Soil Use and Management

Soil Use and Management, March 2015, **31,** 155–164 doi: 10.1111/sum.12160

Effect of mixing soil saprophytic fungi with organic residues on the response of *Solanum lycopersicum* to arbuscular mycorrhizal fungi

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Abstract

The effect of the dual inoculation with arbuscular mycorrhizal (AM) and saprophytic fungi and a combination of wheat straw and sewage sludge residues were studied by determining their effect on dry weight of tomato and on chemical and biochemical properties of soil. Incubation of organic residue (sewage sludge combined with wheat straw) with saprophytic fungi and plant inoculation with mycorrhizal fungi was essential to study plant growth promotion. Soil application of organic residues increased the dry weight of tomato inoculated with *Rhizophagus irregularis*. The greatest shoot dry mass was obtained when the organic residues were incubated with *Trichoderma harzianum* and applied to AM plants. However, the greatest percentage of root length colonized with AM in the presence of the organic residues was obtained with inoculation with *Coriolopsis rigida*. The relative chlorophyll was greatest in mycorrhizal plants regardless of the presence of either saprophytic fungus. The presence of the saprophytic fungi increased soil pH as the incubation time increased. Soil nitrogen and phosphorus contents and acid phosphatase were stimulated by the addition of organic residues, and contents of N and P. Total N and P content in soil increased when the organic residue was incubated with saprobe fungi, but this effect decreased as the incubation period of the residue with saprobe fungi increased. The same trend was observed for soil β-glucosidase and fluorescein diacetate activities. The application of organic residues in the presence of AM and saprophytic fungi seems to be an interesting option as a biofertilizer to improve plant growth and biochemical parameters of soils.

Keywords: *Coriolopsis rigida*, mycorrhizal fungi, sewage sludge, *Trichoderma harzianum*, wheat straw

Introduction

The application of organic amendments improves chemical, physical and biological soil properties increasing soil fertility and crop production (Cuevas *et al.*, 2006). Among the different types of organic residues used as amendments in agroforestry systems, crop residues and sewage sludge from wastewater treatment plants are widely used. The effect of sewage sludge as fertilizer can be improved by applying wheat straw. The input of carbon provided by wheat straw stabilizes the C:N ratio, which is an important parameter in the biodegradation process, avoiding nitrogen losses and also improving fungal colonization and stabilizing pH, improving the development of microorganisms (Barrington *et al.*, 2002; Huang *et al.*, 2004).

Arbuscular mycorrhizal (AM) fungi are an important group of microorganisms that can improve plant growth by enhancing nutrient and water uptake, especially for phosphorus uptake from soils with high adsorption capacity such as Andisols (Smith & Read, 2008; Borie *et al.*, 2010). Saprobe fungi are another important group of micro organisms which, by breaking down cellulosic materials to simple sugars, can provide energy sources for other micro organisms, including AM fungi (Radford *et al.*, 1996). Some saprobe fungi such as *Coriolopsis rigida* and *Trichoderma harzianum* have been shown to increase the effectiveness of root colonization by AM fungi and plant growth (Arriagada *et al.*, 2009a,b). Among saprobe fungi, *C. rigida* is capable of degrading a variety of compounds usually resistant to

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Received January 2014; accepted after revision November 2014

microbial action, such as lignin and other phenolic substances, mainly due to the production of broad-spectrum enzymes (Baldrian, 2008).

The measurement of enzyme activity is an efficient indicator of soil quality. Hydrolysis of fluorescein diacetate (FDA) is used to estimate microbiological activities of soil reflecting the activity of hydrolases involved in organic matter degradation (Sanchez-Monedero *et al.*, 2008). Phosphatase is responsible for the mineralization of organic phosphorus increasing its availability to plants (Amador *et al.*, 1997). β-glucosidase is involved directly in the carbon cycle and degradation of organic matter (Turner *et al.*, 2002). Dehydrogenase is considered to be a general index of biological activity on account of its role in the respiratory metabolism of microorganisms (Delgado *et al.*, 2004).

The aim of this work was to evaluate the effect of the application of wheat straw and sewage sludge on tomato dry weight and on chemical and biochemical properties of rhizosphere soil. The use of AM and saprobe fungi in combination with wheat straw and sewage sludge residues to improve their effect as soil amendment was expected.

Materials and methods

Microorganisms

Native strains of *Coriolopsis rigida* (CECT 20449) and *Trichoderma harzianum* were obtained from the fungal culture collection of the Bioremediation Laboratory, Universidad de La Frontera, Temuco, Chile. Stock cultures of the fungi were stored in PDA slants at 4 °C and periodically subcultured.

