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Improvements in soil quality and performance of mycorrhizal *Cistus albidus* L. seedlings resulting from addition of microbially treated sugar beet residue to a degraded semiarid Mediterranean soil

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Abstract. A field experiment was undertaken to assess the effectiveness of a combined treatment, involving addition of *Aspergillus niger*-treated sugar beet (SB) residue in the presence of rock phosphate and mycorrhizal inoculation of seedlings with *Pisolithus tinctorius*. The aim was to improve the physical, chemical, biochemical and biological properties of a degraded semiarid Mediterranean soil. Short-term effects of such improvements on the establishment of *Cistus albidus* L. seedlings were evaluated. Eight months after planting, macronutrients (NPK), total carbohydrates, water-soluble C, water-soluble carbohydrates, microbial biomass C and enzyme activities (dehydrogenase, urease, protease, acid phosphatase and β-glucosidase) measured in the rhizosphere soil of *C. albidus* were increased greatly by addition of fermented SB residue. Soil structural stability improved only with the fermented SB addition (about 79% higher in the amended soils than in the non-amended soils). The mycorrhizal inoculation was the most effective treatment in improving the growth of *C. albidus* plants, but only slightly improved soil quality. Growth of inoculated plants was about 33% greater than plants grown in the amended soil and about 131% greater than control plants. The combined benefit of mycorrhizal inoculation of seedlings and addition of fermented SB residue to soil on plant growth was similar to that of the treatments applied individually.

Keywords: *Pisolithus tinctorius*, rock phosphate, enzyme activities, microbial biomass carbon, aggregate stability

INTRODUCTION

Establishment of indigenous plant species is a widely used practice for reclaiming degraded lands in semiarid Mediterranean areas. Shrub communities associated with other small woody plants are characteristic of these semiarid ecosystems. In particular, ectomycorrhizal shrub species, such as *Cistus albidus* L., are extremely important because they constitute a reservoir for ectomycorrhizal fungi in the absence of host trees (Torres *et al.* 1995). The roots of Cistaceae may provide a reservoir of ectomycorrhizal fungi, thereby maintaining the diversity and activity of fungi needed for establishment of forest regeneration. Torres *et al.* (1995) confirmed that the survival of some ectomycorrhizal fungi may be enhanced by the shrub canopies of the forest. This is important in revegetation programmes, especially when eroded soils, such as those found in the Mediterranean area, are re-afforested. Thus, re-establishing shrubs is a key step in revegetation strategies. However, in these semiarid areas the low productivity and fertility of the soil and the severe water deficits seriously limit plant growth. To carry out successful re-afforestation, it is necessary to improve soil quality and the ability of the planted species to resist the semiarid environment.

Evidence suggests that mycorrhizas help plants to thrive in arid conditions by increasing their supply of phosphorus and other nutrients (Querejeta *et al.* 1998), also by improving aggregation in eroded soils (Caravaca *et al.* 2002) and reducing water stress (Augé 2001). In degraded soils the mycorrhizal component may disappear or, at least, be severely depleted, so it may be necessary to reinforce or replace it by appropriate inoculation (Requena *et al.* 2001). However, there are no studies concerning mycorrhizal symbiosis with *C. albidus*.

Phosphorus is limiting nutrient for plant growth in degraded semiarid soils and the possibility of using rock phosphate as a fertilizer has received considerable interest in recent years. Rock phosphates are natural, inexpensive and

