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Influence of an organic amendment comprising saprophytic and mycorrhizal fungi on soil quality and growth of *Eucalyptus globulus* in the presence of sewage sludge contaminated with aluminium

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The single application of either sewage sludge with high aluminium concentration, wheat straw or the mixture of both residues to soil did not increase the growth of *Eucalyptus globulus* Labill. However, inoculation with either the arbuscular mycorrhizal (AM) fungus *Rhizophagus irregularis*, the saprobe fungi *Coriolopsis rigida* (Berk. Et Mont.) Murrill and *Phanerochaete chrysosporium* Burds or the combination of each saprobe with the AM fungus increased both the P concentration and dry weight of *E. globulus* shoots. These effects were greater in the presence of wheat straw or sewage sludge, but were greatest in the presence of the mixed residue. *Phanerochaete chrysosporium* had the greatest effect on plant dry weight when co-inoculated with *R. irregularis* in the treatment with mixed residue. The co-inoculation of AM and saprobe fungi increased fluorescein diacetate and β-glucosidase activities in the bulk soil of *E. globulus* grown in the treatment with mixed residue. However, only the AM fungus increased dehydrogenase activity, and phosphatase activity was similar in all treatments tested. Our results showed that sewage sludge with high aluminium concentration could be used as a soil amendment to improve the growth of *E. globulus* when mixed with wheat straw and co-inoculated with saprobe and arbuscular fungi.

Keywords: arbuscular mycorrhizae; biological fertilisers; saprobe fungi; sewage sludge; soil biochemical parameters; wheat straw

Introduction

Agricultural soils are often subject to a decrease in organic matter due to agricultural practices that can alter the soils' physical, chemical and biological properties, and this can lead to a decrease in soil fertility. The use of sewage sludge as an organic soil amendment in agriculture is a widespread practice because the sewage sludge provides high levels of organic matter and plant nutrients, which can increase soil fertility (Hargreaves et al. 2008; Fagnano et al. 2011; Gonzalez-Ubierna et al. 2012). However, the potential presence of heavy metals in sewage sludge and the effects of these contaminants on plant and soil fertility are well known (Tella et al. 2013). High aluminium (Al) concentration has been found in sewage sludge (Busetti et al. 2005; Üstün 2009). In acidic conditions, Al phytotoxicity is one of the major factors limiting crop production (Kochian 1995; Von Uexküll & Mutert 1995).

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The use of soil microorganisms is a suitable method to improve soil fertility and plant protection against the deleterious effect of soil contaminants such as Al (Batty & Dolan 2013). Arbuscular mycorrhizal (AM) fungi are the most extended symbiosis among plant roots and many studies have demonstrated the efficiency of AM fungi in improving plant growth mainly by higher P uptake from soil through their external mycelium (Smith & Read 2008). AM fungi play a crucial role in plant tolerance against environmental stress as metal contamination, as Al resistance in plants has been ascribed to mycorrhizal symbiosis (Kanu et al. 2013; Seguel et al. 2013). Studies have shown that the addition of organic fertiliser can either reduce or increase the growth of AM fungi (Gryndler et al. 2009; Hammer et al. 2011; Eschen et al. 2013). Most studies have found that sewage sludge reduces both the pre-symbiotic and in-plant stages of the development of mycorrhizal fungus (Jacquot-Plumey et al. 2003; Ghanavati et al. 2012). In addition to AM fungi, saprophytic fungi are another important group of microorganisms that can provide energy for other microorganisms, including AM fungi, by breaking down cellulosic materials into simple sugars (Radford et al. 1996). Some saprobe fungi increase the effectiveness of AM fungi in root colonisation and in the promotion of both plant growth and resistance against Al phytotoxicity (Arriagada et al. 2007; Vaz et al. 2012). Saprobe fungi can also increase the metabolic activity of AM fungi inside the root in the presence of sewage sludge (Arriagada et al. 2009).

The effects of sewage sludge as a fertiliser can be improved by applying different doses of wheat straw. In fact, the application of wheat straw to sludge increases N mineralisation and nutrient availability to plants and helps to reduce the toxicity of heavy metals (Jia et al. 2008; Juwarkar & Jambhulkar 2008). Additionally, application of sewage sludge increases the biomass content and the enzymatic activities of microbes in the soil. Soil enzymes such as fluorescein diacetate hydrolase (FDA), β-glucosidase, phosphatase and dehydrogenase can be used as indicators of soil quality and microbial activity, because these enzymes are highly sensitive to alterations in soil management (Paz-Ferreiro et al. 2012).

Because of the positive impacts of mycorrhizal and saprobe fungi on soil fertility and plant growth, the AM fungus *Rhizophagus irregularis* and the saprobe fungi *Coriolopsis rigida* and *Phanerochaete chrysosporium* were examined for beneficial effects on FDA, β-glucosidase, dehydrogenase and phosphatase activities in the soil and on the growth and nutrient concentrations of *Eucalyptus globulus* shoots. The effects of these fungi were also analysed together with the application of sewage sludge with high Al concentration, wheat straw or a combination of sewage sludge and wheat straw, because these components have also been shown to have positive impacts on soil fertility and plant growth.

Materials and methods

Soil characteristics and measurements

Soil samples were collected at 0–20 cm depth from the Maquehue Experimental Station (38° 50′ S, 72° 41′ W) of La Frontera University, Araucania Region, Southern Chile. The soil is classified as Andisol (Acrudoxic Hapludands) from an agricultural area with pH 5.35. Soil pH was analysed in H_2O suspensions (1:2.5 w:v) at the beginning and end of experiment. Organic matter was estimated by wet digestion using the Walkley and Black (1934) method. The total organic C concentration was determined using dichromate oxidation followed by titration with ferrous ammonium sulphate. Olsen P was measured using the Olsen P test, in which inorganic P was extracted from soil with 0.5 $NaHCO_3$ at

pH 8.5 (Olsen & Sommers 1982); total P was determined using the alkaline oxidation method of Dick and Tabatabai (1977). Total N concentration was obtained using the Kjeldahl method. Exchangeable cations (Ca^{2+}, Na^{+}, Mg^{2+} and K^{+}) were extracted with 1 M ammonium acetate at pH 7.0 (Hendershot et al. 2007) and exchangeable Al was extracted with 1 M KCl analysed by atomic absorption spectroscopy using a Perkin Elmer 3110 atomic absorption spectrometer (Perkin Elmer, Norwalk, CT, USA). Available K and total concentrations of heavy metals were determined as described by Mingorance (2002). The mean values of chemical characteristics for soil samples are listed in Table 1.

Organic residues

Sewage sludge was collected from a wastewater plant (Vilcún, Chile) and stored at 4°C until use. The wastewater plant produces sewage sludge that has been aerobically digested and dried to 19% dry matter. Wheat straw was obtained from crop residues from the Araucanía Region. Wheat straw was mixed with sewage sludge (1:3 w:w) and called mixed residue. Standard methods (Dane & Topp 2002) were used to determine the principal properties of wheat straw, sewage sludge and mixed residue samples; each sample was analysed in triplicate.

Plant species

After surface sterilisation and a thorough rinse with sterilised water, *E. globulus* Labill seeds were sown in moistened sand. After germination, uniform seedlings were planted in 0.3 l pots (measuring 10 cm of height × 8 cm of diameter) filled with a 1:4 (v:v) mixture of sterilised sand and soil (substrate density 0.8 g cm^{-3}). Plants were grown in growth chambers with supplementary light provided by Sylvania incandescent and cool-white

Table 1. Chemical characteristics of soil.