The arbuscular mycorrhizal fungus, *Rhizophagus irregularis* (Krüger *et al.*, 2012), was obtained from the collection of the Bioremediation Laboratory, Facultad de Ciencias Agropecuarias y Forestales, Universidad de La Frontera, Temuco, Chile.

Soil and residues

The experiments were carried out in a soil classified as Andisol (Acrudoxic Hapludands), moderately acidic, that was obtained from Rucamanque field, Temuco (Chile). Wheat straw corresponded to crop residues of Araucanía Region and the stabilized sewage sludge was collected from a municipal wastewater plant ESSAL S.A., Osorno, Chile. Cellulose, hemicellulose and lignin were determined according to Goering *et al.* (1970). Carbon and nitrogen contents were determined using a Flash EA 1112 Series LECO-TRUSPEC elemental analyzer. Sodium, Cu, Zn, Cd and P concentrations were analysed by plasma emission spectroscopy (ICP). Each sample was analysed in triplicate (Table 1).

Substrate inoculation

For *C. rigida* inoculum, one slant of active mycelia from stock culture was diluted in 40 mL of sterile distilled water that was homogenized and strongly agitated; 10 mL of this suspension, equivalent to 70 mg of dry mycelium, was added to the organic residues. *T. harzianum* was grown on a slant and spores were scraped in sterile distilled water; 3 mL of spore suspension, equivalent to 1.8×10^6 spores, was spread over the surface of the organic residues. Sterilized slants were added to noninoculated controls.

Each fungus was added to flasks with 40 g of sterilized organic residues (10 g wheat straw + 30 g sewage sludge), in a 250-mL Erlenmeyer flask autoclaved at 121 °C for 20 min. After the inoculation, the substrate was incubated for 0 (24 h after inoculation), 2 and 4 weeks at 23 °C in the darkness.

Table 1 Chemical characteristics of wheat straw, sewage sludge and soil

	Wheat straw	± SE	Sewage sludge	± SE	Mixture	± SE	Soil	± SE
Cellulose (%)	43	1.1	1.96	0.01	18	0.76	–	–
Hemicellulose (%)	30	1.7	11	0.21	28	1.9	–	–
Lignin (%)	9	0.2	1.3	0.01	5.4	0.39	–	–
C:N ratio	87	2.9	8.5	0.7	11.9	0.91	–	–
pH	5.5	0.12	12	0.59	8.3	0.81	5.7	0.1
Organic matter (%)	–	–	81	3.7	–	–	12.3	0.73
Carbon (%)	46	1.3	34	2.3	26	2.4	10.2	0.83
Total N (%)	0.5	0.07	0.6	0.14	0.8	0.18	0.002	0.00001
Total P (mg/kg)	1.5	0.01	19 500	227	5000	107.2	8.0[a]	0.67
Na (cmol/kg)	191	6.4	1612	47	819	26.1	0.2	0.001
Cu (mg/kg)	2.5	0.09	113	3.7	39	2.3	3.4	0.01
Zn (mg/kg)	5.8	0.18	399	6.1	128	3.9	2.9	0.11
Cd (mg/kg)	<0.025	0.0001	0.60	0.01	<0.025	0.001	<0.025	0.001

Data are means ± standard error. [a]Olsen P.

Greenhouse experiments

The experiments were carried out using tomato (*Solanum lycopersicum* L.) as test plant. Seeds were surface-sterilized with NaClO at 0.5% for 15 min, thoroughly rinsed with sterilized water and then sown in a seedling bed. After 15 days, seedlings were inoculated with 8 g of inoculum of *R. irregularis* 10 days before transplanting to 0.3-L pots. The AM fungal inoculum was a root and soil mixture consisting of rhizosphere soil containing spores and colonized root fragments of *Medicago sativa* L. with high levels of root colonization. Plants noninoculated with AM fungi were given a filtrate (Whatman no. 1 paper) of the inoculum containing the common soil microflora, but free of AM propagules. The plants were grown in a greenhouse with supplementary light provided by Sylvania incandescent and cool-white lamps, 400 E/m^2/s, 400–700 nm, with a 16/8 h day/night cycle at 25/19 °C and 50% relative humidity.