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easily obtainable fertilizers but their solubility is very low in non-acidic soils. One attractive approach for increasing the solubility of rock phosphate is the application of micro-organisms that are able to excrete organic acids, which increases the concentration of phosphorus in solution by mechanisms involving chelation and exchange reactions. For example, Vassilev *et al.* (1995) used a selected strain of *Aspergillus niger* grown on sugar beet (SB) residue for increasing the solubilization of rock phosphate in agricultural soils and thereby improved crop growth and nutrition (Vassilev *et al.* 1996). The use of agro-wastes that are high in lignocellulose as substrates changes pH, conductivity and nutrient levels (Rodríguez *et al.* 1999), which may induce positive or negative effects on rhizosphere microorganisms and/or plant growth and nutrition (Boddington & Dodd 2000). Previous studies demonstrated that the fungal (*A. niger*) solubilization of rock phosphate on media based on agro-wastes was compatible with AM fungi and *Rhizobium* strains, when supplied as amendments to the soil/plant systems (Vassilev *et al.* 1996; Rodríguez *et al.* 1999). However, no information is available on the use of such materials in revegetation programmes.

In semiarid Mediterranean areas, the establishment of plants is made difficult by the severe climate characterized by little and irregular precipitation and frequent drought. Under these conditions, the first stages of growth are most critical for predicting the success of soil re-afforestation. For this reason, we investigated the relationships between mycorrhizae and soil properties only 8 months after planting. The objectives of this study were: (1) to assess the effect of the combined addition of *A. niger*-treated SB residue in the presence of rock phosphate plus mycorrhizal inoculation of seedlings on soil properties and (2) to determine the influence of such improvements on the establishment of *C. albidus* seedlings.

MATERIALS AND METHODS

Study sites

The experimental area was located in Los Cuadros in the Province of Murcia (southeast Spain) (coordinates: 1°05′W, 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 evapo-transpiration reaches 1000 mm yr^{-1}. The loam soil used was a Typic Haplocalcid (Soil Survey Staff 1999) developed from Quaternary sediments (Table 1).

Materials

Sugar beet (SB) residue, a lignocellulosic material, was dried at 60 °C and ground to pass a 2-mm pore screen. Portions of 5 g of SB residue were mixed with 50 ml of Czapek solution (agar, 15.0 g L^{-1}; di-potassium hydrogen phosphate, 1.0 g L^{-1}; iron(II) sulphate heptahydrate, 0.01 g L^{-1}; potassium chloride, 0.5 g L^{-1}; magnesium sulphate heptahydrate, 0.5 g L^{-1}; sodium nitrate, 3.0 g L^{-1}; sucrose, 30.0 g L^{-1}; pH = 7.3) for static fermentation in 250 ml Erlenmeyer flasks. Rock phosphate (Morocco fluorapatite, 12.8% P, 1 mm mesh), was added at a rate of 0.75 g per flask. Media were sterilized by autoclaving at 120 °C for 30 min. A suspension of *Aspergillus niger* NB2 (1.2×10^7 spores) was spread carefully over the surface of the media. The mixture was allowed to ferment at 30 °C for 20 days without shaking. The characteristics of the SB after fermentation were: pH, 3.0; total P, 0.2%; total N, 1.2%; cellulose, 11.3%; hemicellulose, 3.1%; lignin, 4.1% and reducing sugar, 0.25 g L^{-1}.

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

pH (H_2O)	8.5±0.0
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
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
Microbial biomass C (μg g^{-1})	396±11
Aggregate stability (%)	11.5±0.4
Bulk density (g cm^{-3})	1.10±0.02

[a]Mean ± standard error ($n = 6$).

The plant used for the re-afforestation experiment was *Cistus albidus* L., which is a low-growing shrub reaching a height of about 1.5 m and widely distributed in the Mediterranean. It is well adapted to water stress and, therefore, potentially could be used in the revegetation of semiarid degraded lands.

Mycorrhizal inoculation of seedlings

The mycorrhizal fungus used in the experiment was *Pisolithus tinctorius* (Pers.) Coker & Couch, obtained from the herbarium of the DPV-IRTA, Barcelona, Spain. The potting substrate consisted of equal volumes of autoclaved peat and vermiculite (60 min, 120 °C), which was used to fill 160-ml containers. Spores of *P. tinctorius* were added to autoclaved vermiculite (20 min, 120 °C) and then mixed with the potting substrate, at rates of 1 : 10 inoculum : substrate (v/v) at sowing time. The inoculum applied corresponded to 10^6 spores per container. Inoculated and non-inoculated seedlings were grown for 8 months under nursery conditions without fertilizer.

