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INCREASED PLANT GROWTH, NUTRIENT UPTAKE, AND SOIL ENZYMATIC ACTIVITIES IN A DESERTIFIED MEDITERRANEAN SOIL AMENDED WITH TREATED RESIDUES AND INOCULATED WITH NATIVE MYCORRHIZAL FUNGI AND A PLANT GROWTH-PROMOTING YEAST

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The effectiveness of microbial inoculants with different and interactive metabolic abilities, native arbuscular mycorrhizal (AM) fungi and a plant growth-promoting yeast (PGPY) *Yarowia lipolytica*, were assayed in a natural semiarid soil. The soil was either amended or was not amended with microbiologically treated residues, sugar beet (SB), or olive processing waste (OPW). *Dorycnium pentaphyllum*, used for revegetation purposes in semiarid soils, was selected as the test plant. The effects of microbial inoculations and/or OPW and SB amendments were evaluated in terms of plant growth and P nutrition. β-glucosidase and phosphatase activities in the rhizosphere, and other soil characteristics such as aggregate stability, were determined as well. The supply of treated OPW, SB, or both alleviated limiting P conditions in the soil, and inoculations of *Y. lipolytica* or native AM fungi increased plant P acquisition to the highest extent in residue-amended soils. Autochthonous mycorrhizal populations and native *Rhizobium* sp. were not able to colonize roots of *Dorycnium* plants growing in natural soil, but inoculants and the amendments (OPW or SB) applied enhanced the formation of such symbiotic structures. Root AM colonization depended on the AM inoculum applied (enriched population of native AM fungi), but this value was increased considerably by the amendments and by *Y. lipolytica* inoculation. The low nodulation found in this degraded soil was also increased by OPW and/or SB and by microbial inoculations. The application of organic amendments to the soil increased β-glucosidase and phosphatase activities as well as aggregate stability, water-soluble C, and water-soluble carbohydrates. These values were also altered by the microbial inoculants. Treatments increased the biodiversity of AM populations in this arid soil. Application of biologically treated agrowastes was able to increase soil microbial activity and plant development, which are highly depressed in many stressed areas. Thus, these treatments can be used as a valuable strategy in desertified areas. (Soil Science 2004;169:260–270)

Key Words: Desertification, mycorrhizal fungi, soil enzymes, soil phosphorus

STRESSED arid areas may be the result of progressive degradation of both vegetation cover (species diversity) and soil quality, both of which involve soil structure, nutrient availability, and microbial activity (Barea and Jeffries, 1995). Arbuscular mycorrhizal (AM) fungi constitute an important symbiotic association, not only for ameliorating plant nutrition but for enhancing water-stress tolerance (Ruiz-Lozano et al., 1995a, b, 1996) and other soil physiochemical character-

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istics as well (Degens et al., 1996). It is also believed that AM symbiosis plays an important role in soil structure and stability (Caravaca et al., 2002a, b; Wright and Upadhyaya, 1998). Thus, AM symbiosis, which contributes to better soil aggregate formation and plant nutrient acquisition, may be of particular importance in reclamation of desertified ecosystems (Azcón and Barea 1997; Caravaca et al., 2002a and b; Herrera et al., 1993a and b; Requena et al., 1996a and b)

The most effective strategy for reclaiming degraded semiarid areas is the establishment of autochthonous plant species. The degradation of lignocellulasic agrowastes by biological processes provides the organic compounds required as nutrient and energy sources for microbial survival under limited conditions. The use of rock phosphate (RP) as an inexpensive and available fertilizer is also recommended in sustainable systems, but RP solubilization rarely occurs in nonacidic soils. Thus, microorganisms able to excrete organic acids have been used successfully on agrowaste media supplied with RP as amendments for improving soil conditions and plant nutrition (Rodríguez et al., 1999).