Eight grams of incubated residues (equivalent to a field application of 80 000 kg/ha) (inoculated with the saprobe fungi as described before) was mixed with the soil in each pot. The treatments were as follows: (i) noninoculated soil or plants – control, (ii) plants inoculated with *R. irregularis* in noninoculated soil (iii) noninoculated plants and soil plus organic residues, (iv) soil plus organic residue and plants inoculated with *R. irregularis*, (v) noninoculated plants in soil with organic residues incubated with *C. rigida*, (vi) plants inoculated with *R. irregularis* in soil with organic residues incubated with *C. rigida*, (vii) noninoculated plants in soil with organic residue incubated with *T. harzianum* and (viii) plants inoculated with *R. irregularis* in soil with organic residues incubated with *T. harzianum*. Each plant was inoculated with the organic residues incubated with each saprobe fungus for 0, 2 and 4 weeks (Toole, 1971). After 15 days of germination, seedlings were inoculated with the AM fungi. After another 10 days, uniform seedlings were transplanted to 0.3 L pots. One seedling was planted per pot and four replicates were performed per each treatment. The relative chlorophyll content was measured with the Minolta SPAD-502 chlorophyll meter. Plants were harvested after 30 days and dry mass was determined. The biomass of shoots and roots were determined after drying in an air-forced oven (70 °C, 48 h). After the harvest, samples of fresh weight were taken from the entire root system at random, cleared in KOH, stained with trypan blue in lactic acid according to Phillips & Hayman (1970) and examined microscopically to determine AM root length colonization by the grid intersections plate using a stereo microscope (Giovannetti & Mosse, 1980).

Chemical and biochemical postharvest soil analysis

After harvest, soil N and P contents were analysed as described before. Rhizosphere soil samples were collected by shaking the roots gently. β-glucosidase activity was determined by measuring p-nitrophenol released from *p*-nitrophenyl-β-D-glucopyranoside (PNG) according to the method of Tabatabai & Bremner (1969) and expressed as μmol p-nitrophenol/g dry soil/h. Acid phosphatase was measured using the same procedure as β-glucosidase, but using p-nitrophenyl phosphate instead of PNP (Tisserant *et al.*, 1993). Dehydrogenase activity was determined according to the method described by Casida *et al.* (1964) and expressed as μg TPF (triphenyl formazan)/g dry soil/h. The activity of FDA was assessed as described by Adam & Duncan (2001) and expressed as μg fluorescein/g soil.

Statistical analyses

The percentage values were arcsine-transformed for statistical analyses. We studied the following three main factors and their respective levels as follows: AM fungal (control and inoculation with *R. irregularis*), saprobe fungi (control and organic residues incubated with *C. rigida* or *T. harzianum*) and incubation time (amended organic residues incubated during 0, 2, and 4 weeks). We also analysed the interaction between the main factors using a factorial analysis of variance (Sokal & Rohlf, 1981). Statistical significance was determined at $P < 0.05$. Statistical analyses were conducted using SPSS software, version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Plant biomass

There were significant differences in the response of plant and soil variables to the main factors and their interactions (Table 2). Organic residues, and inoculation only with *C. rigida* or *T. harzianum*, did not increase shoot or root dry weight. The inoculation with the arbuscular mycorrhiza *R. irregularis* increased shoot dry weight and their effect was greater in plants grown in the presence of the organic residues in particular when inoculated with *T. harzianum* after 24 h of incubation (0 weeks) (Figure 1). Root biomass and SPAD values were greater in mycorrhizal than in noninoculated plants (Figures 1 and 2). The addition of organic residues further increased the root dry weight of mycorrhizal plants (Figure 1), but not SPAD values (Figure 2). The shoot concentration of N increased with the application of organic residues, and in inoculated plants, while P increased in all treatments compared with control. (Table 3).

Chemical parameters of rhizosphere soil

Total N content in soil was greater in all treatments, with the exception of soil inoculated with *C. rigida* after 4 weeks

Table 2 Significance of the main treatment effects and their interactions based on factorial ANOVA

	SF	IT	AM	SF × IT	SF × AM	IT × AM	SF × IT × AM
F-values							
Shoot dry weight	31.421**	0.813 n.s	387.6**	0.773 n.s	25.82**	0.043 n.s	0.118 n.s
Root dry weight	11.198**	1.021 n.s	89.247**	2.78 n.s	10.911**	0.090 n.s	1.642 n.s
R:S Ratio	1.135 n.s	2.890**	44.026**	1.711 n.s	0.722 n.s	3.469*	1.363 n.s
Nitrogen	0.010 n.s	30.23**	2.617 n.s	0.040 n.s	0.028 n.s	0.433 n.s	0.045 n.s
P-Olsen	1.747 n.s	20.65**	0.313 n.s	0.275 n.s	0.786 n.s	2.017 n.s	0.101 n.s
pH	107.88**	173.37**	3.391 n.s	31.295**	2.915*	0.760 n.s	9.939**
β-glucosidase	28.15**	17.703**	0.741 n.s	6.239**	2.744*	1.266 n.s	0.686 n.s
Dehydrogenase	30.093**	1.528 n.s	0.558 n.s	1.382 n.s	23.835**	0.492 n.s	0.979 n.s
Acid phosphatase	9.918**	2.403 n.s	9.255*	2.005 n.s	3.813*	0.126 n.s	0.113 n.s
Fluorescein diacetate	30.089**	130.44**	2.936 n.s	44.674**	3.215*	1.856 n.s	0.752 n.s
Chlorophyll	4.589 n.s	1.073 n.s	319.196**	0.422 n.s	3.261*	1.101 n.s	0.533 n.s