Experimental design and layout

A factorial design in randomized blocks was used with two factors and five-fold replication. The first factor was the addition of fermented SB residue to the soil, and the second was the inoculation of *C. albidus* plants with *P. tinctorius* in the nursery. More specifically, four treatments were established:

1. Seedlings without mycorrhizal treatment and soil without fermented SB residue addition (control).
2. Seedlings without mycorrhizal treatment and soil with fermented SB residue addition (R).

3. Seedlings inoculated with *P. tinctorius* and soil without SB residue addition (M).
4. Seedlings inoculated with *P. tinctorius* and soil with fermented SB residue addition (R + M).

Each treatment block occupied 180 m^2. Planting holes 40 × 40 cm wide and 30 cm deep were dug manually. In early November 2001, fermented SB residue was added to half of the holes (0–20 cm depth) at a rate of 3% fresh wt. The seedlings (inoculated and non-inoculated) were planted at least 1 m apart between holes, with 3 m between blocks. At least 32 seedlings per block were planted (eight plants × four treatments in each block).

Sampling procedures

Eight months after planting, five soil samples were collected from each treatment (20 soil samples in total). Each sample consisted of eight bulked subsamples (200 cm^3 soil cores), collected randomly at 0–20 cm depth in the rhizospheres of eight individual plants. The sampling was carried out in June 2002, before the dry season and at the end of the growing period, when the highest microbial activity would be expected (Lax *et al.* 1997). At the same time, five plants were harvested from each treatment (one per block).

Plant analyses

Basal stem diameter and height of plants were measured. Fresh and dry (105 °C, 5 h) weight of shoots and roots were recorded and 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 (Plank 1992). Foliar P was determined by colorimetry (Murphy & Riley 1962), foliar N was colorimetrically measured after Kjeldhal digestion (Page *et al.* 1982) and foliar K was estimated by flame photometry (Schollemberger & Simon 1954).

To assess mycorrhizal colonization, methods described in Grand & Harvey (1982) and Amaranthus & Perry (1989) were followed. Roots were subsampled in three 2-cm cross-sections of the upper, middle and lower root systems. Root tips in these sections appearing mycorrhizal and active were counted and the results expressed as a percentage of all the root tips.

Physical, chemical and biochemical analyses of soil

Soil pH and electrical conductivity were measured in a 1 : 5 (w/v) aqueous solution. Total nitrogen was determined by the Kjeldhal method and total organic C by oxidation with $K_2Cr_2O_7$ in a sulphuric medium; excess dichromate was evaluated using Mohr's salt (Yeomans & Bremner 1988). In aqueous extracts of soil, water-soluble carbon was determined by wet oxidation with $K_2Cr_2O_7$ and measured by absorbance at 590 nm (Sims & Haby 1971). Water-soluble carbohydrates and total carbohydrates were determined by the method of Brink *et al.* (1960). Available phosphorus, extracted with 0.5 M $NaHCO_3$, was determined by colorimetry according to Murphy & Riley (1962). Potassium extractable by ammonium acetate was determined by flame photometry.

Microbial biomass carbon was determined using a fumigation–extraction method (Vance *et al.* 1987). Dehydrogenase activity was determined according to García *et al.* (1997). Urease and *N*-α-benzoyl-L-arginina-mide (BAA) hydrolysing 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. Aliquots of 2 mL of buffer and 0.5 mL of substrate were added to 0.5 g of sample followed by incubation for 90 min at 30 °C (for urease) or 39°C (for protease). Both activities were determined as the NH_3 released in the hydrolysis reaction (Nannipieri *et al.* 1980).

