Microbial Ecology

Establishment of Two Ectomycorrhizal Shrub Species in a Semiarid Site after *in Situ* Amendment with Sugar Beet, Rock Phosphate, and *Aspergillus niger*

F. Caravaca[1], M.M. Alguacil[1], R. Azcón[2], J. Parladé[3], P. Torres[4] and A. Roldán[1]

(1) CSIC-Centro de Edafología y Biología Aplicada del Segura, Department of Soil and Water Conservation, P.O. Box 164, Campus de Espinardo, 30100 Murcia, Spain
(2) CSIC-Estación Experimental del Zaidín, Microbiology Department, Profesor Albareda, 1. 18008-Granada, Spain
(3) IRTA—Centre de Cabrils, Department of Plant Pathology, Ctra. de Cabrils s/n, 08348-Cabrils, Barcelona, Spain
(4) Universidad Miguel Hernńndez de Elche, Department of Applied Biology, Avda. Ferrocarril, s/n, Edf. Laboratories—03202-Elche, Alicante, Spain

Received: 18 June 2003 / Accepted: 26 September 2003 / Online publication: 24 January 2005

Abstract

A field experiment was carried out to assess the effectiveness of the addition of sugar beet, rock phosphate, and *Aspergillus niger* directly into the planting hole, and the mycorrhizal inoculation of seedlings with *Scleroderma verrucosum*, for promotion of plant growth of *Cistus albidus* L. and *Quercus coccifera* L. and enhancement of soil physicochemical, biochemical, and biological properties, in a degraded semiarid Mediterranean area. One year after planting, the available phosphorus content in the amended soils of both species was about fourfold higher than in the nonamended soil. Amendment addition increased the aggregate stability of the rhizosphere of *C. albidus* (by 56% with respect to control soil) while the mycorrhizal inoculation increased only the aggregate stability of the rhizosphere of *Q. coccifera* (by 13% with respect to control soil). Biomass C content and enzyme activities (dehydrogenase, urease, protease-BAA, acid phosphatase, and β-glucosidase) of the rhizosphere of *C. albidus* were increased by amendment addition but not by mycorrhizal inoculation. Both treatments increased enzyme activities of the rhizosphere of *Q. coccifera*. The mycorrhizal inoculation of the seedlings with *S. verrucosum* was the most effective treatment for stimulating the growth of *C. albidus* (by 469% with respect to control plants) and *Q. coccifera* (by 74% with respect to control plants). The combined treatment, involving mycorrhizal inoculation of seedlings and addition of the amendment directly into soil, had no additive effect on the growth of either shrub species.

Correspondence to: F. Caravaca; E-mail: fcb@cebas.csic.es

Introduction

The establishment of a plant cover based on autochthonous plant species constitutes the most effective strategy for reclaiming degraded lands in Mediterranean semiarid areas and for restoring the characteristic biodiversity of such areas. Shrub species such as *Cistus albidus* L. and *Quercus coccifera* L., associated with other small woody plants, are characteristic of the plant communities in these semiarid ecosystems. In semiarid Mediterranean areas, the establishment of plants is made difficult by low soil fertility and the severe climate, characterized by low and irregular precipitation and frequent drought periods. Thus, it is necessary to apply methods which improve soil quality and the ability of the planted species to resist semiarid environmental conditions.

Mycorrhizas represent key ecological factors governing the cycles of major plant nutrients and have a significant influence on plant health and productivity [15, 32]. There is evidence that mycorrhizas help plants to thrive in arid conditions, by increasing the supply of P (particularly arbuscular mycorrhizas) and N, especially organic forms (particularly ectomycorrhizas) to the plant [30], improving soil aggregation in eroded soils [6], reducing water stress [3], and defending the seedlings against pathogenic agents. To date, several studies have reported that the mycorrhizal potential of degraded semiarid Mediterranean ecosystems is low [21]. Thus, to carry out successful reafforestation programs, it is necessary to apply mycorrhizal inoculation technologies which reinforce or replace mycorrhizal potential in these degraded areas. *C. albidus* and *Q. coccifera* are extremely important because they constitute a reservoir for ecto-

mycorrhizal fungi in the absence of host trees [8, 31, 40]. Although there are no direct studies concerning mycorrhizal symbiosis with *C. albidus*, ectomycorrhizas do occur in the congeners *C. ladanifer* [40] and *C. incanus* [45]. Recent studies have shown that mycorrhizal inoculation of *Q. coccifera* with *Pisolithus tinctorius* did not improve the seedling survival in a semiarid degraded steppe [20].

The use of rock phosphate (RP) as a fertilizer for P-deficient soils has received significant interest in recent years. Rock phosphates are natural, inexpensive, and available fertilizers, but their solubilization rarely occurs in nonacidic soils. The solubilization of rock phosphate by microorganisms able to excrete organic acids has been tested in fermentation conditions [33]. Some ectomycorrhizal fungi etch micropores in feldspar, horblende, and other soil minerals by release of organic acids, and then use and transport these nutrients to their mycorrhizal host plants. Some decomposer fungi such as lichen fungi may also attack rocks for their mineral nutrients, especially P [16]. The addition of microbiologically solubilized phosphate and microbiologically treated agrowastes to agricultural soils has been used for improving plant growth and nutrition [43, 44]. However, the effectiveness of rock phosphate solubilization on agrowastes media by microorganisms inoculated directly into the soil under field conditions is unclear because of the possible refixation of phosphate ions on their way to the root surface. In addition, no information is available on the use of such materials in revegetation programs.

The objectives of this study were (1) to determine the viability of using mycorrhizal *C. albidus* and *Q. coccifera* seedlings in soil revegetation programs in a semiarid Mediterranean area, and (2) to assess the effectiveness of the addition of sugar beet, rock phosphate, and *Aspergillus niger* directly into the planting hole with respect to promotion of plant growth and enhancement of soil properties.

Materials and Methods

Study Sites. The experimental area was located in Los Cuadros, in the Province of Murcia (southeast Spain) (coordinates: 1°05'W and 38°10'N). The climate is semiarid Mediterranean with an average annual rainfall of 300 mm and a mean annual temperature of 19.2°C; the potential evapotranspiration reaches 1000 mm y^{-1}. The soil used was a Typic Haplocalcid [37] developed from Quaternary sediments with a loam texture. The analytical characteristics of the soil are shown in Table 1.