SF, saprophytic Fungi; IT, incubation time; AM, arbuscular mycorrhiza, n.s., not significant. *$P < 95\%$. **$P < 99\%$.

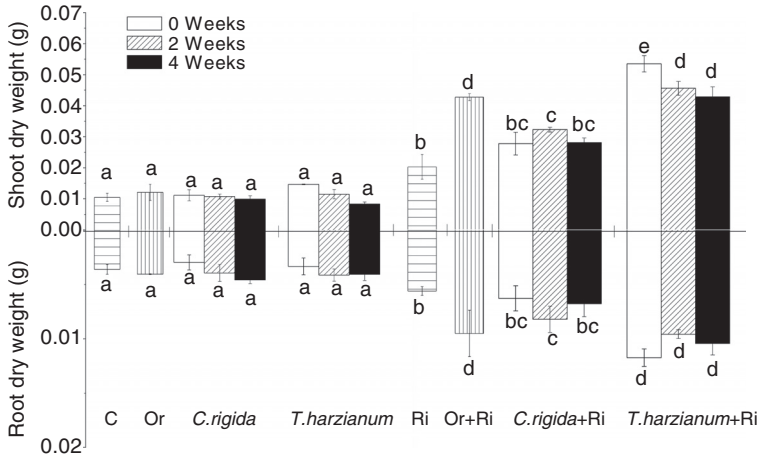

Figure 1 Shoot and root dry weight (g) of *S. lycopersicum* inoculated with *R. irregularis* and grown in the presence of organic residues incubated with the saprobe fungi for 0, 2 and 4 weeks. C, control; Or, organic residue without fungi; *C. rigida*, organic residue incubated with *C. rigida*; *T. harzianum*, organic residue incubated with *T. harzianum*; Ri, *R. irregularis*; Or + Ri, organic residue + *R. irregularis*; *C. rigida* + Ri, organic residue incubated with *C. rigida* + *R. irregularis*; *T. harzianum* + Ri, organic residue incubated with *T. harzianum* + *R. irregularis*. Data are means ± standard error. Bars with the same letter are not significantly different ($P < 0.05$).

of incubation (Figure 3). The greatest total N content was observed when the organic residues were incubated for 24 h with *T. harzianum* or *C. rigida* in the presence of mycorrhizal plants (Figure 3). Addition of organic residues also increased available soil P content; the greatest value was obtained when these were not inoculated. The addition of *T. harzianum* or *C. rigida* to the residues led to a decrease of soil P, especially when the incubation period was longer (Figure 4).

Soil pH increased in all treatments compared with control. Organic residues incubated for 4 weeks with *T. harzianum* or *C. rigida* and plants inoculated with *R. irregularis* led to a greater soil pH than when residues were incubated for 24 h (Figure 5).

Biochemical parameters of rhizosphere

Acid phosphatase and dehydrogenase activities were stimulated in rhizosphere soil when organic residues were added, but the presence of saprobe and arbuscular fungi did not influence these enzymatic activities (Table 4). *β*-glucosidase activity was only stimulated by the addition of organic residues incubated for a short period with *C. rigida* or *T. harzianum* compared with treatments without saprobe fungi. Fluorescein diacetate activity was increased by the inoculation with saprobe fungi with an incubation for 24 h with *T. harzianum* or *C. rigida* (0 weeks of incubating). However, this enzymatic activity decreased with the addition of organic residues incubated with both saprobe fungi for 2 and 4 weeks (Table 4).

Root colonization

The AM root length colonization was increased by the application of the organic residues. The percentage of AM root colonization was significantly greater when the organic residues were incubated for 0 and 4 weeks with *C. rigida*, but not when incubated for 2 weeks. The incubation of the organic residue with *T. harzianum* did not increase the percentage of AM colonization (Figure 6).