Parameter	Amount
Soil organic matter (%)	12
pH (H_2O)	5.35
C (g kg^{-1})	60
Total P (mg kg^{-1})	1270
Olsen-P (mg kg^{-1})	8.01
Available-K (mg kg^{-1})	414
Total N (g kg^{-1})	2.3
Exchangeable Ca (cmol kg^{-1})	30.87
Exchangeable Na (cmol kg^{-1})	0.24
Exchangeable Mg (cmol kg^{-1})	5.97
Exchangeable K (cmol kg^{-1})	1.12
Exchangeable Al (cmol kg^{-1})	0.03
CEC* (cmol kg^{-1})	38.23
Al-saturation (%)	0.06
Extractable Al (mg kg^{-1})	1891
Total Fe (mg kg^{-1})	48.1
Total Cu (mg kg^{-1})	3.4
Total Zn (mg kg^{-1})	2.9

Note: *Cationic exchange capacity = Σ(K, Ca, Mg, Na and Al).

lamps (400 E m^{-2} s^{-1}; 400–700 nm) with a 16/8 h day/night cycle at 25/19°C and 50% relative humidity.

AM inoculation

The mycorrhizal fungus *R. irregularis* was used for inoculation (Krüger et al. 2012). The mycorrhizal fungal inoculum used in these experiments was a mixture of soil spores and root fragments of *Medicago sativa* L. grown in pots. The inoculum was applied in the amount of 2.7 g per 100 g of soil, which has been previously determined to achieve high levels of root colonisation (approximately 1000 spores per 100 g). Non-AM-fungus-inoculated pots received a water filtrate (Whatman no. 1 paper) of the AM inoculum which contained common soil microflora but were free of AM fungal propagules.

Saprobe fungi

Two saprobe fungi were used, *C. rigida* (Berk. Et Mont.) Murrill (obtained from the Culture Collection of the Centro de Investigaciones Biológicas (CIB) in Madrid) and *P. chrysosporium* Burds (obtained from the fungal collection of the Laboratorio de Biorremediación, Facultad de Ciencias Agropecuarias y Forestales, Universidad de La Frontera, Chile). The strains were stored on malt extract agar (MEA) plates at 4°C and periodically sub-cultured. To prepare inoculum, barley seeds (as substrate to form fungal mycelium) were inoculated with MEA (disks of 1 cm^2), withdrawn from 14-day-old fungal cultures grown at 28°C. Once a dense culture of fungal mycelium was generated, 10 barley seeds completely colonised by the fungal mycelium were added per pot to inoculate the soil.

Experimental design

The treatments consisted of

(1) uninoculated controls,
(2) soil inoculated with *C. rigida* or *P. chrysosporium* with or without sewage sludge or wheat straw or mixed residue,
(3) soil inoculated with *R. irregularis* with or without sewage sludge or wheat straw or mixed residue and
(4) soil inoculated with *C. rigida* or *P. chrysosporium* with or without *R. irregularis* and with or without sewage sludge or wheat straw or mixed residue.

The plants were inoculated with AM (inoculum obtained from *M. sativa*) and saprobe fungi (inoculated on barley seeds) when they were transplanted (after 4 weeks of growth). One seedling was planted per pot and six replicates were performed per treatment. Sewage sludge was applied to *Eucalyptus* pots at a concentration of 4 g per 100 g of soil (dry matter basis). Wheat straw was chopped into 1–2 cm pieces and applied at a concentration of 1.5 g per 100 g of soil. The mixed residue was applied at a concentration of 1.5 g wheat straw and 4 g of sewage sludge per 100 g of soil.

Plant analysis

The *Eucalyptus* plants were harvested after 16 weeks and dry biomass (root and shoot) was determined. For determination of nutrient concentration, shoot was ground to pass through a 0.5-mm sieve and digested in a H_2SO_4–H_2O_2 mixture. Phosphorus and N concentrations in the digest were determined as described by Jackson (1973). Potassium (K), magnesium (Mg), calcium (Ca), iron (Fe) concentrations were determined as described by Mingorance (2002).

Fungi analysis

After harvest, two fresh root samples were randomly taken from the entire root system. One of the two root samples was used to determine the percentage of root length colonised by AM fungi using the gridline intersect method (Giovannetti & Mosse 1980) after the sample had been cleared and stained with trypan blue (Phillips & Hayman 1970). The second root sample was used to measure the succinate dehydrogenase (SDH) activity (EC 1.3.99.1) in fungal mycelia through the reduction of tetrazolium salts at the expense of added succinate (MacDonald & Lewis 1978); the percentage of AM fungal mycelia with SDH activity was determined under a compound microscope Nikon Eclipse E200 (Nikon, Tokyo, Japan) (Ocampo & Barea 1985).

Biochemical determinations

In each pot six samples were randomly collected for biochemical analyses, approximately 10 g of soil per pot was used. FDA was assessed as described by Adam and Duncan (2001) and expressed as μg fluorescein g^{-1} soil. β-glucosidase activity was determined by measuring *p*-nitrophenol released from *p*-nitrophenyl-β-D-glucopyranoside (PNG) according to the method of Eivazi and Tabatabai (1990) and expressed as μmol *p*-nitrophenol g^{-1} dry soil h^{-1}. Acid phosphatase was measured using the same procedure as β-glucosidase, but using *p*-nitrophenyl phosphate (PNPP) instead of PNG (Sannino & Gianfreda 2001). Dehydrogenase activity was determined according to the method described by Casida et al. (1964) and expressed as μg TPF (triphenyl formazan) g^{-1} dry soil h^{-1}. The average and standard deviations of the enzymatic activities from triplicate cultures are reported.

Statistical analysis

All data expressed as a percentage were arcsine-square root transformed before statistical analysis. The data were analysed using factorial design analysis of variance with AM fungi treatment (control, *R. irregularis*), saprobe fungi treatment (control, *C. rigida* and *P. chrysosporium*), organic residue treatment (control, sewage sludge, wheat straw and mixed residue) and their interactions as sources of variation. Statistical procedures were carried out with the software package SPSS 11.0 for Windows (Aranaz 1996). Means were compared using Tukey's multiple range test. Statistical significance was determined at $p < 0.05$. Prior to statistical analysis, data were tested for normality and homogeneous variances.

Results

The nutritional values of the organic residues applied to the soil are summarised in Table 2. After 16 weeks of application of wheat straw, sewage sludge or mixed residue to the soil, the pH of the soil changed from 5.35 (without amendment) to 5.43, 6.16 and 6.63, respectively. The statistical results of the factorial analyses can be found in Table 3. Application of wheat straw, sewage sludge or mixed residue to the soil without fungal inoculation did not increase the dry weight of *E. globulus* shoots (Figure 1). Inoculation of *R. irregularis* increased the dry weight of *E. globulus* shoots in all treatments, with the highest increase obtained from the addition of mixed residue. Inoculation with the saprobe fungi *C. rigida* or *P. chrysosporium* increased the dry weight of plants when inoculated in the presence of sewage sludge or mixed residue. Inoculation with *C. rigida* or *P. chrysosporium* also increased the dry weight of *E. globulus* shoots when colonised together with *R. irregularis*. The effects of each saprobe fungus on the dry weight of *E. globulus* shoots colonised with *R. irregularis* were increased in the presence of wheat straw, sewage sludge or mixed residue. The most dramatic effect on the dry weight of *E. globulus* shoots was observed when *P. chrysosporium* was co-inoculated with *R. irregularis* in the presence of mixed residue (Figure 1).

The dry weight of *Eucalyptus* roots increased when grown in soil amended with sewage sludge, whether or not it was inoculated with *R. irregularis* (Figure 2). *Coriolopsis rigida* had no effect on the dry weight of roots in any of the treatments; however, dual treatment of *C. rigida* together with *R. irregularis* increased the dry weight of *E. globulus* roots when plants were grown in the presence of sewage sludge or mixed residue. In contrast to *C. rigida*, inoculation with *P. chrysosporium* alone had a stimulating effect on the growth of *E. globulus* roots when grown in the presence of sewage sludge. Dual treatment of *P. chrysosporium* together with *R. irregularis* also increased the dry weight of *E. globulus* roots when plants were grown in the presence of sewage sludge or mixed residue.