Table 1. Chemical, biochemical, microbiological, and physical characteristics of the soil used in the experiment

pH (H_2O)	8.5 ± 0.0[a]
EC (1:5, μS cm^{-1})	225 ± 2
Texture	Loam
Total organic C (g kg^{-1})	10.3 ± 0.3
Total carbohydrates (μg g^{-1})	552 ± 20
Water soluble C (μg g^{-1})	100 ± 1
Water soluble carbohydrates (μg g^{-1})	8 ± 0
Total N (g kg^{-1})	0.95 ± 0.02
Available P (μg g^{-1})	7 ± 0
Extractable K (μg g^{-1})	222 ± 4
Microbial biomass C (μg g^{-1})	396 ± 11
Dehydrogenase (μg INTF g^{-1})	51 ± 1
Urease (μmol NH_3 g^{-1} h^{-1})	0.31± 0.03
Protease-BAA (μmol NH_3 g^{-1} h^{-1})	0.60 ± 0.04
Phosphatase (μmol PNP g^{-1} h^{-1})	0.28 ± 0.02
β-Glucosidase (μmol PNP g^{-1} h^{-1})	0.46 ± 0.01
Aggregate stability (%)	11.5 ± 0.4

[a]Mean ± standard error (n = 6).

Materials. The sugar beet (SB) residue selected was dried in a 60°C oven and then ground in an electrical grinder to 2-mm fragments. The SB is a lignocellulosic material characterised by cellulose, 29%; hemicellulose, 23%; lignin, 5%, total C, 55% and total N, 1.7%.

A strain of *Aspergillus niger* NB2 was used in this study. It had previously been selected as producing citric acid on complex substrates [42].

Rock phosphate (RP), i.e., fluorapatite from Morocco with 12.8% P (1 mm mesh), was also used in this study.

The plants used for the reafforestation experiment were *Cistus albidus* L. and *Quercus coccifera* L., which are low-growing shrubs reaching a height of 1.0 m and 3.0 m, respectively, and widely distributed in the Mediterranean area. They are also well adapted to water stress conditions [20] and, therefore, potentially could be used in the reafforestation of semiarid disturbed lands.

Mycorrhizal Inoculation of Seedlings. Fungal material was fresh *Scleroderma verrucosum* (Bull.:Pers.) Pers. (Scaly Earthball) fruitbodies collected under an *Abies alba* plantation at the Montseny range (northeast Spain). This fungus is a gasteromycete, and the inner hymenium of nondamaged fruitbodies is usually free of microbial contamination.

After using a portion of fruitbodies, the rest was kept at the herbarium of the DPV-IRTA, Barcelona (Spain). Spores were suspended in sterile tap water with two drops of Tween 20 as surfactant. Then, the spore suspension was mixed with autoclaved vermiculite (20 min, 120°C).

A potting substrate containing equal volumes of peat and vermiculite, autoclaved (60 min, 120°C), was used to fill containers of 160-mL capacity. Fungal inoculum was mixed with the potting substrate, at rates of 1:10 inoculum:substrate (v:v) at seed sowing time. The doses of

inoculum applied corresponded to 10^6 spores per container. *C. albidus* and *Q. coccifera* seedlings (inoculated and noninoculated) were grown for 8 months under nursery conditions without any fertilization treatment.

Experimental Design and Layout. This study was conducted in two independent experiments. We initiated a two-factor design (one per plant species) with five replicate blocks. The first factor was addition of SB+*A. niger*+RP to the soil, and the second, inoculation with *S. verrucosum*; plants were inoculated in the nursery before planting out. Four treatments were established: (1) seedlings without mycorrhizal treatment and soil without SB+*A. niger*+RP addition (control, C); (2) seedlings without mycorrhizal treatment and soil with SB+*A. niger*+RP addition (A); (3) seedlings inoculated with *S. verrucosum* and soil without SB+*A. niger*+RP addition (M); and (4) seedlings inoculated with *S. verrucosum* and soil with SB+*A. niger*+RP addition (AM). Two adjacent plots of 1000 m^2 were established on a flat surface. Planting holes 40 cm wide, 40 cm long, and 30 cm deep were manually dug. In early December 2001, 75 g of SB, 3.75 g of rock phosphate (RP), and 10 mL of a spore suspension of *A. niger* (1.2×10^7) were added to half of the holes (0–20 cm depth), corresponding with a rate of 3% by weight, following the randomized design. The seedlings (inoculated and noninoculated) were planted ≥1 m apart between holes, with 3 m between blocks. At least 32 seedlings per replication block were planted (eight plants × four treatments in each block).

Sampling Procedures. One year after planting, soil samples of each treatment and replication block were collected (20 soil samples per species and 40 soil samples in total). Each sample consisted of eight bulked subsamples (200 cm^3 soil cores), collected randomly at 0–20 cm in the rhizospheres of eight individual plants. The sampling was carried out in early December after the autumn rainy season, when the highest microbial activity would be expected [19]. At the same time, five plants of each plant species (one per block) and of each treatment were also harvested.

Plant Analyses. Basal stem diameters and heights of plants were measured with calipers and rules. Fresh and dry (105°C, 5 h) weights of shoots and roots were recorded. Plant tissues were ground before chemical analysis. The foliar concentrations of N, P, and K were determined after digestion in nitric–perchloric acid (5:3) for 6 h [29]. The foliar P was determined by colorimetry [23], foliar N was colorimetrically measured after Kjeldahl digestion [27], and foliar K was estimated by flame photometry [35].

To assess mycorrhizal colonization, methods described in Grand and Harvey [11] and Amaranthus and Perry [1] were followed. Roots were subsampled in three 2-cm cross-sections of the upper, middle, and lower root system. Root tips in these sections appearing mycorrhizal and active were counted, and the results were expressed as percentage of mycorrhizal short lateral roots.

Soil Physicochemical, Chemical, and Biochemical Analyses. Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous solution. Total nitrogen was determined by colorimetry after Kjeldahl digestion. In soil aqueous extracts, water-soluble carbon was determined by wet oxidation with $K_2\ Cr_2O_7$ and measurement of the absorbance at 590 nm [36]. Water-soluble carbohydrates and total carbohydrates were determined by the method of Brink et al. [5]. Available P, extracted with 0.5 M $NaHCO_3$, was determined by colorimetry according to Murphy and Riley [23]. Extractable (with ammonium acetate) K was determined by flame photometry.

Microbial biomass C was determined using a fumigation–extraction method [41]. Ten g of soil at 60% of its field capacity was fumigated in a 125-mL Erlenmeyer flask with purified $CHCl_3$ for 48 h. After removal of residual $CHC1_3$, 40 mL of 0.5 M K_2SO_4 solution was added and the sample was 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 was calculated as the difference between fumigated and nonfumigated samples.

Dehydrogenase activity was determined according to García et al. [10]. 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 (iodonitrotetrazolium formazan) formed was extracted with 10 mL of methanol by shaking vigorously for 1 min and filtering through a Whatman No. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

Urease and *N*-α-benzoyl-L-arginine amide (BAA) hydrolyzing protease activities were determined in 0.1 M phosphate buffer at pH 7; 1 M urea and 0.03 M BAA were used as substrates, respectively. Two mL of buffer and 0.5 mL of substrate were added to 0.5 g of sample, which was incubated at 30°C (for urease) or 39°C (for protease) for 90 min. Both activities were determined as the NH_4^+ released in the hydrolysis reaction [25].