Acid phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate. For the assay, 2 mL of 0.5 M sodium acetate buffer adjusted to pH 5.5 using acetic acid (Naseby & 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 2mL of 0.5 M NaOH were added and the mixture centrifuged at 4000 rpm for 5 min. The *p*-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm (Tabatabai & Bremner 1969). Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

β-Glucosidase was determined using 0.05 M *p*-nitrophe-nyl-β-D-glucopyranoside (PNG) as substrate (Masciandaro *et al.* 1994). For this assay, based on the release and detection of PNP, 2 mL of 0.1 M maleate buffer at 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 aminomethano (THAM) according to Tabatabai (1982). The amount of PNP was determined by spectrophotometry at 398 nm (Tabatabai & Bremner 1969).

Physical analysis

The percentage of stable aggregates was determined by the method described by Lax *et al.* (1994). A 4 g aliquot of sieved (0.2–4 mm) soil was placed on a small 0.250 mm sieve and wetted by spraying. After 15 min the soil was subjected to artificial rainfall of 150 mL with energy of 270 J m^{-2}. The soil remaining on the sieve was placed in a weighed capsule (T), dried at 105 °C and weighed (P1). Then the soil was soaked in distilled water for 2 h and passed through the same 0.250 mm sieve with the assistance of a small stick to break up 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 was calculated as (P1 - P2) × 100/(4 - P2 + T).

Bulk density was determined after maintaining soil moisture at 60% of field capacity for one month using the paraffin method described by Barahona & Santos (1981)

Statistical analysis

Aggregate stability and percentage colonization were arcsin-transformed, and the other parameters were log-transformed to compensate for variance heterogeneity before analysis of variance. The effects of residue addition and mycorrhizal inoculation and their interactions on measured variables were tested by a two-way analysis of variance. Statistical

Table 2. Changes in physicochemical properties of the soil in response to either mycorrhizal inoculation (M) and fermented SB residue addition (R) or both (R + M) compared to control soil.[a]

	Control	R	M	R + M
pH (H_2O)	8.7±0.0	8.3±0.0	8.5±0.0	8.3±0.0
EC (1:5, $\mu S\,cm^{-1}$)	255±2	294±4	273±4	310±7
TOC ($g\,kg^{-1}$)	10.0±0.4	13.3±0.2	10.1±0.3	13.5±0.2
Total CH ($\mu g\,g^{-1}$)	423±10	695±28	478±17	759±32
Water-soluble C ($\mu g\,g^{-1}$)	87±30	152±5	101±5	168±10
Water-soluble CH ($\mu g\,g^{-1}$)	2±0	10±1	4±0	13±1
Total N ($g\,kg^{-1}$)	0.9±0.0	1.3±0.0	0.8±0.0	1.3±0.1
Available P ($\mu g\,g^{-1}$)	1±0	10±1	2±0	10±1
Extractable K ($\mu g\,g^{-1}$)	128±000	179±3	164±4	165±4
Aggregate stability (%)	18.4±1.00	30.5±0.9	17.4±0.6	33.6±1.3
Bulk density ($g\,cm^{-3}$)	1.10±0.01	1.03±0.01	1.13±0.01	1.04±0.01

[a]Mean ± standard error ($n = 5$). For statistical differences between treatments, see Table 3.
EC, electrical conductivity; TOC, total organic carbon; CH, carbohydrates.

Table 3. Two factor ANOVA for mycorrhizal inoculation (M) and fermented SB residue addition (R) and their interaction (R × M) for physicochemical, biological and biochemical parameters of the soil, given as F values (P values).