When oil is extracted from the olive fruit, large amounts of dry olive cake waste (OPW) are produced. Such material, because of its a lignocellulosic composition, can be used, after biotransformation processes, as an organic amendment. Another very suitable approach is rock phosphate (RP) solubilization by acid-producing microorganisms such as *Aspergillus niger* grown on agro-industrial residues (Vassilev et al., 1995, 1996). Biotechnological schemes based on microbial treatment of agro-industrial wastes, such as SB or OPW residues, and RP solubilization can be used in sustainable low-input systems. But the importance of microbial activities is not always obvious in the soil/plant system inasmuch as they are modified by microbial interactions in the rhizosphere, soil and plant characteristics, and the quantity/quality of the amended product (Rodríguez et al., 1999). These factors may induce positive or negative effects on rhizosphere microorganisms and/or plant growth.

Phosphorus mineral fertilizer, normally used in agricultural practices, may be replaced by these treated compounds for plant P nutrition. Nevertheless, organic amendments, in addition to increasing soil properties, are able to enhance the capacity of the natural soil to initiate AM symbiosis (Mäder et al., 2000a and b).

The use of these treated agrowastes creates changes in mineral nutrients and soil physiochemical characteristics (Li et al., 1997). Moreover, the breakdown of such materials provides simple sugars that can be used as energy sources for heterotrophic microorganisms, which require such compounds for growth and metabolic activities (Bowen and Rovira, 1999). The effects of these treated products, assayed as amendments in sterilized agricultural soils, on plant growth and nutrition were maximized when applied with beneficial microorganisms (Rodríguez et al., 1999; Vassilev et al., 1995, 1996).

In previous studies by our group, sugar beet (SB) waste and olive processing waste (OPW), treated with *Aspergillus niger* and supplemented with rock phosphate (RP), were used in agricultural soil microcosm experiments because they are convenient materials for a low-input system, particularly in interaction with rhizobial strains and AM fungi (Rodríguez et al., 1999; Vassilev et al., 1995, 1996, 1997a and b, 1998).

In a logical next step for evaluation of the practical application of these treated compounds, we determined their effectiveness in a natural soil from a Mediterranean arid and degraded zone using an autochthonous legume plant species belonging to the natural succession in the area. Moreover, we considered it to be of particular interest to test the influence of amendments and/or microbial inoculations not only on plant growth and nutrition but also on the biological and physical soil properties related to soil fertility.

Therefore, in the present study, we evaluated the effect of these fermented organic amendments on natural degraded soil in relation to a reclamation strategy for revegetation of desertified areas. Such degraded soils are convenient model soils for such studies because their organic matter content is very low and, as there is no inherent soil structure, the polysaccharide components of the agrowastes are able to stabilize the soil structure. Soil enzyme activities are responsible for changes in soil management that increase soil productivity. Such activities provide knowledge of the rate and extent of the recovery of fertility (Caravaca et al., 2002c). *Dorycnium pentaphyllum*, a small, woody, N_2-fixing legume of the shrubland community, currently thrives in such desertified natural systems, but improvement of its performance is still needed. Therefore, it was used here as a test plant.

The effectiveness of amendments (OPW or SB treated with *Aspergillus niger* and supplemented with RP) was assayed, in the presence or absence of autochthonous AM fungi and/or *Yarowia lipolytica* inoculum, using *Dorycnium* plants grow-

ing in pots with natural degraded soil. *Yarowia lipolytica* was selected as an interesting microorganism since in previous studies it was shown to have plant growth-promoting (PGP) abilities in arid systems (Vassileva et al., 2000). The mixture of different microbial groups possessing different characteristics was assayed to determine interactive benefits for plant growth. Moreover, in this study we evaluated the influence of organic amendments and/or microbial inocula in the biological (enzymatic activities and biodiversity of AM populations) and physical (aggregate stability) soil properties related to soil fertility.

MATERIALS AND METHODS

Experimental Design

The experiment consisted of a two-factor randomized complete block design with the following factors: (i) microbial treatment, including assays with two micoorganisms (*Yarowia lipolytica*, AM fungi, or both) and one noninoculated control treatment; and (ii) the addition or lack of treated agrowaste (SB, OPW, or SB + OPW). Each treatment was repeated seven times.