Acid phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate. Two mL of 0.5 M sodium acetate buffer at pH 5.5 using acetic acid [26] 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

Table 2. Changes in physicochemical properties of rhizosphere soil of *C. albidus* and *Q. coccifera* in response to mycorrhizal inoculation and SB+*A. niger*+RP addition 1 year after planting ($n = 5$)

	pH (H_2O)	*EC* (1:5, $\mu S\ cm^{-1}$)	*Total N* ($g\ kg^{-1}$)	*Avail. P* ($\mu g\ g^{-1}$)	*Extract. K* ($\mu g\ g^{-1}$)	*Total CH* ($\mu g\ g^{-1}$)	*Water-soluble C* ($\mu g\ g^{-1}$)	*Water-soluble CH* ($\mu g\ g^{-1}$)	*Aggregate stability (%)*
C. albidus									
C	8.15 ± 0.01b	401 ± 12a	1.16 ± 0.05a	2 ± 0a	180 ± 7a	621 ± 36a	144 ± 13a	8 ± 1a	23.5 ± 0.5a
A	7.40 ± 0.05a	522 ± 17b	1.38 ± 0.02b	7 ± 0b	260 ± 12b	931 ± 40b	317 ± 4c	21 ± 2b	36.6 ± 3.0b
M	8.17 ± 0.01b	410 ± 4a	1.02 ± 0.02a	2 ± 0a	163 ± 5a	676 ± 21a	101 ± 2a	10 ± 1a	24.8 ± 1.5a
AM	7.65 ± 0.01a	467 ± 5b	1.52 ± 0.02c	5 ± 0b	240 ± 4b	1145 ± 36c	187 ± 7b	14 ± 1a	38.8 ± 2.3b
Q. coccifera									
C	8.18 ± 0.00b	358 ± 10a	1.00 ± 0.06a	1 ± 0a	178 ± 6	704 ± 3a	105 ± 2a	6 ± 0a	33.1 ± 0.5a
A	8.10 ± 0.01a	410 ± 8b	1.08 ± 0.00ab	4 ± 1b	180 ± 3	731 ± 2b	224 ± 19b	22 ± 1b	33.1 ± 1.5a
M	8.17 ± 0.01b	383 ± 15a	1.16 ± 0.03ab	1 ± 0a	240 ± 13	672 ± 14a	107 ± 1a	7 ± 0a	37.1 ± 0.9b
AM	8.00 ± 0.03a	437 ± 2b	1.37 ± 0.05b	3 ± 0b	182 ± 1	994 ± 20c	209 ± 4b	16 ± 2ab	37.8 ± 1.2b

C: Control; A: SB+*A. niger*+RP addition; M: mycorrhizal inoculation; AM: SB+*A. niger*+RP addition and mycorrhizal inoculation; CH: carbohydrates. Mean ± standard error. For each species, values in columns followed by the same letter do not differ significantly ($p < 0.05$) as determined by the LSD test.

was determined by spectrophotometry at 398 nm [39]. Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

β-Glucosidase was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M; [22]) as substrate. This assay is based on the release and detection of PNP. Two mL 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-hydroxymethylaminomethane (THAM) according to Tabatabai [38]. The amount of PNP was determined by spectrophotometry at 398 nm [39].

Physical Analysis. The percentage of stable aggregates was determined by the method described by Lax et al. [17]. A 4-g aliquot of sieved (0.2–4 mm) soil was placed on a small 0.250-mm sieve and wetted by spray. After 15 min the soil was subjected to an artificial rainfall of 150 mL with energy of 270 J m^{-2}. The remaining soil on the sieve was placed in a previously weighed capsule (T), dried at 105°C, and weighed (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 $(P1-P2) \times 100/(4-P2 + T)$.

Statistical Analysis. Aggregate stability and percentage colonization were arcsine-transformed, and the other parameters were log-transformed to compensate for variance heterogeneity before analysis of variance. The effects of SB+*A. niger*+RP addition and mycorrhizal inoculation, and their interactions, on measured variables were tested by a two-way analysis of variance and comparisons among means were made using the least significant difference (LSD) test, calculated at $p < 0.05$. Statistical procedures were carried out with the software package SPSS 10.0 for Windows.

Results

Physicochemical Parameters. One year after planting, only the (SB+ *A. niger*+RP) amendment had significantly decreased pH and increased electrical conductivity of the rhizosphere soil of both shrub species considered (Table 2). It is worth noting the sharp decrease in pH values in the rhizosphere soil of *C. albidus* amended with SB+*A. niger*+RP (0.75 units with respect to control soil), whereas in the rhizosphere of *Q. coccifera* the decrease was 0.08 units. However, neither mycorrhizal inoculation nor the interaction of amendment × mycorrhizal inoculation had any significant effect on soil physicochemical parameters in the rhizosphere of either species (Table 3).

The addition of the amendment significantly increased the total N, available P and extractable K contents of the rhizosphere soil of *C. albidus* and the total N and available P contents of the rhizosphere soil of *Q. coccifera* (Tables 2 and 3). The greatest increase in response to the addition of the amendment was observed in the soil available P content of both shrub species. Thus, the available phosphorus content in the amended soils was about fourfold higher than in the nonamended soils. The mycorrhizal inoculation treatment only had significant effects on the total N and extractable K contents of the rhizosphere soil of *Q. coccifera*.

In the revegetation experiments with *C. albidus* and *Q. coccifera* seedlings, the addition of the amendment increased the total carbohydrates and soluble C-fraction (water-soluble C and water-soluble carbohydrates) values in the rhizosphere of both species (Tables 2 and 3). The interaction of amendment with mycorrhizal inoculation had a positive significant effect on the total carbohydrates concentration of the rhizosphere soil of *Q. coccifera*. However, the mycorrhizal inoculation decreased significantly the total carbohydrates of the rhizosphere soil of

Table 3. Two-factor ANOVA (mycorrhizal inoculation and SB+*A. niger*+RP addition) for all parameters studied in the rhizosphere soil of *C. albidus* and *Q. coccifera* seedlings 1 year after planting [*F* values (significance level)]