	R	M	R × M
pH (H_2O)	21.603 (<0.001)	3.418 (0.083)	1.396 (0.255)
EC	13.513 (0.002)	2.816 (0.113)	0.069 (0.797)
TOC	24.115 (<0.001)	0.054 (0.820)	0.000 (0.994)
Total CH	35.861 (<0.001)	1.644 (0.218)	0.030 (0.865)
Water-soluble C	25.108 (<0.001)	1.049 (0.321)	0.124 (0.729)
Water-soluble CH	47.892 (<0.001)	3.947 (0.064)	0.891 (0.359)
Total N	58.902 (<0.001)	0.154 (0.700)	0.353 (0.561)
Available P	93.998 (<0.001)	3.076 (0.099)	1.950 (0.182)
Extractable K	16.075 (0.001)	3.488 (0.080)	15.261 (0.001)
Microbial biomass C	40.286 (<0.001)	0.601 (0.450)	0.074 (0.789)
Dehydrogenase	93.821 (<0.001)	1.360 (0.261)	0.093 (0.764)
Urease	78.169 (<0.001)	4.523 (0.049)	0.648 (0.433)
Protease-BAA	22.018 (<0.001)	1.281 (0.274)	1.062 (0.318)
Acid phosphatase	44.449 (<0.001)	1.493 (0.239)	1.390 (0.256)
β-glucosidase	44.271 (<0.001)	1.896 (0.187)	0.768 (0.394)
Aggregate stability	41.809 (<0.001)	0.266 (0.613)	0.904 (0.356)
Bulk density	16.981 (0.001)	0.877 (0.363)	0.094 (0.763)

procedures were carried out with the software package SPSS 10.0 for Windows.

RESULTS AND DISCUSSION

Physicochemical parameters

Only the fermented SB residue significantly decreased soil pH and increased soil electrical conductivity (Tables 2 and 3). However, neither mycorrhizal inoculation nor the interaction between fermented SB residue and mycorrhizal inoculation had any significant effect on the physicochemical parameters of the soil (Table 3). The decreases observed in soil pH could be due to citric acid produced by *A. niger* during the mineralization processing of the SB residue (Vassilev *et al.* 1995).

Fermented SB residue was more effective than mycorrhizal treatment in increasing total soil organic carbon (SOC) and all the C fractions in soil with growing *C. albidus*, as shown in Tables 2 and 3. Eight months after planting, the total organic carbon (TOC) content of the soil had increased by about 33% over the control soil.

Polysaccharides, which are mostly by-products of the microbial activity developed in the C-containing substrate, are considered as the main temporary aggregate-stabilizing agents (Roldán *et al.* 1994). The addition of the fermented SB residue increased the total carbohydrate levels of the soil, probably because it contained simple sugars produced during the biological transformation process.

The water-soluble organic matter fraction consists of a heterogeneous mixture of components of varying molecular weight, such as mono- and polysaccharides, polyphenols, proteins and organic acids of low molecular weight. This fraction can be used as carbon and energy sources by soil microflora (Roldán *et al.* 1994); the carbohydrate fraction may also aggregate soil particles. The increased soluble C fraction values (water-soluble C and water-soluble carbohydrates) were mainly due to the fermented SB residue added to the soil.

The addition of the fermented SB residue significantly increased the total N, available P and extractable K contents of the soil (Tables 2 and 3). The greatest increase in response to the addition of SB residue was observed in the available P content. Thus, the available P content in fermented SB-amended soils (R and R + M) was about ten times greater than in non-amended soils (control and M). This increase in soil available P, which was associated also with a decrease in soil pH, could be related to the ability of *A. niger* in solubilizing rock phosphate (Vassilev *et al.* 1995; Rodríguez *et al.* 1999) and supports the use of such a biosystem to improve P bioavailability in P-deficient soils. The mycorrhizal inoculation treatment had no significant effect on the nutrient (NPK) contents of the soil.

Physical parameters

Soil structure largely determines soil quality and fertility, which in turn favours the establishment and viability of a stable plant cover (Caravaca *et al.* 2002). Soil structural stability was significantly improved by the addition of fermented SB residue; on average, by about 79% compared with the non-amended soils (Tables 2 and 3). Dinel *et al.* (1992) found that the restoration of soil structure may depend on the amount and nature of the organic matter added. As already mentioned, the biological transformations that the SB residue underwent during fermentation increased the quantity of aggregate-stabilizing agents. It is worth noting that this type of residue is, in the short-term, more effective than other residues, such as sewage sludge and urban waste, that are widely used for improving soil structure under semiarid Mediterranean conditions (Caravaca *et al.* 2002).