The percentage of root length colonisation of *E. globulus* by AM decreased when plants were grown in the presence of sewage sludge (Figure 3). Inoculation with either saprobe fungi did not have a significant effect on the percentage of root length colonisation of *E. globulus* by AM. The co-inoculation of both arbuscular and saprobe fungi increased the percentage of AM root length colonisation in the presence of the mixed residue. Similar levels of SDH activity in *E. globulus* roots were observed whether or not residues were applied (Figure 3). However, internal AM mycelium in the root of

Table 2. Chemical characteristics of mixed residue.

Parameter	Amount
pH	8.1
C (%)	36.5
Total N (%)	2.15
Total P (mg kg^{-1})	1124
Total K (mg kg^{-1})	2780
Extractable Al (mg kg^{-1})	9679
Total Zn (mg kg^{-1})	733
Total Cu (mg kg^{-1})	750
Total Mn (mg kg^{-1})	187
Total As (mg kg^{-1})	2.5
Total Pb (mg kg^{-1})	59.7

Table 3. Significance of the main treatment effects and their interaction based on factor ANOVA.

	F-values						
	AM	OR	SF	AM × OR	AM × SF	OR × SF	AM × OR × SF
Shoot dry weight	73.24**	221.93**	1.63**	17.19**	14.25**	1.08*	23.19**
Root dry weight	20.35 (ns)	152.7*	0.53 (ns)	6.27 (ns)	5.31 (ns)	0.53 (ns)	8.61*
β-glucosidase	35.19 (ns)	116.1 (ns)	0.95*	8.37 (ns)	10.13*	0.82*	9.27*
Fluorescein diacetate	83.26**	97.18 (ns)	0.65 (ns)	7.61 (ns)	7.33 (ns)	0.49 (ns)	8.63*
Dehydrogenase	110.1*	104.82 (ns)	0.71 (ns)	4.20 (ns)	6.12 (ns)	0.37 (ns)	6.24*
Phosphatase	19.33 (ns)	86.69 (ns)	0.47 (ns)	3.97 (ns)	5.71 (ns)	0.33 (ns)	5.99*

Notes: AM, arbuscular mycorrhiza; OR, organic residue; SF, saprobe fungi.
(ns), not significant; *significant at $p < 0.05$; **significant at $p < 0.01$.

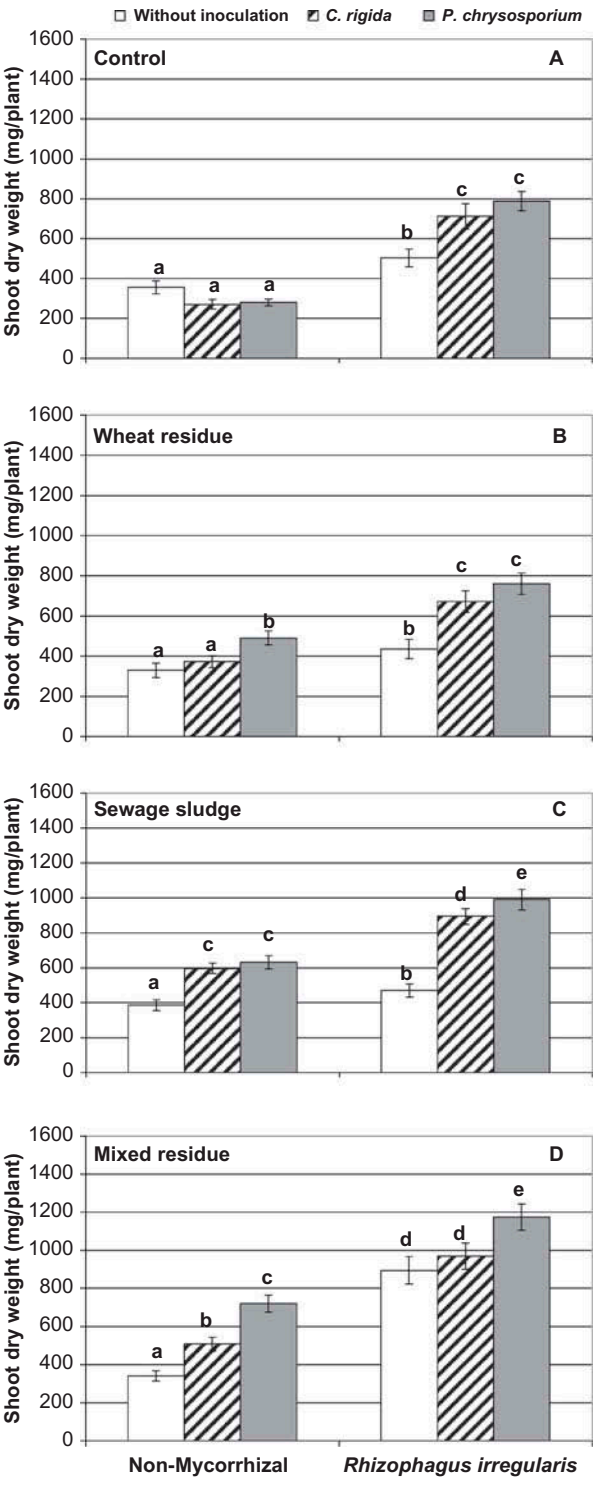

Figure 1. Shoot dry weight of *Eucalyptus globulus* inoculated with the AM fungus *Rhizophagus irregularis* and the saprobe fungi *Coriolopsis rigida* and *Phanerochaete chrysosporium* in soil amended with wheat straw, sewage sludge or mixed residue. Residue type: A = Without residue; B = Wheat straw; C = Sewage sludge; and D = Mixed residue. The data are the means ± standard errors of means ($n = 6$). Bars with the same letter are not significantly different ($p < 0.05$).