	Amendment (A)	*Mycorrhiza (M)*	*Interaction (A × M)*
C. albidus			
pH	112.9 (<0.001)	3.3 (0.096)	2.3 (0.155)
Electrical conductivity	6.3 (0.027)	0.2 (0.693)	1.6 (0.229)
Total N	66.1 (<0.001)	0.2 (0.662)	10.2 (0.008)
Available P	44.9 (<0.001)	5.7 (0.098)	0.2 (0.627)
Extractable K	58.7 (<0.001)	3.3 (0.092)	0.1 (0.791)
Total carbohydrates	64.1 (<0.001)	6.1 (0.030)	1.1 (0.316)
Water-soluble C	109.4 (<0.001)	41.2 (<0.001)	1.9 (0.193)
Water-soluble carbohydrates	19.3 (0.001)	0.5 (0.511)	4.2 (0.063)
Aggregate stability	19.6 (0.001)	0.3 (0.582)	0.1 (0.817)
Biomass C	54.0 (<0.001)	0.0 (0.959)	63.9 (<0.001)
Dehydrogenase	28.1 (<0.001)	3.8 (0.073)	3.8 (0.074)
Urease	6.3 (0.028)	1.7 (0.214)	1.1 (0.318)
Protease	146.5 (<0.001)	29.9 (<0.001)	22.9 (<0.001)
Phosphatase	150.3 (<0.001)	9.7 (0.009)	8.1 (0.015)
β-Glucosidase	78.8 (<0.001)	1.9 (0.195)	18.4 (0.001)
Q. coccifera			
pH	6.0 (0.039)	0.8 (0.402)	0.3 (0.606)
Electrical conductivity	9.3 (0.016)	2.2 (0.179)	0.0 (0.989)
Total N	4.2 (0.074)	9.9 (0.014)	0.5 (0.517)
Available P	96.8 (<0.001)	2.4 (0.163)	0.3 (0.587)
Extractable K	4.6 (0.065)	6.6 (0.033)	5.6 (0.045)
Total carbohydrates	69.9 (<0.001)	25.4 (0.001)	47.2 (<0.001)
Water-soluble C	83.9 (<0.001)	0.0 (0.837)	0.2 (0.661)
Water-soluble carbohydrates	98.9 (<0.001)	2.5 (0.155)	2.8 (0.134)
Aggregate stability	0.0 (0.937)	5.5 (0.047)	0.0 (0.820)
Biomass C	0.7 (0.419)	2.2 (0.175)	4.2 (0.075)
Dehydrogenase	9.1 (0.017)	5.9 (0.041)	1.3 (0.281)
Urease	38.4 (<0.001)	0.0 (0.972)	2.4 (0.159)
Protease	21.2 (0.002)	33.5 (<0.001)	9.6 (0.015)
Phosphatase	10.9 (0.011)	41.9 (<0.001)	0.8 (0.397)
β-Glucosidase	5.0 (0.056)	9.0 (0.017)	0.0 (0.920)

Q. coccifera and the water-soluble C of the rhizosphere soil of *C. albidus*.

One year after planting *C. albidus*, the (SB+ *A. niger*+RP) amendment had increased the percentage of stable aggregates of rhizosphere soil by about 56% compared to the control soil (Table 2). However, neither mycorrhizal inoculation nor the interaction of amendment × mycorrhizal inoculation had any significant effect on soil aggregate stability (Table 3). The mycorrhizal inoculation with *S. verrucosum* was the only treatment which increased the aggregate stability of the rhizosphere soil of *Q. coccifera* (by about 13% with respect to soil control), as shown in Tables 2 and 3.

Biological and Biochemical Parameters. In the revegetation experiment with *C. albidus* seedlings, the C-biomass values of the amended soil were higher than for the non-amended soil (Table 4). The increases in microbial biomass of the rhizosphere soil of *Q. coccifera* produced by the addition of the amendment and mycorrhizal inoculation were not statistically significant (Tables 3 and 4). The combined treatment involving mycorrhizal inoculation of seedlings and addition of the amendment to soil increased the microbial biomass of the rhizosphere soil of both shrub species by less than each treatment applied individually.

Enzyme activities of the rhizosphere soil of *C. albidus* were significantly increased by the application of the amendment but not by mycorrhizal inoculation (Table 4). In addition, there was a positive interaction between the addition of the amendment and the mycorrhizal inoculation for most enzyme activities. Both treatments increased enzyme activities of the rhizosphere soil of *Q. coccifera*, except for urease activity, which was increased only by the addition of the amendment (Tables 3 and 4).

Foliar Nutrients, Growth Parameters of C. albidus and Q. coccifera, and Mycorrhizal Infection. At the time of planting, foliar N and K contents of noninoculated *C. albidus* and *Q. coccifera* plants and foliar P of noninoculated *C. albidus* plants were higher than for inoculated plants (Table 5). At the time of planting, the inoculated *C. albidus* and *Q. coccifera* plants exhibited averages of 12% and 17%, respectively, of short lateral roots colonized by *S. verrucosum*. The roots of the noninoculated *C.*

Table 4. Changes in biological and biochemical properties of rhizosphere soil of *C. albidus* and *Q. coccifera* in response to mycorrhizal inoculation and SB+*A. niger*+RP addition 1 year after planting ($n = 5$)

	Biomass C ($\mu g\ g^{-1}$)	*Dhase* ($\mu g\ INTF\ g^{-1}\ soil$)	*Urease* ($\mu mol\ NH_3\ g^{-1}\ h^{-1}$)	*Protease* ($\mu mol\ NH_3\ g^{-1}\ h^{-1}$)	*Phosphatase* ($\mu mol\ PNP\ g^{-1}\ h^{-1}$)	β-*Glucosidase* ($\mu mol\ PNP\ g^{-1}\ h^{-1}$)
C. albidus						
C	528 ± 8a	45.2 ± 1.5a	0.71 ± 0.03a	0.44 ± 0.02a	0.32 ± 0.00a	0.81 ± 0.03a
A	848 ± 5b	51.8 ± 2.7b	0.78 ± 0.00ab	0.65 ± 0.02b	0.61 ± 0.03b	1.03 ± 0.01b
M	677 ± 3ab	45.1 ± 1.1a	0.72 ± 0.02a	0.46 ± 0.02a	0.33 ± 0.01a	0.60 ± 0.02a
AM	663 ± 8ab	60.2 ± 0.7c	0.95 ± 0.02b	1.13 ± 0.03c	0.93 ± 0.05c	1.22 ± 0.05b
Q. coccifera						
C	482 ± 4a	42.7 ± 1.3a	0.52 ± 0.01a	0.33 ± 0.00a	0.38 ± 0.02a	0.61 ± 0.02a
A	515 ± 5a	50.3 ± 3.3b	0.76 ± 0.03b	0.40 ± 0.00b	0.53 ± 0.00b	0.84 ± 0.05b
M	579 ± 3a	47.5 ± 1.5a	0.46 ± 0.00a	0.49 ± 0.07b	0.56 ± 0.01b	0.96 ± 0.05c
AM	496 ± 1a	67.4 ± 3.7c	0.87 ± 0.04c	1.22 ± 0.07c	0.76 ± 0.02c	1.24 ± 0.05c

C: Control; A: SB+*A. niger*+RP addition; M: Mycorrhizal inoculation; AM: SB+*A. niger*+RP addition and mycorrhizal inoculation; Dhase: Dehydrogenase. Mean ± standard error. For each species, values in columns followed by the same letter do not differ significantly ($p < 0.05$) as determined by the LSD test.

albidus and *Q. coccifera* plants were not colonized by mycorrhizal fungi (Table 5).