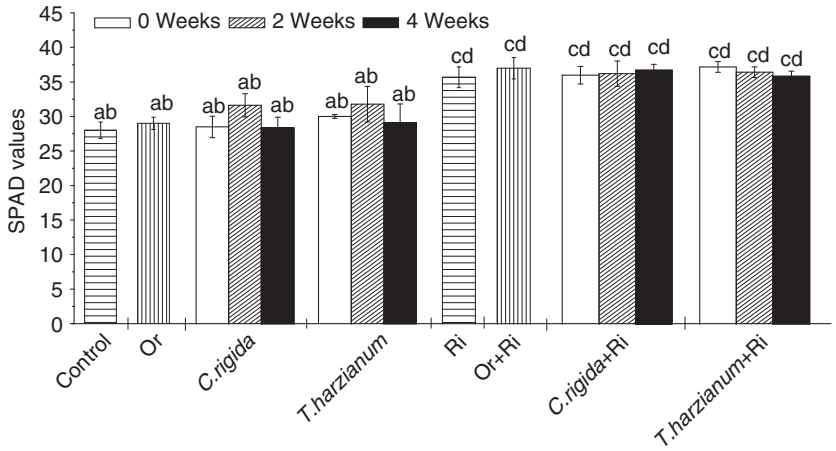

Figure 2 SPAD relative values of *S. lycopersicum* inoculated with *R. irregularis* and grown in the presence of organic residues incubated with the saprobe fungi for 0, 2 and 4 weeks. Control; Or, organic residue without fungi; *C. rigida*, organic residue incubated with *C. rigida*; *T. harzianum*, organic residue incubated with *T. harzianum*; Ri, *R. irregularis*; Or + Ri, organic residue + *R. irregularis*; *C. rigida* + Ri, organic residue incubated with *C. rigida* + *R. irregularis*; *T. harzianum* + Ri, organic residue incubated with *T. harzianum* + *R. irregularis*. Data are means ± standard error. Bars with the same letter are not significantly different ($P < 0.05$).

Table 3 Mineral concentration in *Solanum lycopersicum* plants (mg/kg) inoculated with *Rhizophagus irregularis* and organic residues incubated with saprobe fungi (0, 2 and 4 weeks)

	N	SE	P	SE
Control	9600	956 a	900	85 a
Rhizophagus irregularis	15 600	1589 b	1800	179 b
Or	17 000	1500 b	1857	166 b
Or + Ri	18 500	1790 b	2530	246 c
C. rigida 0 weeks	11 600	1150 a	1600	156 b
C. rigida 2 weeks	11 000	1100 a	1550	144 b
C. rigida 4 weeks	9440	933 a	1500	149 b
C. rigida + Ri 0 weeks	19 000	1870 bc	1700	166 b
C. rigida +Ri 2 weeks	22 800	2200 c	1799	175 b
C. rigida + Ri 4 weeks	23 540	2110 c	1840	175 b
T. harzianum 0 weeks	11 600	1230 a	1574	157 b
T. harzianum 2 weeks	11 200	1321 a	1402	140 b
T. harzianum 4 weeks	10 100	9809 a	1385	128 b
T. harzianum + Ri 0 weeks	25 200	1460 d	1414	141 b
T. harzianum + Ri 2 weeks	27 900	1690 d	1547	143 b
T. harzianum + Ri 4 weeks	28 300	1730 d	1684	166 b

SE, standard error; Or, organic residue without fungi; Or + Ri, organic residue + *R. irregularis*; *C. rigida*, organic residue incubated with *C. rigida*; *C. rigida* + Ri, organic residue incubated with *C. rigida* + *R. irregularis*; *T. harzianum*, organic residue incubated with *T. harzianum*; *T. harzianum* + Ri, organic residue incubated with *T. harzianum* + *R. irregularis*. Data are means ± standard error. Columns with the same letter are not significantly different ($P < 0.05$).