Fermentation Process

An acid-producing filamentous fungus, *Aspergillus niger* NB2, used throughout this study, was maintained on potato-dextrose agar slants.

Sugar beet waste (SB) and dry olive cake (OPW) characterics were: cellulose (29% (SB) and 18% (OPW)), hemicellulose (23% (SB) and 16% (OPW)), and lignin (5% (SB) and 26% (OPW)); %organic matter (88 (SB) and 74.2 (OPW)); %C (51.1 (SB) and 43.1 (OPW)); %N (1.73 (SB) and 1.64 (OPW)); $\mu g\ g^{-1}$ P (51.1 (SB) and 8.7 (OPW)); and pH (6.4 (SB) and 8.3 (OPW)). The agrowastes were ground to 2-mm fragments in an electrical grinder. Portions of 15 g of each solid substrate and a 1:1 mixture (w/w) of them were mixed in 250-mL Erlenmeyer flasks with 40 mL of Czapeck DOX solution (0.01g L^{-1}, $FeSO_4 \cdot 7H_2O$, 0.5g L^{-1} Mg $SO_4 \cdot 7H_2O$; 0.5 gL^{-1} KCl; 3 g L^{-1} $NaNO_3$; 1.0 g L^{-1} K_2HPO_4 and 30.0 g L^{-1} sucrose) at pH 7.3.

Rock phosphate, at a concentration of 0.75 g/flask, was added to all treatments. After sterilization at 120 °C/30 min, each flask was inoculated with 3 mL of *A. niger* spore suspension (1.2×10^6 spores mL^{-1}).

The fermentation process was performed at 30 °C, for 20 days (previously defined as the best conditions for optimizing characteristics of the end products, Rodríguez et al., 1999).

Soil-Plant Experiment

The experiment consisted of four treatments based on soil amendment: Control (soil without amendment) and SB+RP, OPW+RP, and (SB+OPW) + RP, preincubated with *A. niger*.

The agro-industrial residues were mixed with desertified natural Mediterranean soil-sand mixture (5/2, v/v) at a rate of 3% per pot and left to equilibrate for 4 weeks at room temperature. The soil used was the top 0–20 cm of Murcia province (Spain) soil, with a pH of 7.80, and containing 1.36 $\mu g\ P\ g^{-1}$ (Olsen et al., 1954), organic carbon 0.94%, total N 0.22%, and E.C. 1.55. Soils were not sterilized.

Dorycnium penthaphylum was used as the test plant. Two-week-old seedlings, obtained from surface sterilized seeds grown in seed pots, were transplanted to 500-mL capacity pots (one per pot). They either were or were not inoculated with a mixture of autochthonous arbuscular mycorrhizal fungi isolates from Murcia soil: *Glomus mosseae, G. microagregatum, G. constrictum, G. albidum, G. sp, G. claroideum,* and *G. coronatum*.

This AM inoculum was selected as most effective in a previous assay with *Dorycnium* plants (unpublished results). This AM inoculum (5 g) consisted of spores, mycelium, and mycorrhizal root fragments and was introduced to each pot by inoculation at the bottom of a 5-cm-deep hole below the seedlings where the plants were transplanted. The P-solubilizing microorganism *Yarrowia lipolytica* was similarly introduced, or not, into soil-plant systems at a rate of 1 mL per pot (1.7×10^6 cfu mL^{-1}). This yeast had been maintained on potato dextrose agar and was grown in a medium described previously (Vassileva et al., 2000).

Seven replicate pots were prepared for each treatment. Plants were grown in a controlled environmental chamber at 50% relative humidity, day and night temperatures of 27 °C and 18 °C, respectively, and a photo period of 14 h. Photosynthetic photon flux density (PPFD) was 503 $\mu mol/m^2/s$, as measured with a lightmeter (LICOR, model LI-188B). Water was weighed daily and supplied as needed to maintain the required water level.

Plants were harvested after 2 months of growth. At harvest, the root system was separated from the shoot, and dry weights were determined for the shoot and root tissues.