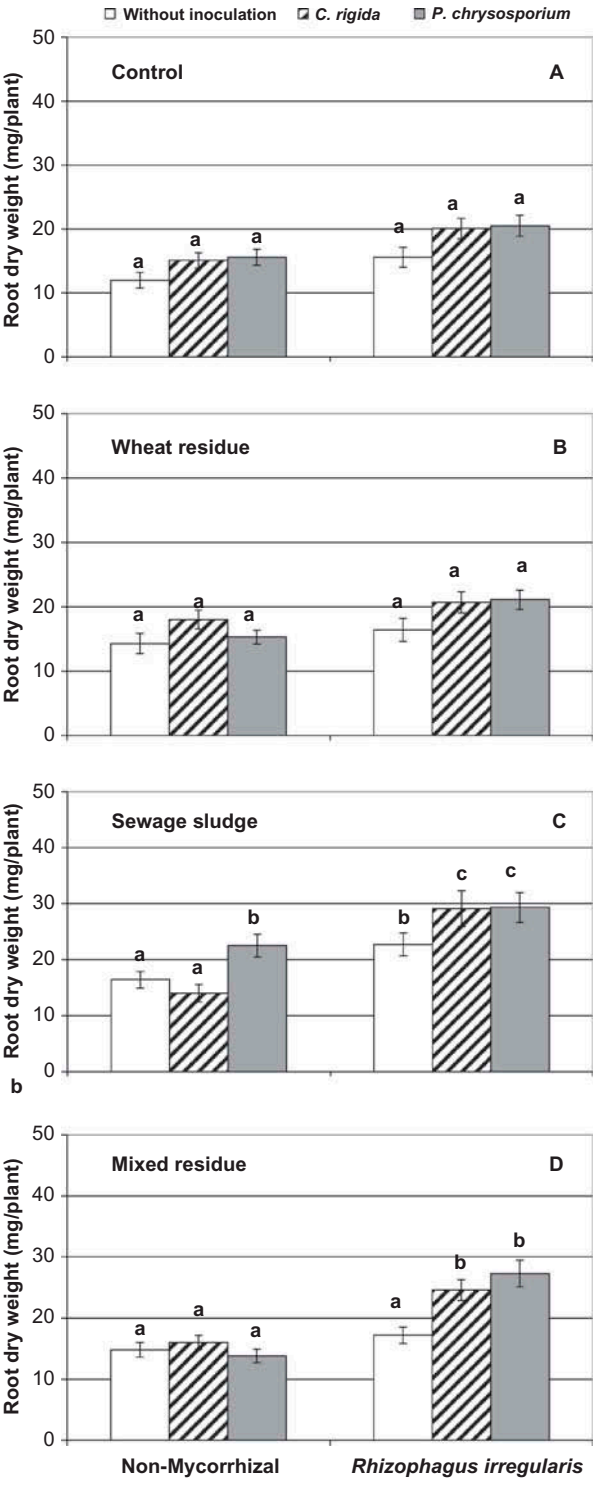

Figure 2. Root dry weight of *Eucalyptus globulus* inoculated with the AM fungus *Rhizophagus irregularis* and the saprobe fungi *Coriolopsis rigida* and *Phanerochaete chrysosporium* in soil amended with wheat straw, sewage sludge or mixed residue. Residue type: A = Without residue; B = Wheat straw; C = Sewage sludge; and D = Mixed residue. The data are the means ± standard errors of means ($n = 6$). Bars with the same letter are not significantly different ($p < 0.05$).

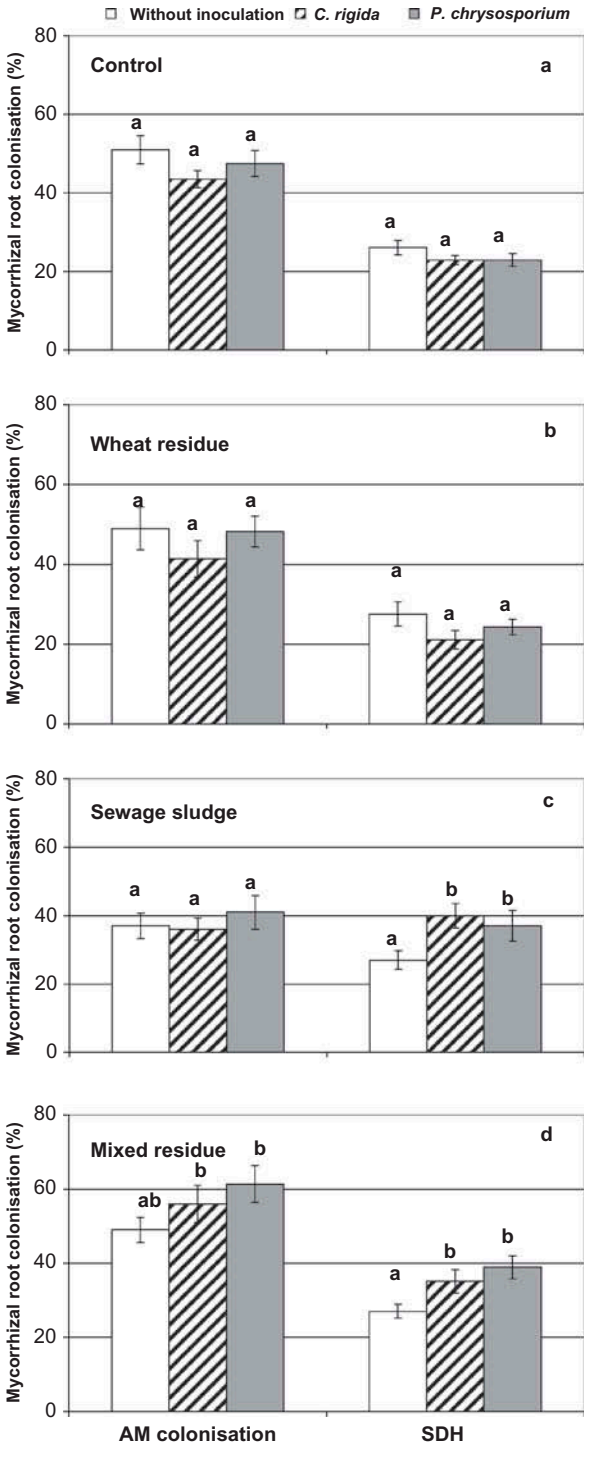

Figure 3. Arbuscular mycorrhizal root length colonisation and arbuscular mycorrhizal mycelium with succinate dehydrogenase (SDH) activity of *Eucalyptus globulus* inoculated with the AM fungus *Rhizophagus irregularis* and the saprobe fungi *Coriolopsis rigida* and *Phanerochaete chrysosporium* in soil amended with wheat straw, sewage sludge or mixed residue. Residue type: A = Without residue; B = Wheat straw; C = Sewage sludge; and D = Mixed residue. The data are the means ± standard errors of means (n = 6). Bars with the same letter are not significantly different ($p < 0.05$).

E. globulus had higher SDH activity when co-inoculated with saprobe fungi in the presence of sewage sludge or mixed residue (Figure 3).

The application of wheat straw, sewage sludge or mixed residue to the soil did not increase the FDA (Figure 4). Inoculation with *R. irregularis* increased the FDA activity in the bulk soil of *E. globulus* in the presence of all organic residues, but no significant differences between them were observed. The co-inoculation of *C. rigida* or *P. chrysosporium* together with *R. irregularis* in the presence of mixed residue was the most effective in increasing the FDA.

Increased levels of β-glucosidase activity in the bulk soil of *E. globulus* were observed with the addition of wheat straw, sewage sludge or mixed residue. Inoculation of the soil with *R. irregularis* alone did not increase the β-glucosidase activity in the presence of any of the three residues in soil without saprobe fungi. Inoculation of the soil with AM fungi combined with *C. rigida* or *P. chrysosporium* increased the β-glucosidase activity in the soil samples of plants grown in the presence of wheat straw or mixed residue. Inoculation with either *C. rigida* or *P. chrysosporium* saprobe fungi together with *R. irregularis* in the presence of the mixed residue induced the greatest increase in the β-glucosidase activity of soil samples (Figure 5).

The addition of the different organic residues to the soil had a negligible effect on phosphatase activity. Similarly, inoculation of the soil with the AM fungi *R. irregularis* or the saprobe *C. rigida* and *P. chrysosporium* did not induce any change in phosphatase activity (Figure 6).

The addition of the different organic residues to the soil did not increase the dehydrogenase activity. Inoculation of the soil with *R. irregularis* increased dehydrogenase activity compared to the non-AM-fungus-inoculated control, but the saprobe fungi had no significant effect in any of the treatments tested (Figure 7).

Nutrient analysis of *E. globulus* showed that the inoculation of the AM fungus alone or together with the saprobe fungi *C. rigida* or *P. chrysosporium* increased the shoot concentrations of N, P, K, Ca, Mg and Fe in comparison to non-inoculated controls. In addition, shoots of *E. globulus* had the highest concentration of P when co-inoculated with AM and saprobe fungi in the presence of the mixed residue (Table 4).

Discussion

Studies about the use of sewage sludge as an organic fertiliser have been shown to benefit plant growth (Khan et al. 2013). However, in the present study, no beneficial effects of sewage sludge were observed on either the dry plant matter or the nutrient concentration in shoots of *E. globulus.* Sewage sludge from different backgrounds may have different compositions. The sewage sludge used in these experiments contained high levels of Al. It is known that the application of 600 mg kg^{-1} of Al in soil decreases the weight of *E. globulus* (Arriagada et al. 2007). When sewage sludge was applied to the soil pots, the amount of Al reached 290 mg kg^{-1} of soil. Therefore, the high concentration of Al in the sewage sludge may be responsible for the lack of increases in the dry weight and nutrient concentrations of *E. globulus*. In addition, while the application of wheat straw and sewage sludge has been shown to be beneficial to plant growth by increasing the total N supply of the mixture (Jia et al. 2008), the application of wheat straw mixed with sewage sludge had no beneficial effects on either the dry weight or nutrient concentration in the shoots of *E. globulus* in this study.