One year after planting, the N, P, and K contents in shoot tissues of both shrub species were increased significantly by addition of the amendment and mycorrhizal inoculation, being higher in shoot tissue of *C. albidus* than *Q. coccifera* (Tables 5 and 6). The highest levels of mycorrhizal colonization were recorded in the inoculated *C. albidus* and *Q. coccifera* seedlings grown in the amended soil, followed by the inoculated plants grown in the nonamended soil. There was no natural colonization in the noninoculated seedlings of either plant species.

At the time of planting, shoot dry weight and foliar area of noninoculated *C. albidus* and *Q. coccifera* plants were slightly greater than for inoculated plants (Fig. 1). One year after planting, there were no statistically significant differences in plant survival between treatments for *C. albidus*, ~85% of the plants survived, irrespective of the treatment. For *Q. coccifera*, the percentages of plant survival were ~40% for noninoculated plants and 60% for inoculated plants. Both the addition of the amendment and mycorrhizal inoculation stimulated significantly the production of shoot biomass and foliar area of *C. albidus* and *Q. coccifera* with respect to the control plants (Table 6), mycorrhizal inoculation (469 and 74% greater, respectively, compared to control plants) being more effective than the addition of the amendment alone to soil (155 and 64% greater, respectively, compared to control plants). In the amended soil, mycorrhizal inoc-

Table 5. Foliar nutrients and root infection of *C. albidus* and *Q. coccifera* seedlings in response to mycorrhizal inoculation and SB+*A. niger*+RP addition previous to planting (0 months) and 1 year after planting ($n = 5$) (12 months)

	C. albidus		*Q. coccifera*	
Parameters	*0*	*12*	*0*	*12*
Nitrogen ($mg\ plant^{-1}$)				
C	15.9 ± 0.4b	11.8 ± 1.3a	14.4 ± 0.2b	22.3 ± 0.9a
A	15.9 ± 0.4b	50.9 ± 3.9b	14.4 ± 0.2b	43.2 ± 0.7b
M	12.5 ± 0.2a	111.5 ± 10.2c	10.1 ± 0.1a	54.5 ± 0.3c
AM	12.5 ± 0.2a	76.5 ± 1.6b	10.1 ± 0.1a	28.5 ± 1.3a
Phosphorus ($mg\ plant^{-1}$)				
C	1.21 ± 0.03b	0.58 ± 0.02a	1.42 ± 0.05a	1.60 ± 0.14a
A	1.21 ± 0.03b	3.03 ± 0.22b	1.42 ± 0.05a	2.70 ± 0.28b
M	1.13 ± 0.01a	6.63 ± 0.55c	1.64 ± 0.05a	2.68 ± 0.11b
AM	1.13 ± 0.01a	4.24 ± 0.10b	1.64 ± 0.05a	2.89 ± 0.20b
Phosphorus ($mg\ plant^{-1}$)				
C	16.1 ± 0.4b	6.3 ± 0.6a	5.4 ± 0.3b	8.1 ± 0.2a
A	16.1 ± 0.4b	31.5 ± 3.1b	5.4 ± 0.3b	16.2 ± 0.4b
M	10.9 ± 0.3a	56.2 ± 5.0c	3.8 ± 0.1a	13.9 ± 0.9ab
AM	10.9 ± 0.3a	37.6 ± 1.5b	3.8 ± 0.1a	10.9 ± 0.4a
Colonization (%)				
C	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
A	0.0 ± 0.0a	1.0 ± 0.6a	0.0 ± 0.0a	2.3 ± 0.7a
M	12.1 ± 0.7b	8.6 ± 0.7b	16.8 ± 0.6b	8.1 ± 0.8b
AM	12.1 ± 0.7b	24.5 ± 1.4c	16.8 ± 0.6b	20.4 ± 2.0c

C: Control; A: SB+*A. niger*+RP addition; M: mycorrhizal inoculation; AM: SB+*A. niger*+RP addition and mycorrhizal inoculation.
Mean ± standard error. For each species, values in columns followed by the same letter do not differ significantly ($p < 0.05$) as determined by the LSD test.

Table 6. Two factor ANOVA (mycorrhizal inoculation and SB+*A. niger*+RP addition) for growth parameters and mycorrhizal colonization of *C. albidus* and *Q. coccifera* seedlings 1 year after planting [*F* values (significance level)]

	Amendment (A)	*Mycorrhiza (M)*	*Interaction (A × M)*
C. albidus			
Height	0.0 (0.963)	11.9 (0.005)	16.8 (0.001)
Basal diameter	0.0 (0.899)	29.3 (<0.001)	5.1 (0.043)
Shoot	17.4 (0.001)	119.7 (<0.001)	48.2 (<0.001)
Root	4.5 (0.056)	14.1 (0.003)	0.9 (0.357)
Foliar area	2248.2 (<0.001)	11177.0 (<0.001)	4675.1 (<0.001)
Foliar N	20.9 (0.001)	111.7 (<0.001)	52.3 (<0.001)
Foliar P	37.6 (<0.001)	183.2 (<0.001)	102.7 (<0.001)
Foliar K	23.9 (<0.001)	85.8 (<0.001)	60.4 (<0.001)
Mycorrhizal roots	27.3 (<0.001)	97.0 (<0.001)	21.5 (0.001)
Q. coccifera			
Height	3.3 (0.108)	3.3 (0.105)	2.0 (0.191)
Basal diameter	0.5 (0.515)	2.5 (0.150)	17.4 (0.003)
Shoot	3.2 (0.112)	6.4 (0.035)	32.5 (<0.001)
Root	0.2 (0.653)	0.2 (0.687)	5.7 (0.044)
Foliar area	35.4 (<0.001)	102.9 (<0.001)	570.5 (<0.001)
Foliar N	0.0 (0.925)	19.6 (0.002)	151.2 (<0.001)
Foliar P	4.7 (0.063)	5.2 (0.053)	2.7 (0.137)
Foliar K	11.0 (0.011)	1.1 (0.329)	44.2 (<0.001)
Mycorrhizal roots	13.6 (0.006)	43.6 (<0.001)	6.4 (0.036)

ulation of *C. albidus* and *Q. coccifera* plants produced smaller increases than in the nonamended soil.