Physical, Chemical, Biological, and Biochemical Analyses

Plant shoots were weighed and dried in a forced draught oven at 70 °C for 1 day and then

ground in a Wiley Mill to pass a 0.5-mm mesh. Shoot P content was determined by the molybdo-vanadote method described by Lachica et al. (1973).

Roots were washed carefully. The percentage of root length colonized by mycorrhizal fungi was estimated by visual observation (40× magnification) of mycorrhizal infection, after clearing washed roots in 10% KOH and staining with 0.05% Trypan blue in lactophenol (v/v), according to Phillips and Hayman (1970)

In aqueous extracts of rhizosphere soil, 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). Water soluble carbohydrates were determined by the method of Brink et al. (1960).

Acid phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 *M*) as the substrate. Two milliliters of 0.5 *M* sodium acetate buffer adjusted to pH 5.5 using acetic acid (Naseby and Lynch 1997) and 0.5 mL of substrate were added to 0.5 g of 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 2287 g for 5 min. The *p*-nitrophenol (PNP) formed was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner 1969). Controls were analyzed in the same way, except that the substrate was added before the $CaCl_2$ and NaOH.

β-glucosidase was determined using p-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 *M*; Masciandaro et al., 1994) as substrate. This assay is also based on the release and detection of PNP. Two milliliters of 0.1 *M* maleate buffer (pH 6.5) and 0.5 mL of substrate were added to 0.5 g of sample and incubated at 37 °C for 90 min. The reaction was stopped with tris-hydroxymethyl aminomethane (THAM) according to Tabatabai (1982). The amount of PNP was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969).

The percentage of stable aggregates was determined by the method of 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 soil remaining on the sieve was put into a previously weighed capsule (T), dried at 105 °C, and weighed (P1). The soil was then 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 comprised 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). The four soil samples of each treatment were analyzed in triplicate for percentage of stable aggregates.

The diversity of AM fungi present in the soil was calculated using the spore counts in the soil samples, following a morphological identification. For that purpose, the Shannon-Weaver diversity index (Shannon and Weaver 1949) was used. This index is calculated as $H^1\ Xi = \sum (Xi/Xo)\ Log\ 2\ (Xi/Xo)$, where Xi is the spore numbers of each individual specie and Xo is the total spore populations.

RESULTS

Microbial inoculations of *Y. lipolytica* or AM fungi did not increase shoot P concentration in soil treated with SB or OPW, but it did affect %P in the mixture of both agrowastes positively (Table 1). However, plant P content was increased considerably by the amendments applied, particularly SB. Inoculation with *Y. lipolytica* and/or AM fungi was effective in increasing plant P content, but this effect was observed only in amended soils. The supply of OPW or SB or both associated with microbial inoculum alleviated and compensated for P-limiting conditions and played an important role in the uptake of this element by *Dorycnium* plants (Table 1).

Mycorrhizal colonization by inoculated autochthonous endophytes positively affected the P content but the mycorrhizal effect on P acquisition by *Dorycnium* plants was more important in residues-treated soil and changed according to the soil amendment applied. In fact, mycorrhizal-colonized plants increased P uptake by 120% (unamended soil), 209% (OPW amended soil), 140% (SB amended soil), or 179% (OPW + SB), respectively. The compatibility of *A. niger* treated amendments and AM fungi affected the colonizing ability of AM inoculum. Dual inoculations were less effective than single inoculation in increasing P content in plants (Table 1).

The autochthonous mycorrhizal populations were not able to colonize roots of *Dorycnium* plants growing on noninoculated control natural soil. For AM root colonization, the extra application of native AM inoculum was required, but there were differences in the ability of the AM inoculum to colonize roots, depending on the amendment ap-

TABLE 1

Phosphorus concentration (%) and content (mg) in *Dorycnium* sp. plants inoculated with *Yarowia lipolytica* and/or AM fungi in soils amended or unamended with dry olive cake (OPW), sugar beet (SB), or both (OPW + SB) treated agrowastes