Several authors have described an inhibition of the effectiveness of AM fungi in improving plant growth from constant application of sewage sludge, most likely due to the accumulation of Al in the soil (Kanu et al. 2013; Seguel et al. 2013). The high Al concentration in the sewage sludge in these studies may explain the observed decrease in

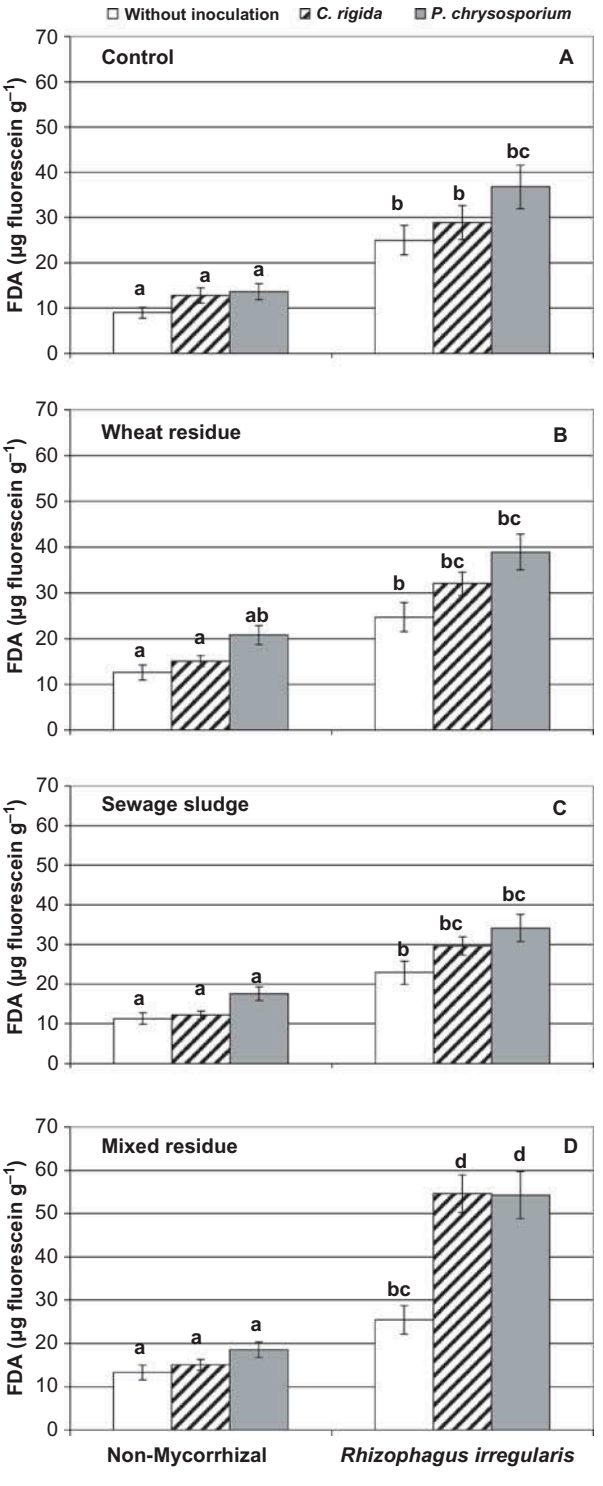

Figure 4. Fluorescein diacetate hydrolase (FDA) activity in the bulk soil of *Eucalyptus globulus* inoculated with the AM fungus *Rhizophagus irregularis* and the saprobe fungi *Coriolopsis rigida* and *Phanerochaete chrysosporium* in soil amended with wheat straw, sewage sludge or mixed residue. Residue type: A = Without residue; B = Wheat straw; C = Sewage sludge; and D = Mixed residue. The data are the means ± standard errors of means (n = 6). Bars with the same letter are not significantly different ($p < 0.05$).

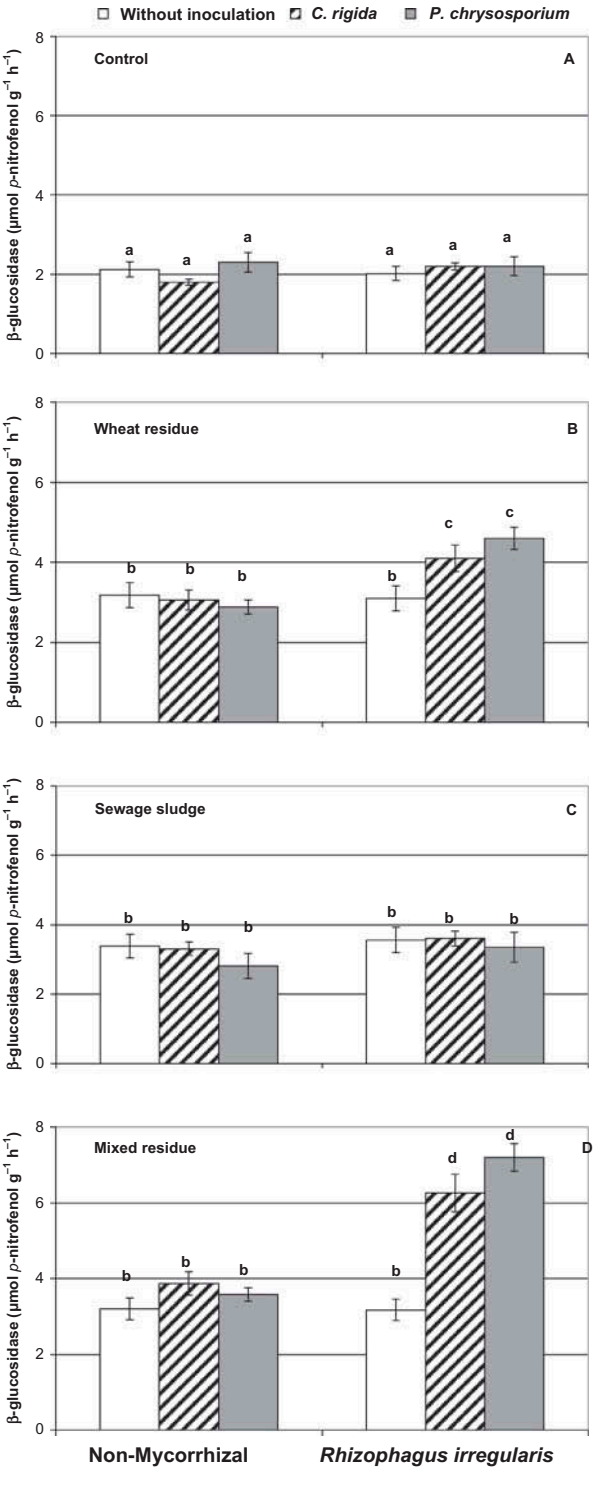

Figure 5. β-glucosidase activity in the bulk soil of *Eucalyptus globulus* inoculated with the AM fungus *Rhizophagus irregularis* and the saprobe fungi *Coriolopsis rigida* and *Phanerochaete chrysosporium* in soil amended with wheat straw, sewage sludge or mixed residue. Residue type: A = Without residue; B = Wheat straw; C = Sewage sludge; and D = Mixed residue. The data are the means ± standard errors of means (n = 6). Bars with the same letter are not significantly different ($p < 0.05$).

