labile C fractions of the organic matter are particularly involved in these changes as can be deduced from the great differences observed between the values of the Cmic/WSC ratio in treated and control soils (Table 5).

3.2.2. Basal respiration

In afforestation programs, such as those carried out in semiarid areas, in which organic amendments are needed to improve soil fertility and its capacity for supporting plant development, soil basal respiration is a very important parameter. It has been widely used as an index of soil microbial activity (Nannipieri et al., 1990). The trend of basal respiration values was similar to that of Cmic (Table 5) confirming that the assayed treatments stimulate soil microbial activity. Both ORG addition and mycorrhizal treatments increased basal respiration significantly (Table 3).

3.2.3. Metabolic quotient

The metabolic quotient (qCO_2) is an index of ecosystem stress (Insam and Domsch, 1988; Anderson and Domsch, 1993). In general, three years after the starting of the experiment, the values of qCO_2 in amended soils were smaller than in unamended soils (Table 5). It is logical to infer that from the beginning the amended soils have high qCO_2values due to the organic matter incorporated with the organic waste (Pascual et al., 1998). As time elapses, organic matter becames more stable; the ecosystem matures and thus the qCO_2 decreases.

3.3. Enzymatic activities

Soil enzymes are biological catalysts of specific reactions and these reactions, in turn, depend on a variety of factors (Burns, 1978), such as the presence or absence of inhibitors, climate, type of amendment, crop type, etc. (Skujins, 1976). In general soil enzymes are good markers of soil fertility since they are involved in the cycling of the most important nutrients.

3.3.1. Oxydoreductases

Dehydrogenases are among the main endocellular enzymes and take part in metabolic reactions producing adenosine 5 triphosphate (ATP) through oxidation and fermentation of glucose. Dehydrogenase activity has been frequently used as indicator of soil microbial activity (Garcia et al., 1997b). The ORG treatment stimulated dehydrogenase activity (Tables 3 and 6). The incorporation of organic wastes influences soil enzymatic activities because the added material may contain endo or exocellular enzymes and may also stimulate soil microbial activity (Goyal et al., 1993). The highest values of dehydrogenase activity were obtained with the ORG + M treatment (Table 6). The development of microbial populations favoured by the root exudates of the mycorrhized plants can

Table 5
Biological parameters in the studied plots[a,b]

	Without ORG	With ORG	Without ORG	With ORG
Treatments	Cmic (μg g^{-1}soil[c])		Basal Respiration (μg C–CO_2 g^{-1}soil d^{-1})	
C	170 (17.2)	542 (30.2)	80 (7.9)	210 (16.1)
M	290 (23.7)	669 (29.8)	160 (14.1)	245 (14.9)
F	239 (24.4)	681 (34.9)	149 (14.3)	231 (15.3)
Treatments	qCO_2 (ng C–CO_2 μg Cmic h^{-1})Cmic × 100/Corg			
C	1.8	1.6	1.6	2.6
M	2.3	1.5	2.4	3.0
F	2.6	1.4	1.7	3.5
Treatments	Cmic × 100/WSC[d]			
C	2.6	5.2		
M	3.8	5.8		
F	3.4	5.5		

[a] C: pines without mycorrhizal treatment. M: pines mycorrhized with *P. arhizus*. F: pines planted with forest soil (indirect mycorrhizal treatment.
[b] In parenthesis, standard deviation for each measure ($n = 4$).
[c] Microbial biomass carbon: Cmic.
[d] WSC: Water Soluble Carbon.

Table 6
Enzyme activities in the studied plots[a,b]

	Without ORG	With ORG	Without ORG	With ORG
Treatments	Dehydrogenase activity (μg INTF g^{-1}soil)		Catalase activity (μM O_2 g^{-1} soil h^{-1})	
C	21.5 (0.7)	36.7 (2.4)	16.0 (0.5)	21.2 (0.6)
M	25.0 (2.2)	48.7 (2.2)	18.5 (0.7)	23.5 (0.7)
F	25.0 (2.2)	35.7 (1.2)	18.7 (0.8)	22.7 (1.6)
Treatments	Urease activity (μM NH_3 g^{-1} soil h^{-1})		Protease activity (μM NH_3 g^{-1}soil h^{-1})	
C	1.63(0.039)	2.95 (0.15)	0.21 (0.008)	0.65 (0.09)
M	2.00 (0.12)	3.19 (0.9)	0.35 (0.028)	1.06 (0.06)
F	2.25 (0.18)	3.22 (0.12)	0.51 (0.06)	0.93 (0.02)
Treatments	Phosphatase activity (μM PNP g^{-1}soil h^{-1})			
C	43.5 (2.62)	122 (8.1)		
M	82.0 (9.96)	217 (17.5)		
F	73.0 (6.2)	252 (7.5)		