Discussion

The experiment shows that the addition of sugar beet, rock phosphate, and *A. niger* directly into soil and the inoculation of seedlings can considerably improve the growth of *C. albidus* and *Q. coccifera* in semiarid conditions. For both shrub species, mycorrhizal inoculation on its own was even more effective than the addition of the amendment alone to soil, with respect to improving the performance of the plants. However, *C. albidus* and *Q. coccifera* plants showed different levels of response to inoculation with *S. verrucosum*. Total nutrient content can be taken as a representative parameter of mycorrhizal effectiveness, because it takes into account the well-balanced effects of nutrient acquisition and biomass production. In both shrub species, mycorrhizal inoculation appeared effective in improving nutrient content, particularly in inoculated *C. albidus* plants. The highest contents of N and K in shoot tissue were observed in the inoculated seedlings, which might explain why the growth of *C. albidus* and *Q. coccifera* was greatest in this treatment. The fact that the foliar P contents of plants inoculated with *S. verrucosum* were similar to (in *Q. coccifera*) or even higher than (in *C. albidus*) those of noninoculated plants grown in the amended soil reaffirms the key role of mycorrhizas in sustaining the plant cover in P-deficient soils, as well as showing the necessity of including mycorrhizal inoculation in revegetation programs to guarantee plant performance.

The extent of mycorrhizal infection is of importance when studying the influence of ectomycorrhizal fungi on the host plant. High infection may not be a prerequisite for growth responses in all plants inoculated with ectomycorrhizal fungi. Thus, Roldńn and Albaladejo [34] observed that *Rhizopogon roseolus* was ineffective at promoting growth of *Pinus halepensis* despite colonizing a relatively large percentage of the roots. Inoculated *C. albidus* and *Q. coccifera* grown in the amended soil showed the highest levels of mycorrhizal colonization, although the combined treatment increased the biomass of both shrub species by less than each treatment applied individually. At the end of the growth period, the infection level of *S. verrucosum* was low and similar in the roots of both shrub species, but the increase in shoot biomass was higher in *C. albidus* seedlings than in *Q. coccifera*.

Soil structure largely determines soil quality and fertility, which in turn favor the establishment and viability of a stable plant cover [6]. Increased growth of inoculated *Q. coccifera* plants could be attributed to improved structural stability of the rhizosphere soil. Roots and associated mycorrhizal hyphae may form a three-dimensional network that enmeshes fine particles of soil into aggregates. In addition, the organic C released by roots promotes a dense microbial community in the immediate environment of the root, which, in turn, produces exocellular mucilaginous polysaccharide material that has the capacity to stabilize soil aggregates [14]. Caravaca et al. [6] concluded that improvements in the aggregate stability of rhizosphere soil of *Pinus halepensis* Mill. plants inoculated with an ectomycor-

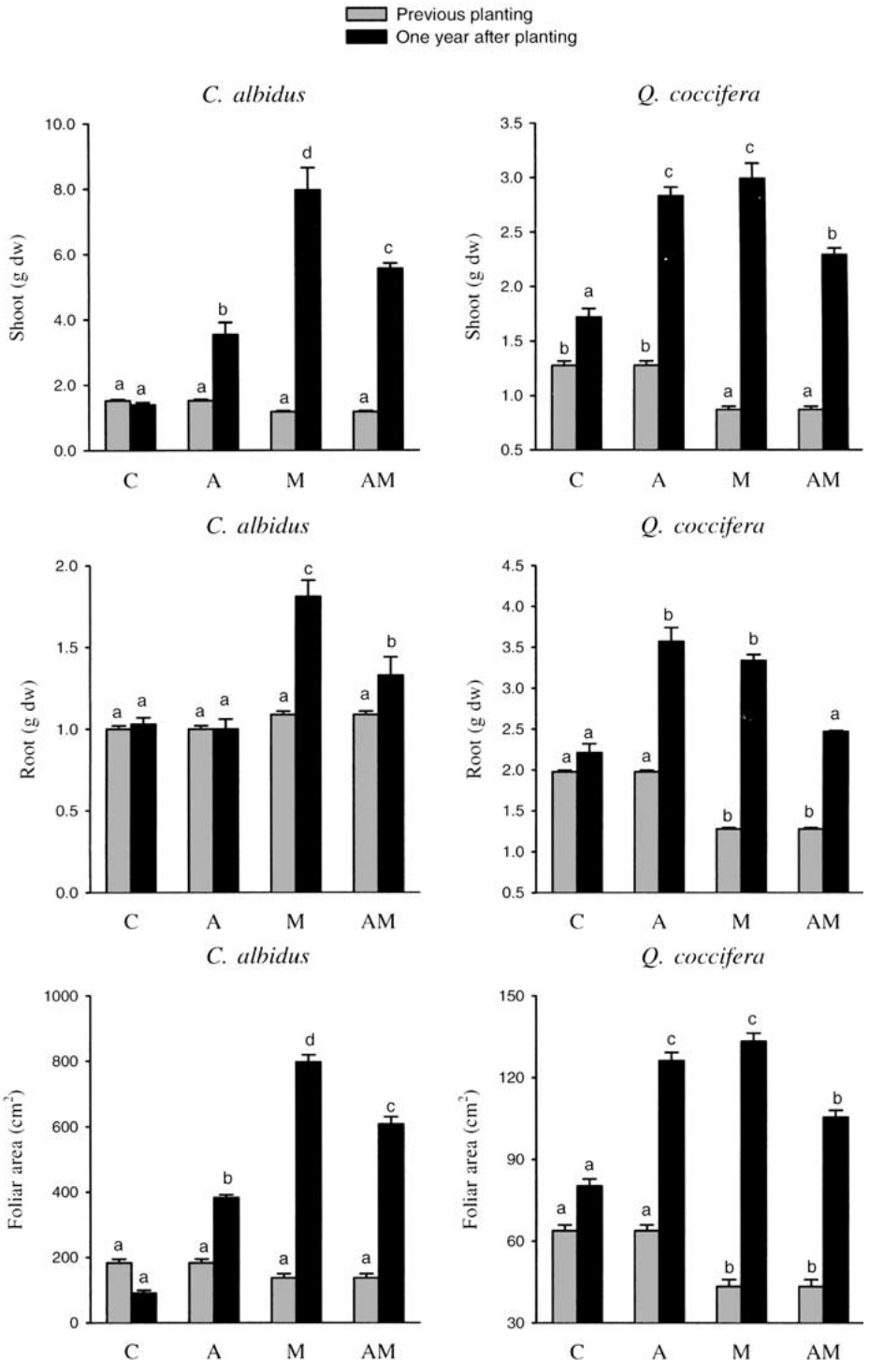

Figure 1. Shoot and root dry weights and foliar area of *C. albidus* and *Q. coccifera* seedlings in response to mycorrhizal inoculation and SB+*A. niger*+RP addition previous to planting and 1 year after planting. Bars represent standard errors. For each sampling, values with the same letter are not significantly different values at $p < 0.05$, according to LSD test.

rhizal fungus, *Pinus arhizus*, six years after planting, were due to a reactivation of microbiological activity. In our experiment, the effect of mycorrhizal inoculation on the stability of aggregates might also be attributed to a greater degree of biological activity in the rhizosphere soil of inoculated *Q. coccifera* plants. In fact, biomass C and dehydrogenase, protease-BAA, acid phosphatase, and β-glucosidase activities were higher in the rhizosphere soil of *Q. coccifera*, and these parameters have frequently been used as indicators of soil microbial activity [7, 10]. Furthermore, the hyphae of ectomycorrhizal fungi may release enzymes involved in the mineralization of organic matter. Thus, a positive correlation has been reported between phosphatase activity and the length of fungal hyphae associated with ectomycorrhizal mantles [12].