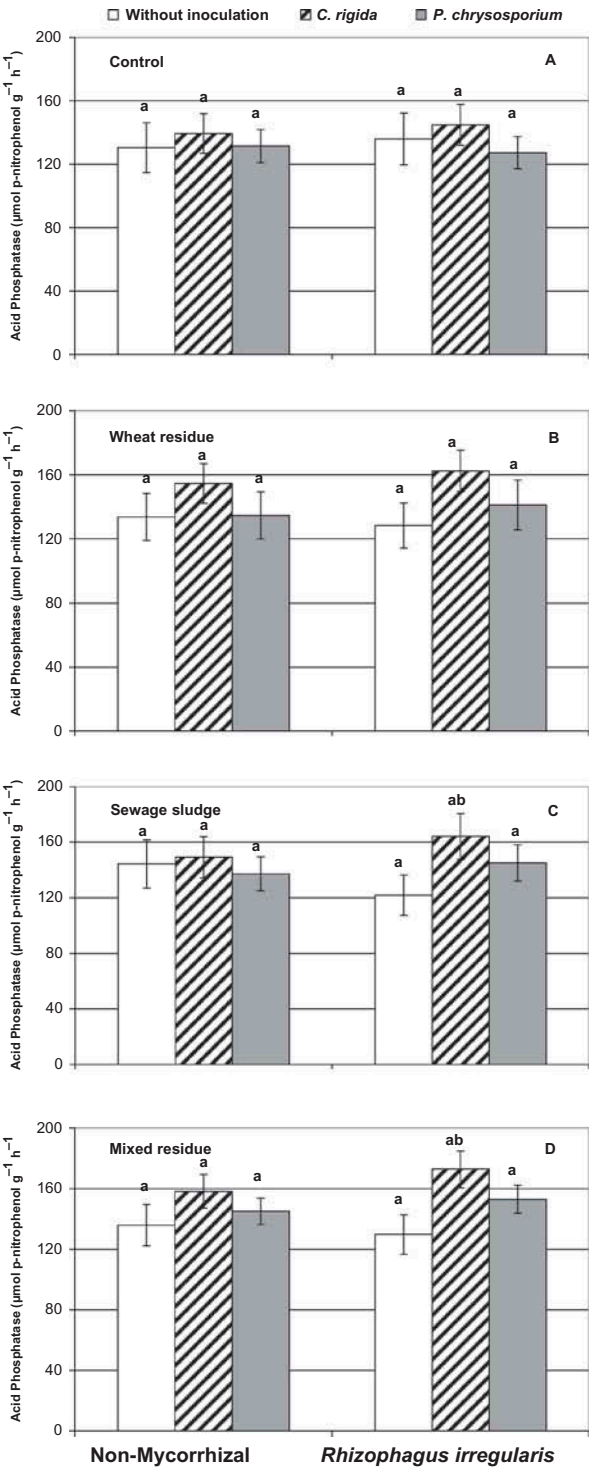

Figure 6. Phosphatase activity in the bulk soil of *Eucalyptus globulus* inoculated with the AM fungus *Rhizophagus irregularis* and the saprobe fungi *Coriolopsis rigida* and *Phanerochaete chrysosporium* in soil amended with wheat straw, sewage sludge or mixed residue. Residue type: A = Without residue; B = Wheat straw; C = Sewage sludge; and D = Mixed residue. The data are the means ± standard errors of means ($n = 6$). Bars with the same letter are not significantly different ($p < 0.05$).

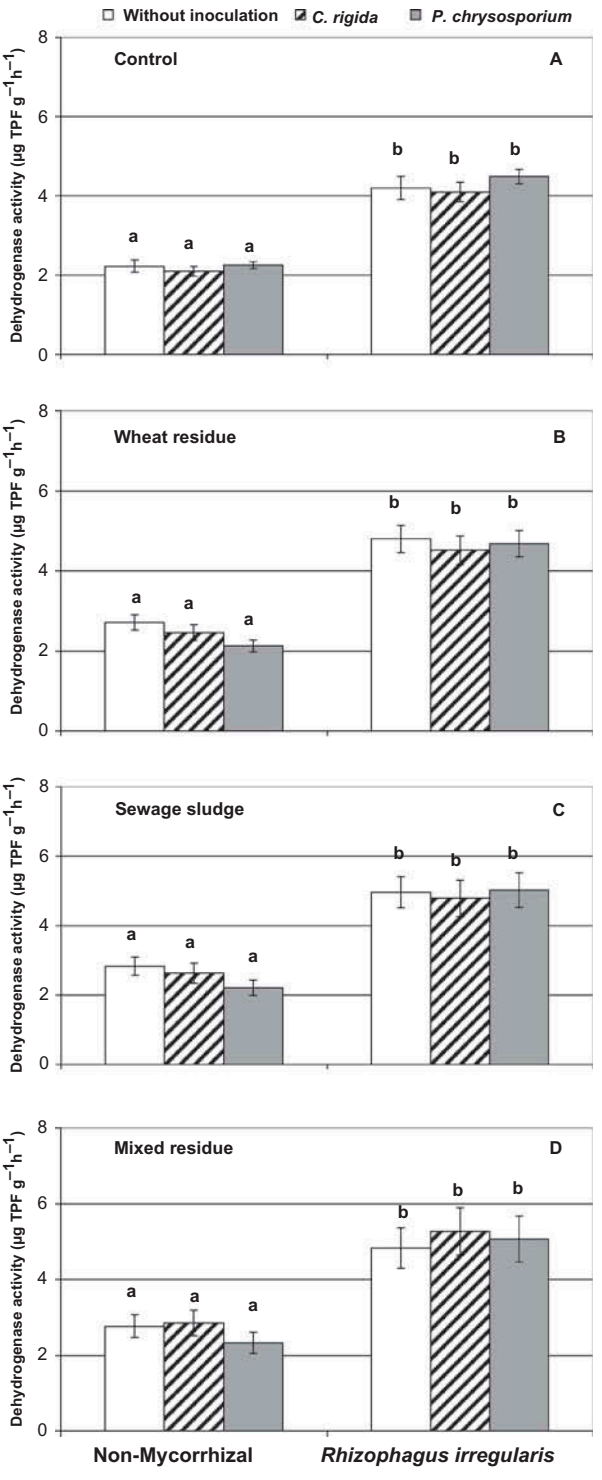

Figure 7. Dehydrogenase activity in the bulk soil of *Eucalyptus globulus* inoculated with the AM fungus *Rhizophagus irregularis* and the saprobe fungi *Coriolopsis rigida* and *Phanerochaete chrysosporium* in soil amended with wheat straw, sewage sludge or mixed residue. Residue type: A = Without residue; B = Wheat straw; C = Sewage sludge; and D = Mixed residue. The data are the means ± standard errors of means ($n = 6$). Bars with the same letter are not significantly different ($p < 0.05$).

Table 4. Mineral concentration (on dry matter basis) in shoots from *Eucalyptus globulus* plants (%) inoculated with the AM fungus *Rhizophagus irregularis* and the saprobe fungi *Coriolopsis rigida* and *Phanerochaete chrysosporium* in soil amended with organic residue. The plants were harvested at 20 weeks after transplanting. Values followed by the same letter are not significantly different as determined by Tukey's multiple range test ($p < 0.05$).