Discussion

Mycorrhizal plants had greater biomass than nonmycorrhizal plants, and these parameters were increased by the presence of the organic residues. The combination of wheat straw with sewage sludge seems to avoid the negative effect of the sewage sludge observed on AM colonization of some plants (Arriagada *et al*., 2009a). The effect of *T. harzianum* on tomato growth was superior to that of *C. rigida* and this can stimulate growth in a broad range of AM-colonized plants including tomato, through several mechanisms, including production or stimulation of hormones or P solubilization (Altomare *et al*., 1999; Hoyos-Carvajal *et al*., 2009). However, *T. harzianum* did not increase the percentage of AM root length colonization of tomato in the presence of the organic residues, while *C. rigida* increased the percentage of AM root length colonization, but decreased the dry weight of the plants compared with organic residues on their own. It is known that saprobe fungi can increase the infectiveness of AM fungi although they do not always increase their effects on plant growth (Aranda *et al*., 2007). Moreover, the absence of a close relationship between AM root length colonization and AM effect on plant growth has been observed before (Treseder, 2013). On the other hand, root biomass was not increased by the AM fungus. It is known that mycorrhizal plants do not need to increase root biomass due to the increase in surface which allows a greater area of absorption to be achieved, provided by the mycelium of mycorrhizal fungi (González-Monterrubio *et al*., 2005).

The increased levels of chlorophyll in leaves of AM-colonized plants (SPAD values) presumably contributed to higher photosynthetic rates in tomato, thus benefiting the development and functionality of the symbiosis. However, the enhancement of the relative chlorophyll in leaves of plants can be independent of environmental and nutritional factors (Baslam & Goicoechea, 2012). In fact, we found that

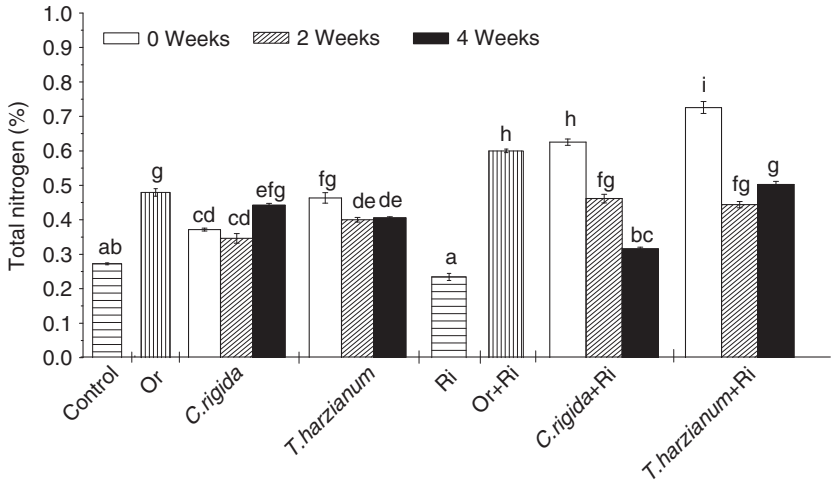

Figure 3 Total nitrogen in soil of *S. lycopersicum* inoculated with *R. irregularis* and grown in the presence of organic residues incubated with the saprobe fungi for 0, 2 and 4 weeks. Control; Or, organic residue without fungi; *C. rigida*, organic residue incubated with *C. rigida*; *T. harzianum*, organic residue incubated with *T. harzianum*; Ri, *R. irregularis*; Or + Ri, organic residue + *R. irregularis*; *C. rigida* + Ri, organic residue incubated with *C. rigida* + *R. irregularis*; *T. harzianum* + Ri, organic residue incubated with *T. harzianum* + *R. irregularis*. Data are means ± standard error. Bars with the same letter are not significantly different ($P < 0.05$).