[a] C: pines without mycorrhizal treatment. M: pines mycorrhized with *P. arhizus*. F: pines planted with forest soil (indirect mycorrhizal treatment).
[b] In parenthesis, standard deviation for each measure ($n = 4$).

also be responsible for the dehydrogenase activity stimulation. The high level of dehydrogenase activity in the soil treated with ORG or ORG + M suggests the availability of a high quantity of biodegradable substrates (which is in agreement with the higher content of labile C observed in these soils, Table 4) and hence an improvement of their microbial activity. The highest metabolic activity of microorganisms was observed when direct mycorrhizal inoculation and organic soil amendment techniques were combined.

Catalase is an oxydoreductase which catalyses the oxidation of organic compounds (amine, phenols) in presence of H_2O_2, and has been considered as an index of soil fertility (Rodriguez-Kavana and Truelove, 1982). As the proportion of recalcitrant compounds (lignine type compounds) in the organic matter increases, soil catalase activity decreases (Garcia et al., 1998). Organic amendment and particularly M-ORG and F-ORG treatments stimulated catalase activity (Table 6). This indicates that the organic matter of the treated soils has higher proportion of biodegradable compounds than the control soil.

3.3.2. Hydrolases

Ureases are enzymes involved in the N cycle that hydrolyse urea to CO_2 and NH_3. Urease activities were stimulated by both organic amendment and direct mycorrhizal inoculation (Tables 3 and 6). The demand of N by both, plants and soil microorganisms is, probably, responsible for the increase of urease activity.

Proteases catalyse the hydrolytic breakdown of proteins. This group of enzymes participate in the turnover of N in soils. The behaviour of this enzyme in the studied soils was quite similar to that of urease (Table 6). Thus, it is clear that functioning of N cycle is improved in the treated soils. The stimulation of these two enzymatic activities related with the N cycle suggests either that the treatment used does not include compounds toxic for this activity, or that the increase due to microbial growth and/or the addition of microbial cells or enzymes with the amendment counteract any inhibitory effect due to toxic compounds. Garcia et al. (1994) studied the influence of some toxic compounds (for example, heavy metals), contained in organic amendments such as municipal solid wastes on soil microbial activity in semiarid zones; the positive effect of the organic matter on biological soil quality counteracted the negative effect produced by these toxic compounds. The highest activities of these two enzymes involved in the *N* cycle were found when organic soil amendment was used along with direct mycorrhizal inoculation. As shown by Table 3, these two factors (organic amendment and direct mycorrhizal inoculation) significantly stimulated the activities of these N cycle enzymes.

Phosphatases are a group of enzymes that catalyse the hydrolysis of P organic compounds to phosphate. This is of great importance in revegetation programs since P is an essential element to plants. As indicated above, the assayed treatments increased both plant yields and microbial biomass C (Table 6). The demand of P by plants and soil microorganisms can be responsible for the stimulation of the synthesis of this enzyme (Garcia et al., 1997c). In addition, the processes related to degradation of organic matter may be followed through hydrolases such as phosphatase (Ceccanti and Garcia, 1994).

According to Rao and Tarafdar (1992), increases in phosphatase activity (as we have detected in the treated soils) indicate changes in the quantity and quality of soil phosphoryl substrates. Phosphatase inhibition, caused either by an excess of inorganic P (Nannipieri et al., 1990), or by heavy metals incorporated into the soil with the organic waste, was not observed. As

shown in Table 1, the heavy metal content of the used waste was below the limits admitted for organic fertilisers in Spain legislation (Real Decreto, 1988).

In conclusion, we can indicate that the conbined use of organic amendment and direct mycorrhizal inoculation is a useful revegetation technique to enhance the microbial activity in soils of semiarid regions.

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