	Microbial treatments							
Soil	Control		*Y. lipolytica*		AM fungi		*Y. lipolytica* + AM fungi	
amendments	% P	P (mg)	% P	P (mg)	% P	P (mg)	% P	P (mg)
Control	0.13 e	0.15 bc	0.09 b	0.10 ab	0.21 g	0.18 cd	0.10 c	0.08 a
OPW	0.09 b	0.22 cd	0.10 cd	0.36 ef	0.11 cd	0.46 gh	0.07 a	0.23 d
SB	0.22 i	2.38 k	0.21 gh	2.72 l	0.22 hi	3.33 m	0.14 e	1.86 j
OPW + SB	0.11 d	0.34 e	0.18 f	0.52 h	0.18 f	0.61 i	0.11 d	0.41 fg

Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

plied. Infectivity was greatest with OPW or SB and lowest in the absence of amendment. The percentage of plant infectivity was highest with dual *Y. lipolytica* plus AM fungi inoculation in OPW- or SB- amended soils (Table 2).

Native *Rhizobium* strains also did not produce nodules in nonAM-inoculated or nonamended soil (Table 2). The effect of native AM fungi inoculation on nodule formation and, consequently, N_2-fixation was strongest in SB-amended soil. The inoculation with *Y. lipolytica* favored nodule formation, particularly with OPW + SB or when associated with AM inoculum in OPW-amended soil (Table 2).

The β-glucosidase activity has been used as an index of carbohydrate transformation. It shows that the organic amendments used significantly increase this hydrolytic activity in rhizosphere soil, particularly with SB (an increase of 236% over nonamended soil). *Y. lipolytica* and AM inoculum produce the strongest response on β-glucosidase activity in soil amended with OPW (Table 3).

Phosphatase activity (Table 4) was higher in the rhizosphere of amended soil compared with nonamended soil (311% increase in SB-added soil). SB was the organic residue that increased phosphatase activity to the greatest, except in mycorrhizal-inoculated plants. *Y. lipolytica* did not affect this hydrolase activity.

Quantities of water-soluble carbohydrate-C (Table 5) were highest in OPW-amended soils, but all of the organic amendments assayed increased water-soluble carbohydrate-C as well as water-soluble C in the rhizosphere soil (Table 6). However, microbial inoculations did not increase, or even decreased, both values in the rhizosphere (Table 5 and Table 6).

The application of organic residues significantly increased aggregate stability in the rhizosphere. *Y. lipolytica* did not alter the values, and inoculation of AM fungi was more effective in nonamended soil. AM fungi inoculation was as effective as organic amendments at increasing this parameter (Table 7).

As with the other parameters measured, the biodiversity of the AM population increased in all amendment treatments. *Y. lipolytica* had a greater effect than AM inoculation, with respect to increasing the biodiversity of the mycorrhizal

TABLE 2

AM colonization (percentage of root length AM colonized) and nodule formation in *Dorycnium* sp. plants inoculated with *Yarowia lipolytica* and/or AM fungi in soils amended or unamended with dry olive cake (OPW), sugar beet (SB), or both (OPW + SB) treated agrowastes

	Microbial treatments							
Soil	Control		*Y. lipolytica*		AM fungi		AM fungi + *Y. lipolytica*	
amendments	% AM	Nodule	% AM	Nodule	% AM	Nodule	% AM	Nodule
Control	0.0 f	0.3 c	0.0 f	0.0 d	10.0 d	0.0 d	8.0 d	0.0 d
OPW	0.0 f	3.0 b	1.0 e	5.0 b	45.0 b	4.0 b	56.0 a	17.0 a
SB	0.0 f	0.7 c	1.0 e	4.0 b	50.0 ab	18.0 a	65.0 a	0.3 c
OPW + SB	0.0 f	0.0 d	1.0 e	17.0 a	24.0 c	0.0 d	38.0 b	0.0 d

Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

TABLE 3

β-glucosidase activity (μg PNF $g^{-1}h^{-1}$) in rhizosphere of *Dorycnium* sp. plants inoculated with *Yarowia lipolytica* and/or AM fungi in soils amended or unamended with dry olive cake (OPW), sugar beet (SB), or both (OPW + SB) treated agrowastes