The application of organic materials, precultured with microorganisms in the presence of RP, has been used for increasing soil available phosphorus and improvement of plant growth and nutrition [43, 44]. Rodríguez et al. [33] demonstrated that the effectiveness of these biosystems for increasing the growth and nutrient uptake of *Medicago sativa* L. plants decreased with the time of microbial preincubation of the lignocellulosic substrate. However, we have shown that *A. niger* inoculated directly into soil was highly efficient for solubilizing RP on a medium of SB, which, in turn, increased available P in soil and stimulated the growth of *C. albidus* and *Q. coccifera* plants. Such microorganisms are able to excrete organic acids, which increase the concentration of phosphorus in solution by mechanisms involving chelation and exchange reactions [44]. In fact, we have also recorded that the addition of the amendment caused a decrease in pH of the rhizosphere soil of *C. albidus* and *Q. coccifera*.

The degradation of agrowastes of lignocellulosic nature by biological processes provides soil with an organic matter rich in polysaccharidic compounds [4, 9], which have a cementing effect [18]. In this regard, the amendment significantly improved the structural stability of rhizosphere soil of *C. albidus* and enhanced microbial populations. In the present study, the addition of amendment to soil increased the level of water-soluble C, which can be used as carbon and energy sources for soil microbiota and may also have a structural function [13]. Increased biological activity was also revealed by the variation in biomass C and enzyme activities. The enhanced soil microbiota particularly bacteria and fungi, are principally responsible for the formation of aggregates larger than 0.2 mm [2]. The improvement of the soil structure by the addition of the amendment could have contributed positively to the establishment of the *C. albidus* plants.

Several authors have demonstrated that total percentages of mycorrhizal colonization and sporulation are correlated negatively with the concentration of soluble C fractions [24, 28], indicating that mycorrhizal fungi act as strong sinks for photosynthates. In both shrub species, we also found the highest levels of seedling colonization in inoculated seedlings grown in the amended soil (AM), where concentrations of water-soluble carbon and water-soluble carbohydrates were lower than in amended soil and with noninoculated seedlings (A). However, the combined treatment (addition of amendment and mycorrhizal inoculation) increased the growth of *Q. coccifera* to a lesser extent than each treatment applied individually and increased the growth of *C. albidus* less than did the mycorrhizal inoculation. This result agrees with the widely accepted idea that mycorrhizas present little advantage to seedlings grown in amended soils [46]. Perhaps the release of lignin, as a consequence of mineralization of SB, could have had adverse effects on the functional activity of the ectomycorrhizal fungi [33].

In conclusion, in the short term, the mycorrhizal inoculation of the seedlings with *S. verrucosum* was the most effective treatment for stimulating the growth of *C. albidus* and *Q. coccifera* plants in a semiarid Mediterranean area. The success of revegetation carried out with *Q. coccifera* is based on improvement of soil physical and biological quality. The addition of sugar beet, rock phosphate, and *A. niger* directly into the soil was the most effective treatment for improving the physical, chemical, microbiological, and biochemical quality of the rhizosphere soil of *C. albidus*, with a subsequently enhanced plant growth. Likewise, the P demand of plants can be satisfied by microorganisms, such as *A. niger*, able to solubilize rock phosphate into soil. Finally, the combined treatment, involving mycorrhizal inoculation of seedlings and addition of the amendment directly into the soil, had no additive effect on the plant growth of either shrub species.

Acknowledgment

This research was supported by the EC + CICYT co-financed FEDER programme (REN 2000-1724-CO3-01).

References

1. Amaranthus, MP, Perry, D (1989) Rapid root tip and mycorrhiza formation and increased survival of Douglas-fir seedlings after soil transfer. New Forests 3: 77–82
2. Andrade, G, Mihara, KL, Linderman, RG, Bethlenfalvay, GJ (1998) Soil aggregation status and rhizobacteria in the mycorrhizosphere. Plant Soil 202: 89–96
3. Augé, R (2001) Water relations, drought and vesicular–arbuscular mycorrhizal symbiosis. Mycorrhiza 11: 3–42
4. Borken, W, Muhs, A, Beese, F (2002) Changes in microbial and soil properties following compost treatment of degraded temperate forest soils. Soil Biol Biochem 34: 403–412
5. Brink, RH, Dubar, P, Lynch, DL (1960) Measurement of carbohydrates in soil hydrolysates with anthrone. Soil Sci 89: 157–166
6. Caravaca, F, García, C, Hernńndez, MT, Roldńn, A (2002) Aggregate stability changes after organic amendment addition and mycorrhizal inoculation in the afforestation of a semi-arid site with *Pinus halepensis*. Appl Soil Ecol 19: 199–208
7. Caravaca, F, Masciandaro, G, Ceccanti, B (2002) Land use in relation to soil chemical and biochemical properties in semi-arid Mediterranean environment. Soil Till Res 68: 23–30
8. Dickie, IA, Koide, RT, Fayish, AC (2001) Vesicular–arbuscular mycorrhizal infection of *Quercus rubra* seedlings. New Phytol 151: 257–264
9. García, C, Hernńndez, MT, Roldńn, A, Albaladejo, J, Castillo, V (2000) Organic amendment and mycorrhizal inoculation as a practice in afforestation of soils with *Pinus halepensis* Miller: effect on their microbial activity. Soil Biol Biochem 32: 1173–1181
10. García, C, Hernńndez, MT, Costa, F (1997) Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Commun Soil Sci Plant Anal 28: 123–134
11. Grand, LF, Harvey, AE (1982) "Quantitative measurements of ectomycorrhizae on plant roots." In: Schenk, NC (Ed.) Methods