	N%	P	K	Ca	Mg	Fe
Control						
Without inoculation	1.04 ba	0.04 a	1.14 a	0.61 a	0.17 a	183 a
Rhizophagus irregularis	1.37 b	0.13 b	2.02 b	1.14 b	0.35 b	218 b
Coriolopsis rigida	1.05 a	0.04 a	1.13 a	0.72 a	0.22 a	147 a
Phanerochaete chrysosporium	1.01	0.04	1.16	0.70	0.21	153
R. irregularis + *C. rigida*	1.78 c	0.14 b	2.34 b	1.16 b	0.31 b	315 c
R. irregularis + *P. chrysosporium*	1.77 c	0.14 b	2.37 b	1.11 b	0.30 b	308 c
Sewage sludge						
Without inoculation	1.03 a	0.05 a	1.16 a	0.73 a	0.21 a	179 a
Rhizophagus irregularis	1.35 b	0.17 b	2.21 b	1.15 b	0.36 b	235 b
Coriolopsis rigida	1.18 a	0.07 a	1.39 a	0.79 a	0.26 a	194 a
Phanerochaete chrysosporium	1.17 a	0.06 a	1.45 a	0.81 a	0.27 a	206 ab
R. irregularis + *C. rigida*	1.49 b	0.17 b	2.24 b	1.12 b	0.36 b	374 c
R. irregularis + *P. chrysosporium*	1.54 b	0.18 b	2.21 b	1.15 b	0.37 b	359 c
Wheat straw						
Without inoculation	1.02 a	0.04 a	1.15 a	0.65 a	0.18 a	172 a
Rhizophagus irregularis	1.35 b	0.14 b	2.20 b	1.16 b	0.32 b	224 b
Coriolopsis rigida	1.20 ab	0.11 ab	1.31 a	0.75 a	0.25 ab	213 ab
Phanerochaete chrysosporium	1.12 a	0.09 ab	1.18 a	0.83 a	0.25 ab	189 a
R. irregularis + *C. rigida*	1.50 b	0.18 b	2.20 b	1.18 b	0.36 b	326 c
R. irregularis + *P. chrysosporium*	1.75 b	0.17 b	2.28 b	1.18 b	0.35 b	328 c
Mixed residue						
Without inoculation	0.98 a	0.04 a	1.17 a	0.67 a	0.16 a	164 a
Rhizophagus irregularis	1.36 b	0.15 b	2.13 b	1.17 b	0.30 b	237 b
Coriolopsis rigida	1.21 ab	0.10 ab	1.45 a	0.82 a	0.27 ab	216 ab
Phanerochaete chrysosporium	1.20 ab	0.08 a	1.48 a	0.85 a	0.31 b	229 ab
R. irregularis + *C. rigida*	1.60 c	0.21 c	2.99 c	1.90 c	0.39 bc	427 c
R. irregularis + *P. chrysosporium*	1.76 c	0.25 c	3.23 c	2.23 d	0.45 d	493 c

the percentage of AM root length colonisation and the unchanged dry weight of the plant. However, the negative effect of sewage sludge on AM colonisation and its lack of a beneficial effect on plant dry weight were overcome by the application of wheat straw to the residue. It is well known that increasing organic matter in the soil after sludge application contributes to the beneficial effects of AM fungus on plant growth by reducing the toxicity of metals and heavy metals in AM-colonised plants through their immobilisation. Likewise, organic matter and AM fungi both prevent the translocation of heavy metals to the plant (Juwarkar & Jambhulkar 2008). Additionally, in the presence of Al in the soil, mycorrhizal plants have been shown to have increased growth and P uptake when compared to non-mycorrhizal plants (Seguel et al. 2013). These symbionts play a role critical in the protection of roots by avoiding aluminium stress by altering their bioavailability. In addition, in the soil rhizosphere, the mycorrhizal fungi may extract phosphorus from $AlPO_4$ (Cumming & Weinstein 1990) due to changes in soil pH as an aluminium detoxification mechanism. In our research, changes in soil pH from the application of organic residues may be an important factor in Al tolerance for agricultural plants cropped in acidic Chilean soils.

The synergistic effects of AM fungi and *C. rigida* and *P. chrysosporium* saprobe fungi on both the AM root colonisation and the dry weight of plants have already been observed (Arriagada et al. 2009; Medina et al. 2010). In the present study, the saprobe fungi *C. rigida* and *P. chrysosporium* decreased the toxicity of sewage sludge on AM root length colonisation of *E. globulus*, increased the metabolic activity of AM fungi inside *E. globulus* roots and improved the dry weight of the plants. Addition of wheat straw to the sewage sludge increased the benefits of saprobe fungi even more, as this addition further increased the AM root length colonisation, the SDH activity of AM fungi inside roots and the P concentration and growth of shoots of *E. globulus*. Saprobe fungi are able to produce hydrolytic enzymes which degrade organic matter into compounds accessible for plant uptake (polysaccharidases as endoglucanase, endopolymethylgalacturonase and endoxyloglucanase). In addition, the extraradical hyphae of AM fungi are highly efficient in the acquisition and translocation of inorganic compounds (capture of inorganic N and release of inorganic N as NH_4^+) from organic matter to host plants (Hodge et al. 2001; Tribak et al. 2002). Therefore, the increased availability of organic nutrient compounds (polysaccharidases) from the production of hydrolytic enzymes of saprobe fungi is one reason why saprobe fungi may complement the benefits of AM fungi for plant growth (Aranda et al. 2004). However, in the presence of the mixed residue, the highest plant growth took place when *E. globulus* was co-inoculated with *P. chrysosporium* and *R. irregularis*. Differential synergistic effects of various saprobe fungi on the growth of AM plants have already been documented (Fracchia et al. 1998; Medina et al. 2010).

The enzymatic machinery of the saprobe and AM fungi increased the FDA, β-glucosidase and dehydrogenase activities in the soil where the mixed residue was applied. It is known that production of these enzymes are an indication not only of the ongoing metabolic and biological activities of soil microorganisms but also of the microbial hydrolytic processes involved in the breakdown of organic matter, which is important for nutrient availability (Bandick & Dick 1999; Turner et al. 2002).

Conclusions

The application of sewage sludge to soil as an organic fertiliser does not always improve plant growth, because sewage sludge can have high levels of elements such as Al that is not beneficial for plant growth. The application of wheat straw alone did not increase the benefits of the sewage sludge. However, the co-inoculation of AM and saprobe fungi with a mixture of sewage sludge and wheat straw increased the P concentration and growth of *E. globulus* shoots and FDA and β-glucosidase activities in the soil. The combination of saprobe and arbuscular fungi together with wheat straw may allow the use of sewage sludge, even with high aluminium concentration (12,620 mg kg^{-1} of extractable Al), as a biological fertiliser to improve the growth of plants such as *E. globulus*.

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References

Adam G, Duncan H. 2001. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. Soil Biol Biochem. 33:943–951.

Aranda E, Sampedro I, Ocampo JA, García-Romera I. 2004. Contribution of hydrolytic enzymes produced by saprobe fungi on the decrease of plant toxicity caused by water-soluble substances of olive mill dry residue. Appl Microbiol Biotech. 64:132–135.

Arriagada C, Herrera MA, Borie F, Ocampo JA. 2007. Contribution of arbuscular mycorrhizal and saprobe fungi to the aluminum resistance of *Eucalyptus globulus*. Water Air Soil Pollut. 182:383–394.

Arriagada C, Sampedro I, García-Romera I, Ocampo JA. 2009. Improvement of growth of *Eucalyptus globulus* and soil biological parameters by amendment with sewage sludge and inoculation with arbuscular mycorrhizal and saprobe fungi. Sci Total Environ. 407:4799–4806.

Bandick AK, Dick RP. 1999. Field management effects on soil enzyme activities. Soil Biol Biochem. 31:1471–1479.

Batty LC, Dolan C. 2013. The potential use of phytoremediation for sites with mixed organic and inorganic contamination. Crit Rev Environ Sci Technol. 43:217–259.

Busetti F, Badoer S, Cuomo M, Rubino B, Traverso P. 2005. Occurrence and removal of potentially toxic metals and heavy metals in the wastewater treatment plant of Fusina (Venice, Italy). Ind Eng Chem Res. 44:9264–9272.

Casida L, Klein D, Santoro T. 1964. Soil dehydrogenase activity. Soil Sci. 98:371–376.

Cumming JR, Weinstein L. 1990. Utilization of AlPO4 as a phosphorus source by ectomycorrhizal *Pinus rigida* Mill. seedlings. New Phytol. 116:99–106.