	Microbial treatments			
Soil amendments	Control	*Y. lipolytica*	AM fungi	AM fungi + *Y. lipolytica*
Control	46.97 a	40.5 a	49.74 ab	57.22 abc
OPW	75.67 cde	102.62 fg	104.64 fg	90.74 def
SB	111.45 g	98.65 fg	72.54 cd	93.85 efg
OPW + SB	74.17 cde	67.98 bc	68.13 bc	75.13 cde

Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

TABLE 4

Phosphatase activity (μg PNF $g^{-1}h^{-1}$) *Dorycnium* sp. plants inoculated with *Yarowia lipolytica* and/or AM fungi in soils amended or unamended with dry olive cake (OPW), sugar beet (SB), or both (OPW + SB) treated agrowastes

	Microbial treatments			
Soil amendments	Control	*Y. lipolytica*	AM fungi	AM fungi + *Y. lipolytica*
Control	21.82 a	22.11 a	23.87 a	29.81 ab
OPW	40.04 bc	48.44 cd	52.41 cde	41.09 bc
SB	67.97 f	72.01 f	39.03 bc	60.75 def
OPW + SB	61.24 def	63.73 ef	46.92 cd	58.09 def

Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

TABLE 5

Water-soluble carbohydrates (μg C g^{-1}) in rhizosphere in *Dorycnium* sp. plants inoculated with *Yarowia lipolytica* and/or AM fungi in soils amended or unamended with dry olive cake (OPW), sugar beet (SB), or both (OPW + SB) treated agrowastes

	Microbial treatments			
Soil amendments	Control	*Y. lipolytica*	AM fungi	AM fungi + *Y. lipolytica*
Control	69 ab	59 a	77 ab	79 b
OPW	169 i	161 i	161 i	134 fg
SB	137 fgh	119 def	107 cd	99 c
OPW + SB	155 hi	141 gh	133 efg	115 cde

Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

TABLE 6

Water-soluble C (μg g^{-1}) in rhizosphere of *Dorycnium* sp. plants inoculated with *Yarowia lipolytica* and/or AM fungi in soils amended or unamended with dry olive cake (OPW), sugar beet (SB), or both (OPW + SB) treated agrowastes

	Microbial treatments			
Soil amendments	Control	*Y. lipolytica*	AM fungi	AM fungi + *Y. lipolytica*
Control	12 abc	11 ab	13 abc	11 ab
OPW	22 defg	18 cde	23 efg	11 ab
SB	25 ghi	16 bcd	29 hi	19 def
OPW + SB	26 ghi	31 i	24 fgh	24 fgh

Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

TABLE 7

Aggregate stability (percentage of) in rhizosphere of *Dorycnium* sp. plants inoculated with *Yarowia lipolytica* and/or AM fungi in soils amended or unamended with dry olive cake (OPW), sugar beet (SB), or both (OPW + SB) treated agrowastes

Soil amendments	Microbial treatments			
	Control	*Y. lipolytica*	AM fungi	AM fungi + *Y. lipolytica*
Control	7.6 a	7.8 a	12.1 bc	10.1 ab
OPW	13.7 cd	12.0 bc	13.8 cd	14.0 cd
SB	13.8 cd	12.2 bc	10.8 b	14.8 de
OPW + SB	15.9 def	18.3 f	17.7 f	17.1 ef

Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

spores population (Table 8). The greatest increase over control was found in OPW-treated soil, in which the inoculation of *Y. lipolytica* increased AM biodiversity by 187%.

DISCUSSION

This study demonstrates that the P demand of plants can be satisfied by the activity of microorganisms, such as *A. niger*, that are able to solubilize RP through organic acid production on agrowaste materials. The relevance of microorganisms in the mineralization of organic matter from agrowaste compounds has been recognized previously (Vassilev et al., 1995, 1996, 1997a and b). The impact of these amendments on plant P nutrition and soil enzyme activities is particularly evident when associated with beneficial inoculants such as AM fungi and/or *Y. lipolytica*, which increase the positive effect of these treated compounds.