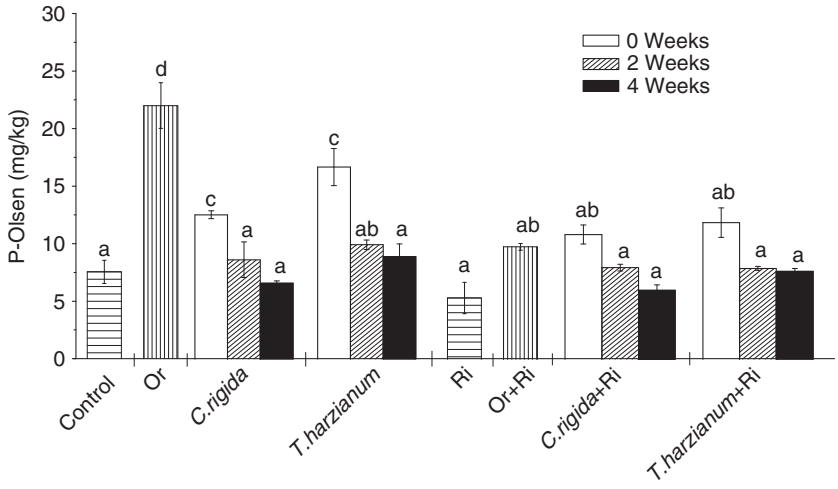

Figure 4 P-Olsen in soil of *S. lycopersicum* inoculated with *R. irregularis* and grown in the presence of organic residues incubated with the saprobe fungi for 0, 2 and 4 weeks. Control; Or, organic residue without fungi; *C. rigida*, organic residue incubated with *C. rigida*; *T. harzianum*, organic residue incubated with *T. harzianum*; Ri, *R. irregularis*; Or + Ri, organic residue + *R. irregularis*; *C. rigida* + Ri, organic residue incubated with *C. rigida* + *R. irregularis*; *T. harzianum* + Ri, organic residue incubated with *T. harzianum* + *R. irregularis*. Data are means ± standard error. Bars with the same letter are not significantly different ($P < 0.05$).

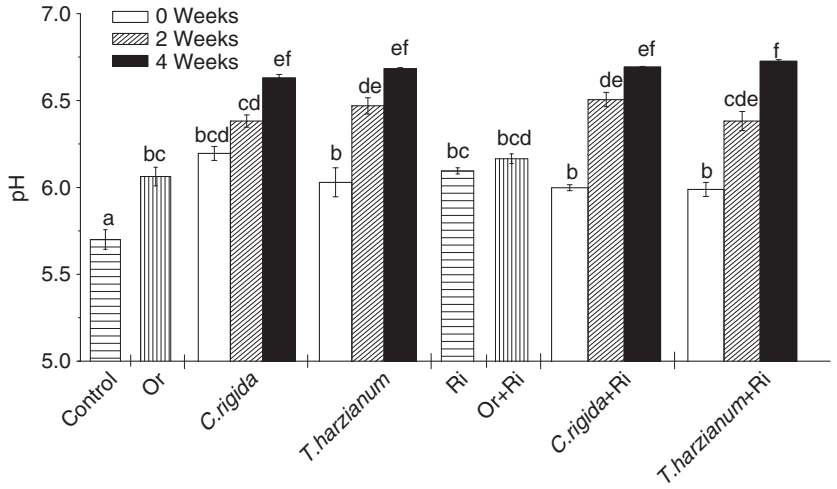

Figure 5 pH of soil of *S. lycopersicum* inoculated with *R. irregularis* and grown in the presence of organic residues incubated with the saprobe fungi for 0, 2 and 4 weeks. Control; Or, organic residue without fungi; *C. rigida*, organic residue incubated with *C. rigida*; *T. harzianum*, organic residue incubated with *T. harzianum*; Ri, *R. irregularis*; Or + Ri, organic residue + *R. irregularis*; *C. rigida* + Ri, organic residue incubated with *C. rigida* + *R. irregularis*; *T. harzianum* + Ri, organic residue incubated with *T. harzianum* + *R. irregularis*. Data are means ± standard error. Bars with the same letter are not significantly different ($P < 0.05$).

R. irregularis increased relative chlorophyll (SPAD values) regardless of the different level of mycorrhization reached in the presence of either saprobe fungi.

Soil N and P contents in soil were stimulated by the addition of the organic residues. This could be due to the large amounts of organic matter and nutrients that the

Table 4 Biochemical parameters of rhizosphere. Activities of β-glucosidase, dehydrogenase, acid phosphatase and fluorescein diacetate in the rhizosphere soil of *Solanum lycopersicum* inoculated with *Rhizophagus irregularis* and the addition of organic residues incubated with saprobe fungi (0, 2 and 4 weeks)