The organic materials are less dense than the mineral fraction of soils and play an important role in improving soil structure. Thus, their application reduces the soil bulk density and leads to an increase in soil porosity. In addition, the organic C of fermented SB residue may affect the bulk density of a soil by improving its structural stability. The addition of organic residue increases the percentage of

Table 4. Changes in biochemical properties of the soil in response to either mycorrhizal inoculation (M) and fermented SB residue addition (R) or both (R + M) compared to control soil.[a]

	Control	R	M	R + M
Microbial biomass C ($\mu g\, g^{-1}$)	532±12[a]	709±14	518±3	679±16
Dehydrogenase activity (μg INTF g^{-1} soil)	60±1	108±3	66±2	114±4
Urease activity ($\mu mol\ NH_3\, g^{-1}\, h^{-1}$)	0.49±0.01	0.99±0.04	0.61±0.01	1.09±0.05
Protease-BAA activity ($\mu mol\ NH_3\, g^{-1}\, h^{-1}$)	0.31±0.01	0.82±0.05	0.46±0.03	0.92±0.12
Acid phosphatase activity (μmol PNP $g^{-1}\, h^{-1}$)	0.29±0.02	0.66±0.03	0.29±0.02	0.55±0.02
β-Glucosidase activity (μmol PNP $g^{-1}\, h^{-1}$)	0.46±0.01	1.24±0.05	0.69±0.02	1.29±0.08
Dehydrogenase:microbial biomass C (μg INTF $\mu g\, C_{mic}^{-1}$)	0.11±0.00	0.15±0.00	0.13±0.00	0.17±0.01
Urease : microbial biomass C ($\mu g\, NH_3\, mg\, C_{mic}^{-1}\, h^{-1}$)	16.0±0.6	23.7±0.6	20.0±0.5	28.0±1.6
Protease : microbial biomass C ($\mu g\ NH_3$ mg $C_{mic}^{-1}\, h^{-1}$)	10.0±0.2	19.5±0.9	15.2±1.0	23.9±3.4
Phosphatase : microbial biomass C (μg PNP mg $C_{mic}^{-1}\, h^{-1}$)	76.0±4.3	128.7±3.8	79.1±5.4	113.7±4.1
β-Glucosidase : microbial biomass C (μg PNP mg $C_{mic}^{-1}\, h^{-1}$)	121.4±4.6	241.6±5.3	186.2±5.0	269.9±19.5

[a]Mean ± standard error ($n = 5$). For statistical differences between treatments, see Table 3.

transmission and storage pores (Pagliai *et al.* 1981). This may explain the reduced bulk density measured in soil amended with fermented SB residue (Table 2).

The positive effect of the symbiosis between arbuscular mycorrhizal fungi and plants on soil structural characteristics has been demonstrated widely (Bearden & Petersen 2000). However, there were no changes in the physical properties of the rhizosphere soil from *C. albidus* plants inoculated with *P. tinctorius* (Table 3), possibly due to the short duration of the experiment. Caravaca *et al.* (2002) recorded improvements in the aggregate stability of rhizosphere soil of *P. halepensis* plants inoculated with an ectomycorrhizal fungus, *Pinus arhizus* at six years after planting.

Biological and biochemical parameters

In amended soils there was more microbial biomass C than in non-amended soils (Table 4). This is in agreement with the results found by García *et al.* (2000) for a soil treated with uncomposted organic residue, although the microbial biomass C increase they reported was greater. The increase in biomass C noted in the amended soil can be attributed to the incorporation of biodegradable organic materials, which stimulated the indigenous microbial activity of the soil, or to incorporation of exogenous microorganisms, because this residue was obtained via highly active microbially-mediated processes (Vassilev *et al.* 1995). The mycorrhizal inoculation treatment did not affect the soil microbial biomass C (Table 3).