The substantially greater P nutrition achieved in mycorrhizal plants in SB-amended soil represent an important contribution to the re-establishment of vegetation and the protection of soil against erosion, which is critical under Mediterranean climatic conditions (Herrera et al., 1993a and b; Requena et al., 1996a and b).

The maximum P uptake in mycorrhizal and nonmycorrhizal *Dorycnium* plants occurred when treated SB waste was applied to the soil/plant system. These results agree well with the P content of this material before its introduction into the soil (Rodríguez et al., 1999). But plant P acquisition in amended soil increased by inocula application. The effect of microbial inoculation with respect to increasing P content in SB-amended soil was 114% (*Y. lipolytica*) or 140% (AM fungi). In the case of AM inoculation, the increased plant P acquisition for this treatment can be related to an increased level of AM colonization, but this was not true for *Y. lipolytica*. The rock-phosphate solubilizing ability of this yeast in the fermentation process on OPW or SB may account for the P results with these amendments. (Vassileva et al., 2000). On the other hand, phosphatase may play a significant role in P availability in the soil. Phosphatase activity was higher in the rhizosphere of SB-supplied plants.

García-Gil et al. (1999) reported that mycorrhizal inoculation decreased phosphatase activity by 43% compared with nonmycorrhizal plants. Phosphatase is an inducible enzyme. The results from our study did not show this effect.

Phosphatase activity, involved in the release of inorganic P from organically bound P, affects

TABLE 8

Biodiversity of AM population (Shannon W. index) in soil growing *Dorycnium* sp. plants inoculated with *Yarowia lipolytica* and/or AM fungi in soils amended or unamended with dry olive cake (OPW), sugar beet (SB), or both (OPW + SB) treated agrowastes

Soil amendments	Microbial treatments			
	Control	*Y. lipolytica*	AM fungi	AM fungi + *Y. lipolytica*
Control	0.94 a	1.88 bc	1.20 ab	1.90 bc
OPW	1.90 bc	2.05 c	1.76 bc	1.41 abc
SB	1.64 abc	1.72 bc	1.98 bc	1.68 abc
OPW + SB	1.78 bc	1.49 abc	1.70 abc	1.88 bc

Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

the rate of phosphorus cycling. P-cycle enzyme activities are commonly inversely related to soil P availability (Tadano et al., 1993). Our results here unexpectedly show high phosphatase activity in SB-amended soil. It was not, however, significantly increased by AM-colonization, as reported previously (Li et al., 1997; Tarafdar and Claassen, 1988; Tarafdar and Marschner, 1994), and it was decreased by AM colonization in SB medium, the most efficient treatment for plant P-uptake. Phosphorus increasing through breakdown of the residues, particularly SB, and rock-phosphate by the microbial treatments is very important to improving soil productivity (Aristovskaya, 1988).

The results from this study indicate that the application of treated agrowastes to degraded lands is an appropriate practice because of the high content of organic and inorganic products in these materials. The treated amendments used here seem to be useful tools for increasing the microbial status (as symbiotic structures developed in plant roots) and availabilities of nutrients in soils. Therefore, the application of these treated amendments to this degraded and nutrient-limited soil was critical for the success of plant development, the formation of symbiotic structures (nodulation and AM colonization), and, of course, for revegetation purposes.

The use of amendments to improve symbiotic development is of great importance for legume growth in poor and desertified soils.

Autochthonous AM fungi and *Rhizobium* sp. did not produce colonization in noninoculated and nonresidue-amended soil. The AM inoculation induced AM colonization as well as nodule formation, but these effects were strongest in soil amended with SB or OPW. *Y. lipolytica* inoculation favored nodule formation in amended soil and only enhanced AM colonization in SB- or OPW-treated plants.

Under the limiting P conditions found in this soil, biological processes are the driving forces determining P availability for plant growth. The use of enzymatic activities as a microbial index of changes in soil biological properties and fertility is widely recognized (Caravaca et al., 2002c; Dormaar et al., 1984).