and Principles of Mycorrhizal Research, American Phytopathological Society, St Paul, MN, pp 157–164
12. Haussling, M, Marschner, H (1989) Organic and inorganic soil phosphates and acid phosphatase activity in the rhizosphere of 80-year-old Norway spruce [*Picea abies* (L.) Karst.] trees. Biol Fertil Soils 8: 128–133
13. Haynes, RJ, Swift, RS (1990) Stability of soil aggregates in relation to organic constituents and soil water content. J Soil Sci 41: 73–83
14. Jastrow, JD, Miller, RM, Lussenhop, J (1998) Contributions of interacting biological mechanisms to soil aggregates stabilization in restored prairie. Soil Biol Biochem 30: 905–916
15. Jeffries, P, Gianinazzi, S, Perotto, S, Turnau, K, Barea, JM (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils 37: 1–16
16. Jongmans, AG, van Breemen, N, Lundström, U, van Hees, PAW, Finlay, RD, Srinivasan, M, Unestam, T, Giesler, R, Melkerud, PA, Olsson, M (1997) Rock-eating fungi. Nature 389: 682–683
17. Lax, A, Díaz, E, Castillo, V, Albaladejo, J (1994) Reclamation of physical and chemical properties of a salinized soil by organic amendment. Arid Soil Res Rehab 8: 9–17
18. Lax, A, García-Orenes, F (1993) Carbohydrates from municipal solid wastes as aggregation factor of soil. Soil Technol 6: 157–162
19. Lax, A, Roldńn, A, Caravaca, F, García-Orenes, F (1997) ''Relationships between aggregate improvement, microbiological activity and organo-mineral complex formation in soils from semiarid areas.'' In: Pandalai, SG (Ed.) Recent Research Developments in Soil Biology and Biochemistry, Trivandrum India, pp 77–92
20. Maestre, FT, Bautista, S, Cortina, J, Díaz, G, Honrubia, M, Vallejo, R (2002) Microsite and mycorrhizal inoculum effects on the establishment of *Quercus coccifera* in a semi-arid degraded steppe. Ecol Eng 19: 289–295
21. Maremammani, A, Bedini, S, Matosevic, I, Tomei, PE, Giovannetti, M (2003) Type of mycorrhizal associations in two coastal nature reserves of Mediterranean basin. Mycorrhiza 13: 33–40
22. Masciandaro, G, Ceccanti, B, García, C (1994) Anaerobic digestion of straw and piggery wastewater: II. Optimization of the process. Agrochimica 3: 195–203
23. Murphy, J, Riley, JP (1962) A modified single solution method for determination of phosphate in natural waters. Anal Chim Acta 27: 31–36
24. Muthukumar, T, Udaiyan, K (2000) Influence of organic manures on arbuscular mycorrhizal fungi associated with *Vigna unguiculata* (L.) Walp. in relation to tissue nutrients and soluble carbohydrate in roots under field conditions. Biol Fertil Soils 31: 114–120
25. Nannipieri, P, Ceccanti, B, Cervelli, S, Matarese, E (1980) Extraction of phosphatase, urease, protease, organic carbon and nitrogen from soil. Soil Sci Soc Am J 44: 1011–1016
26. Naseby, DC, Lynch, JM (1997) Rhizosphere soil enzymes as indicators of perturbation caused by a genetically modified strain of *Pseudomonas fluorescens* on wheat seed. Soil Biol Biochem 29: 1353–1362
27. Page, AL, Miller, RH, Keeney, DR (1982) Methods of Soil Analysis, Part II. American Society of Agronomy, Madison, WI
28. Pearson, JN, Abbot, LK, Jasper, DA (1994) Phosphorus soluble carbohydrates and the competition between two arbuscular mycorrhizal fungi colonizing subterranean clover. New Phytol 127: 101–106
29. Plank, CO (1992) Reference Plant Analysis Procedures for the Southern Region of the United States. Southern Co-operative Series, Bulletin No. 368
30. Querejeta, JI, Roldńn, A, Albaladejo, J, Castillo, V (1998) The role of mycorrhizae, site preparation, and organic amendment in the afforestation of a semi-arid Mediterranean site with *Pinus halepensis*. Forest Sci 43: 203–211
31. Read, DJ (1992) ''The mycorrhizal mycelium.'' In: Allen, MJ (Ed.) Mycorrhizal Functioning. An Integrative Plant–Fungal Process, Chapman & Hall, London, pp 102–133
32. Requena, N, Pérez-Solis, E, Azcón-Aguilar, C, Jeffries, P, Barea, JM (2001) Management of indigenous plant–microbe symbioses aids restoration of desertified ecosystems. Appl Environ Microbiol 67: 495–498
33. Rodríguez, R, Vassilev, N, Azcón, R (1999) Increases in growth and nutrient uptake of alfalfa grown in soil amended with microbially-treated sugar beet waste. Appl Soil Ecol 11: 9–15
34. Roldán, A, Albaladejo, J (1994) Effect of mycorrhizal inoculation and soil restoration on the growth of *Pinus halepensis* seedlings in a semiarid soil. Biol Fertil Soils 18: 143–149
35. Schollemberger, CJ, Simon, RH (1954) Determination of exchange capacity and exchangeable bases in soils. Soil Sci 59: 13–24
36. Sims, J, Haby, V (1971) Simplified colorimetric determination of soil organic matter. Soil Sci 112: 137–141
37. Soil Survey Staff (1999) Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys. USDA Natural Resources Conservation Service, Agric Hdbk 436. US Government Printing Office, Washington, DC
38. Tabatabai, MA (1982) ''Soil enzymes.'' In: Page, AL, Miller, EM, Keeney, DR (Eds.) Methods of Soil Analysis, Part II, ASA and SSSA, Madison, pp 501–538
39. Tabatabai, MA, Bremner, JM (1969) Use of *p*-nitrophenol phosphate in assay of soil phosphatase activity. Soil Biol Biochem 1: 301–307
40. Torres, P, Roldán, A, Lansac, AR, Martín, A (1995) Ectomycorrhizal formation between *Cistus ladanifer* and *Laccaria laccata*. Nova Hedwigia 60: 311–315
41. Vance, ED, Brookes, PC, Jenkinson, D (1987) An extraction method for measuring microbial biomass carbon. Soil Biol Biochem 19: 703–707
42. Vassilev, N, Baca, MT, Vassileva, M, Franco, I, Azcón, R (1995) Rock phosphate solubilization by *Aspergillus niger* grown on sugar-beet waste medium. Appl Microbiol Biotechnol 44: 546–549
43. Vassilev, N, Baca, MT, Vassileva, M, Franco, I, De Nobili, M (1998) ''Mineralization of three agro-industrial wastes by an acid-producing strain of *Aspergillus niger*.'' In: De Bertoldi, M, Sequi, P, Lenunes, B, Papi, T (Eds.) The Science of Composting, Chapman and Hall, London, pp 1375–1379
44. Vassilev, N, Franco, I, Vassileva, M, Azcón, R (1996) Improved plant growth with rock phosphate solubilized by *Aspergillus niger* grown on sugar beet waste. Bioresource Technol 55: 237–241
45. Wenkart, S, Roth-Bejerano, N, Mills, D, Kagan-Zur, V (2001) Mycorrhizal associations between *Tuber melanosporum* mycelia and transformed roots of *Cistus incanus*. Plant Cell Reprod 20: 369–373
46. Yanai, RD, Fahey, TJ, Miller, SL (1995) ''Efficiency of nutrient acquisition by fine roots and mycorrhizae.'' In: Smith, WK, Hinckley, TM (Eds.) Resource Physiology of Conifers: Acquisition, Allocation, and Utilization, Academic Press, London, pp 75–103