Dehydrogenase activity has been considered as an indicator of total microbial activity in soil (García *et al.* 1997), and it has been proposed as a valid biomarker to indicate changes in microbial activity caused by changes in soil management under different agronomic practices and climates (Ceccanti *et al.* 1994). Thus, the increases observed in dehydrogenase activity indicate a greater microbiological activity (García *et al.* 1997) as a consequence of the organic amendment (Table 3). De Luca & Keeney (1993) defined the water-soluble C content as a reflection of soil microbial activity, and in our experiment there was a significant correlation ($P < 0.05$) between the availability of labile and easily-mineralizable organic matter and the activity of microbial populations. Mycorrhizal inoculation had no significant effect on the microbial activity of the soil (Table 3).

Measurement of soil hydrolases provides an early indication of changes in soil fertility, since they are involved in the mineralization of important nutrient elements such as N, P and C. Many researchers have found that soil hydrolase activities are enhanced by the addition of organic materials (García *et al.* 2000) and can remain active in an extracellular soil environment. We also found that urease, protease-BAA, acid phosphatase and β-glucosidase activities were higher in the soil amended with the fermented SB residue (Table 4). β-Glucosidase catalyses the hydrolysis of the ends of unreduced chains of β-D-glucosides releasing β-D-glucose, which indicates the potential for SOM decomposition. The largest enzyme activity increase in the amended soil was β-glucosidase, suggesting an enrichment in materials of a cellulolitic nature acting as substrates (Table 4).

Urease and β-glucosidase activities were enhanced also by the mycorrhizal treatment. The hyphae of ectomycorrhizal fungi may release enzymes into soil. For all the measured enzymes, the enzyme activity : microbial biomass ratio was increased to a greater extent by the addition of fermented SB residue than by mycorrhizal treatment, which indicates a greater enrichment of enzyme activity relative to microbial biomass in the amended soil (Landi *et al.* 2000).

Growth parameters of C. albidus *and mycorrhizal infection*

At the time of planting, the height, basal diameter, shoot dry weight and foliar N content of non-inoculated *C. albidus* plants were slightly greater than for inoculated plants (Table 5). There were no significant differences in foliar P or K content between non-inoculated and inoculated *C. albidus* seedlings previous to planting in the field. At the time of planting, the inoculated plants had an average of 21% of their short lateral roots colonized by *P. tinctorius*. The roots of the non-inoculated plants were not colonized by mycorrhizal fungi (Table 5).

Eight months after planting, addition of both fermented SB and mycorrhizal inoculation had stimulated significantly the production of shoot biomass with respect to the control plants (Tables 5 and 6). It is important to emphasize that mycorrhizal inoculation alone was even more effective than the addition of fermented SB residue alone in improving the

Table 5. Characterization of the plants prior to planting and changes in growth parameters and mycorrhizal colonization of *C. albidus* in response to either mycorrhizal inoculation (M) and fermented SB residue addition (R) or both (R + M) compared to control soil.[a]