Soil enzyme activity is responsive to changes in soil management (Speir, 1977), increasing soil productivity. Moreover, in degraded soils, enzyme activities provide knowledge of the rate and extent of recovery of fertility. Based on our results, the organic matter applied provided a source of substrates and energy for enzyme-producing microorganisms. Treated OPW and SB, used here as amendments, enhanced soil enzymes such as β-glucosidase and phosphatase by increasing microbial and plant development. Therefore, the results of this study indicate that an alternative strategy for coping with environmental deficiencies in the degraded soil is the addition of these treated amendments (OPW or SB), particularly when associated with appropriate microbial inocula such as adapted AM fungi or *Y. lipolytica*.

Soil enzymes, mediators of biochemical transformations in soil, are greatly affected by organic compounds, nutrients, and microbial inoculations. Glucosidase activities are widely distributed in nature, and their hydrolysis products are important sources of energy for soil microorganisms. Ready available carbon is the most limiting substrate to microbial growth in soil. Thus, the rhizosphere is recognized as the zone with the greatest microbial activity, because of the root exudates (Lynch and Whipps, 1990). Here the increased activity produced by SB or OPW incorporation indicates an extra C supply but, in this study, the highest β-glucosidase activity was related to the amendment (SB or OPW) and to the inocula applied in OPW-amended soil (*Y. lipolytica* or AM fungi). These values did not agree well with degree of microbial (symbiotic) development.

There has been increasing interest in using these biological indices to assess soil characteristics that impact on soil fertility and soil quality. Measurements of soil enzymes, such as β-glucosidase and phosphatase, help to show the potential of an experimental soil to degrade substrates and also indicate how efficiently a soil can carry out important steps in nutrient cycling.

As the results show, AM infection and nodulation in degraded soil are extremely low, as reported also by Jasper et al. (1991). The effectiveness of the amendments used here, with respect to stimulating AM colonization and nodulation is a very important consideration for soil recovery in degraded areas. Thus, restoration practices are needed to improve these symbiotic associations. The treatments applied also improve the biodiversity of the AM population, and this is very important ecologically.

Aggregate stability, water-soluble C, and water-soluble carbohydrate-C were increased more by amendments than by microbial inoculations. Soil β-glucosidase and soil phosphatase activities also reacted more sensitively to the amendments than to the microbial inocula applied. Nevertheless, the effect of inoculants on

these enzymatic activities were expressed only in residue-amended soils.

The dual amendment applications (OPW+SB) were not an improvement over single applications for some values, such as β-glucosidase and phosphatase activities, mycorrhizal colonization and nodulation, and biodiversity of AM propagules. Nevertheless, aggregate stability was increased by the OPW+SB mixture in all of the treatments.

Aggregate stability is an important criterion of a healthy, managed ecosystem (Miller and Jastrow, 1992a; Tisdall and Oades, 1980). Studies indicate that AM fungi stabilize aggregates (Caravaca et al., 2002a and b; Miller and Jastrow 1992b; Wright and Upadhyaya, 1996, 1998). This effect was found here only in mycorrhizal, nonamended soil.

In this managed soil, an improvement in soil biological activity yielded an increase in the diversity and activity of the mycorrhizal community. However, relationships between microbial activity and diversity of disturbed ecosystems are still poorly understood (Kennedy and Smith, 1995). As in the present study, substrate availability was the most important factor affecting the activity of soil microorganisms.

Inoculation with AM fungi and other soil microorganisms, such as *Y. lipolytica*, can affect the microbial communities in the rhizosphere in both qualitative and quantitative ways. Changes in enzyme activities are influenced by microbial populations; thus, enzyme activity can be used as an indicator of microbial functioning in soil.

In Mediterranean areas, such as SE Spain, degradation and desertification processes have led to a decrease in soil fertility, but amendment with treated agrowaste residues can increase soil biological status and, thus, its productive capacity, as has been demonstrated here using plant P acquisition and indicators of symbiosis as representative parameters.

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