Dane JH, Topp C. 2002. Methods of soil analysis. Part 4. Physical Methods Book Series No 5. Madison (WI): Soil Science Society of America.

Dick WA, Tabatabai MA. 1977. Determination of orthophosphate in aqueous solutions containing labile organic and inorganic phosphorus compounds. J Environ Qual. 6:82–85.

Eivazi F, Tabatabai MA. 1990. Factors affecting glucosidase and galactosidase activities in soils. Soil Biol Biochem. 22:891–897.

Eschen R, Müller-Schärer H, Schaffner U. 2013. Plant interspecific differences in arbuscular mycorrhizal colonization as a result of soil carbon addition. Mycorrhiza. 23:61–70.

Fagnano M, Adamo P, Zampella M, Fiorentino N. 2011. Environmental and agronomic impact of fertilization with composted organic fraction from municipal solid waste: a case study in the region of Naples, Italy. Agr Ecosyst Environ. 141:100–107.

Ferrán Aranaz M. 1996. SPSS para Windows. Programación y análisis estadístico. Madrid: McGraw-Hill Interamericana.

Fracchia S, Mujica MT, García-Romera I, García Garrido JM, Martín J, Ocampo JA, Godeas A. 1998. Interaction between *Glomus mosseae* and arbuscular mycorrhizal sporocarps-associate saprophytic fungi. Plant Soil. 200:131–137.

Ghanavati N, Nadian HA, Mohecí AA, Rejali F. 2012. Effects of sewage sludge on growth and nutrients uptake by *Hordum vulgare* as affected by two species of arbuscular-mycorrhizal fungi. Adv Environ Biol. 6:612–617.

Giovannetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84:489–500.

Gonzalez-Ubierna S, Mardomingo IJ, Carrero-González B, de la Cruz MT, Casermeiro MA. 2012. Soil organic matter evolution after the application of high doses of organic amendments in a Mediterranean calcareous soil. J Soils Sediments. 12:1257–1268.

Gryndler M, Hršelová H, Cajthaml T, Havránková M, Řezáčová V, Gryndlerová H, Larsen J. 2009. Influence of soil organic matter decomposition on arbuscular mycorrhizal fungi in terms of asymbiotic hyphal growth and root colonization. Mycorrhiza. 19:255–266.

Hammer EC, Nasr H, Wallander H. 2011. Effects of different organic materials and mineral nutrients on arbuscular mycorrhizal fungal growth in a Mediterranean saline dryland. Soil Biol Biochem. 43:2332–2337.

Hargreaves JC, Adl MS, Warman PR. 2008. A review of the use of composted municipal solid waste in agriculture. Agric Ecosyst Environ. 123:1–14.

Hendershot WH, Lalande H, Duquette M. 2007. Ion exchange and exchangeable cations. In: Carter MR, Gregorich EG, editors. Soil sampling and methods of analysis. Boca Raton (FL): CRC Press; p. 197–206.

Hodge A, Campbell CD, Fitter AH. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature. 413:297–299.
Jackson MI. 1973. Soil chemical analysis. New Delhi: Prentice Hall.
Jacquot-Plumey E, Caussanel JP, Gianinazzi S, Van Tuinen D, Gianinazzi-Pearson V. 2003. Heavy metals in sewage sludge contribute to their adverse effects on the arbuscular mycorrhizal fungus *Glomus mosseae*. Folia Geobot. 38:167–176.
Jia C, Zhang Z, Zhang Y. 2008. Transformation of nitrogen forms during co-composting of sewage sludge and wheat straw. Acta Scien Circum. 28:2269–2276.
Juwarkar AA, Jambhulkar HP. 2008. Phytoremediation of coal mine spoil dump through integrated biotechnological approach. Bioresour Technol. 99:4732–4741.
Kanu SA, Okonkwo JO, Dakora FD. 2013. *Aspalathus linearis* (Rooibos tea) as potential phytoremediation agent: a review on tolerance mechanisms for aluminum uptake. Environ Rev. 21:85–92.
Khan S, Chao C, Waqas M, Arp HPH, Zhu YG. 2013. Sewage sludge biochar influence upon rice (*Oryza sativa* L.) yield, metal bioaccumulation and greenhouse gas emissions from acidic paddy soil. Environ Sci Technol. 47:8624–8632.
Kochian LV. 1995. Cellular mechanisms of aluminum toxicity and resistance in plants. Annu Rev Plant Physiol Plant Mol Biol. 46:237–260.
Krüger M, Krüger C, Walker C, Stockinger H, Schüβler A. 2012. Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytol. 193:970–984.
MacDonald RM, Lewis M. 1978. The occurrence of some acid-phosphatases and dehydrogenases in the vesicular-arbuscular mycorrhizal fungus *G. mosseae*. New Phytol. 80:135–141.
Medina A, Roldán A, Azcón R. 2010. The effectiveness of arbuscular-mycorrhizal fungi and *Aspergillus niger* or *Phanerochaete chrysosporium* treated organic amendments from olive residues upon plant growth in a semi-arid degraded soil. J Environ Manage. 91:2547–2553.
Mingorance MD. 2002. Focused microwave-assisted digestion of vegetal materials for the determination of essential mineral nutrients. Anal Bioanal Chem. 373:153–158.
Ocampo JA, Barea JM. 1985. Effect of carbamate herbicides on VA mycorrhizal infection and plant growth. Plant Soil. 85:375–383.
Olsen SR, Sommers LE. 1982. Phosphorus. In: Page AL, editor. Methods of soil analysis. Part 2. Madison (WI): American Society of Agronomy; p. 403–430.
Paz-Ferreiro J, Gascó G, Gutiérrez B, Méndez A. 2012. Soil biochemical activities and the geometric mean of enzyme activities after application of sewage sludge and sewage sludge biochar to soil. Biol Fertil Soil. 48:511–517.
Phillips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc. 55:158–161.
Radford A, Stone PJ, Taleb F. 1996. Cellulase and amylase complexes. In: Haigler CH, Weimer PJ, editors. Biosynthesis and biodegradation of cellulose. New York (NY): Marcel Dekker; p. 535–597.
Sannino F, Gianfreda L. 2001. Pesticide influence on soil enzymatic activities. Chemosphere. 22:1– 9.
Seguel A, Cumming JR, Klugh-Stewart K, Cornejo P, Borie F. 2013. The role of arbuscular mycorrhizas in decreasing aluminium phytotoxicity in acidic soils: a review. Mycorrhiza. 23:167–183.
Smith S, Read D. 2008. Mycorrhizal symbiosis. 3rd ed. London: Academic Press.
Tella M, Doelsch E, Letourmy P, Chataing S, Cuoq F, Bravin MN, Saint Macary H. 2013. Investigation of potentially toxic heavy metals in different organic wastes used to fertilize market garden crops. Waste Manag. 33:184–192.
Tribak M, Ocampo JA, García-Romera I. 2002. Production of xyloglucanolytic enzymes by *Trichoderma viridae*, *Pleurotus ostreatus*, *Paecilomyces farinosus* and *Wardomyces inflatus*. Mycologia. 94:404–410.
Turner BL, Hopkins DW, Haygarth PM, Ostle N. 2002. β-glucosidase activity in pasture soils. App Soil Ecol. 20:157–162.
Üstün GE. 2009. Occurrence and removal of metals in urban wastewater treatment plants. J Hazard Mater. 172:833–838.

Vaz ABM, Sampedro I, Siles J, Vasquez JA, García-Romera I, Vierheilig H, Rosa CA, Ocampo JA. 2012. Arbuscular mycorrhizal colonization of *Sorghum vulgare* in presence of root endophytic fungi of *Myrtus communis*. App Soil Ecol. 61:288–294.

Von Uexküll HR, Mutert E. 1995. Global extent, development and economic-impact of acid soils. Plant Soil. 171:1–15.

Walkley A, Black IA. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37:29–38.