	Plants prior to planting		Control	R	M	R + M
	Non-inoculated	Inoculated				
Height (cm)	18.4±0.1	15.4±0.2	30.0±0.5*	34.3±0.2	41.1±1.7	35.7±1.6
Basal diameter (mm)	2.8±0.0	2.4±0.1	2.9±0.0	4.0±0.1	4.2±0.1	4.2±0.2
Shoot (g dry wt)	1.53±0.08	1.39±0.07	3.55±0.12	6.17±0.14	8.21±0.33	7.58±0.53
Root (g dry wt)	1.30±0.01	0.84±0.01	0.91±0.02	1.45±0.04	1.61±0.06	1.87±0.13
Shoot/root ratio	1.2±0.1	1.7±0.1	3.8±0.1	4.3±0.2	5.2±0.2	6.1±0.6
Nitrogen (mg plant^{-1})	15.9±0.3	12.0±0.3	41.3±2.3	68.9±0.6	89.2±3.3	88.1±7.2
Phosphorus (mg plant^{-1})	1.2±0.0	1.2±0.0	2.4±0.1	6.9±0.5	5.7±0.3	6.8±0.5
Potassium (mg plant^{-1})	16.0±0.4	16.2±0.5	25.6±0.6	43.1±1.8	55.5±1.9	52.6±2.7
Mycorrhizal roots (%)	0.0±0.0	21.1±0.9	0.0±0.0	0.0±0.0	18.4±1.5	22.8±1.8

[a]Mean ± standard error ($n = 5$). For statistical analysis of differences between treatments, see Table 6.

Table 6. Two factor ANOVA for fermented SB residue addition (R) and mycorrhizal inoculation (M) and their interaction (R × M) for growth parameters and mycorrhizal colonization of *C. albidus* plants given as *F* values (*P* values).

	R	M	R × M
Height	0.004 (0.948)	5.147 (0.037)	3.992 (0.063)
Basal diameter	5.251 (0.036)	10.905 (0.004)	8.079 (0.012)
Shoot	4.792 (0.044)	23.773 (<0.001)	10.868 (0.005)
Root	8.541 (0.010)	15.898 (0.001)	3.253 (0.090)
Foliar N	4.291 (0.050)	17.351 (0.001)	6.726 (0.020)
Foliar P	16.105 (0.001)	8.287 (0.011)	9.594 (0.007)
Foliar K	6.085 (0.025)	28.952 (<0.001)	10.645 (0.005)
Mycorrhizal roots	0.729 (0.406)	62.487 (<0.001)	0.729 (0.406)

performance of *C. albidus* plants, even though there was more available P in the rhizosphere soil treated with fermented SB residue than that of plants inoculated with *P. tinctorius*. Thus, the growth of inoculated plants was about 33% greater than that of plants grown in the amended soil and about 131% greater than control plants (Table 5). Greater mycorrhizal colonization in the inoculated seedlings grown in amended and non-amended soils contributed to an improvement of the performance of the *C. albidus* plants (Table 5). In contrast, there was no natural colonization in the non-inoculated seedlings.

As suggested by several authors, mycorrhizal fungi may improve the performance of seedlings, either by stimulating water uptake (Augé 2001), by producing growth-promoting substances or by increasing nutrient uptake (Querejeta *et al.* 1998). The most N and K in shoot tissue was observed in the inoculated seedlings, which might explain why the growth of *C. albidus* was best with this treatment. The fact that the foliar P content of plants inoculated with *P. tinctorius* and non-inoculated plants grown in the amended soil were similar, reaffirms the key role of mycorrhizae in sustaining the plant cover in P-deficient soils, as well as showing the necessity of including mycorrhizal inoculation to guarantee plant performance in revegetation programmes. On the other hand, the benefit of the combined treatment on growth of *C. albidus* seedlings was not additive and was similar to that from each treatment individually. This result agrees with the widely accepted idea that mycorrhizae present little advantage to seedlings grown in P-enriched soils (Yanai *et al.* 1995).

CONCLUSION

In the short-term, the addition of the fermented SB residue in the presence of rock phosphate was the most effective treatment for improving physical, chemical, microbiological and biochemical qualities of the soil, and led to enhanced plant growth. The mycorrhizal inoculation alone was not sufficient to restore soil quality but was the most effective treatment for improving the performance of *C. albidus* plants. Finally, the combined treatment of mycorrhizal inoculation of seedlings and addition of fermented SB residue to the soil produced effects on plant growth similar to each treatment applied individually, and effects on soil quality similar to the addition of fermented SB residue alone.

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