Treatments	β-glucosidase (μmol PNG/g/h)	Dehydrogenase (μg TPF/g/h)	Acid phosphatase (μmol PNP/g/h)	Fluorescein diacetate (μg fluorescein/g)
Control	99.8 (8.0) a	3.60 (0.73) ab	178.0 (14.00) ab	52.0 (2.60) a
Rhizophagus irregularis	104.5 (9.0) a	2.85 (0.26) a	170.0 (12.03) ab	56.5 (4.13) a
Or	120.7 (7.8) ab	5.50 (0.40) b	217.0 (24.88) c	64.8 (1.68) b
Or + Ri	122.5 (6.3) ab	4.08 (0.44) ab	167.0 (18.63) ab	64.1 (3.93) b
C. rigida 0 weeks	156.6 (20.0) cd	3.10 (0.12) ab	170.0 (19.14) ab	79.0 (2.56) c
C. rigida 2 weeks	139.0 (17.0) cd	3.30 (0.52) ab	157.7 (5.60) ab	30.1 (2.60) b
C. rigida 4 weeks	123.6 (7.5) abc	3.29 (0.28) ab	164.4 (11.97) ab	45.6 (4.93) bc
C. rigida + Ri 0 weeks	148.2 (12.0) cd	4.40 (0.41) ab	157.0 (13.18) ab	81.0 (4.62) c
C. rigida + Ri 2 weeks	108.9 (13.0) ab	4.63 (0.50) ab	169.0 (8.17) ab	18.3 (0.90) a
C. rigida + Ri 4 weeks	118.2 (19.0) abc	3.50 (0.20) ab	154.5 (7.17) ab	34.9 (2.56) a
T. harzianum 0 weeks	169.0 (15.0) cd	3.20 (0.41) ab	183.0 (9.93) ab	81.0 (1.16) c
T. harzianum 2 weeks	132.0 (8.1) cd	3.10 (0.25) ab	154.0 (17.86) ab	36.8 (2.43) bc
T. harzianum 4 weeks	108.4 (15.0) ab	3.27 (0.32) ab	126.3 (5.63) a	35.5 (3.69) a
T. harzianum + Ri 0 weeks	157.0 (16.0) cd	4.62 (0.38) ab	175.0 (17.51) ab	80.0 (1.40) c
T. harzianum + Ri 2 weeks	127.8 (9.2) cd	4.30 (0.26) ab	148.5 (7.80) ab	23.4 (1.65) ab
T. harzianum + Ri 4 weeks	122.3 (12.1) abc	4.30 (0.60) ab	121.2 (7.78) a	30.4 (3.27) a

Or, organic residue without fungi; Or + Ri, organic residue + *R. irregularis*; *C. rigida*, organic residue incubated with *C. rigida*; *C. rigida* + Ri, organic residue incubated with *C. rigida* + *R. irregularis*; *T. harzianum*, organic residue incubated with *T. harzianum*; *T. harzianum* + Ri, organic residue incubated with *T. harzianum* + *R. irregularis*. Data are means ± standard error. Columns with the same letter are not significantly different ($P < 0.05$).

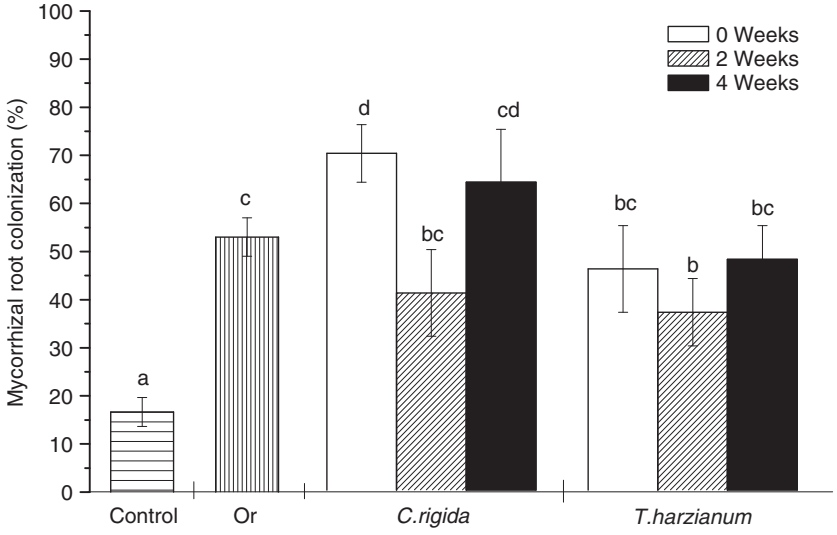

Figure 6 Percentage of root length colonization of *S. lycopersicum* inoculated with *R. irregularis* and grown in the presence of organic residues incubated with the saprobe fungi for 0, 2 and 4 weeks. Control; Or, organic residue; *C. rigida*, organic residue incubated with *C. rigida*; *T. harzianum*, organic residue incubated with *T. harzianum*. Data are means ± standard error. Bars with the same letter are not significantly different ($P < 0.05$).

organic residues contain mainly because of the input of sewage sludge (Montemurro & Maiorana, 2008). Both soil N and P contents were decreased by the saprobe fungi, especially as the time of incubation of the residue increased. This would suggest increased degradation of organic matter and mineralization of N and P (Barbarick & Ippolito, 2007) increasing the bioavailability of these nutrients for plant uptake, thereby reducing the amounts N and P in the soil (Diacono & Montemurro, 2010), but if so, the effect was not enough to significantly increase plant growth or the concentrations of N and P in tomato. Several authors have indicated that the addition of organic soil amendments can enhance plant nutrient uptake and so increase their plant biomass through the inoculation with AM fungi (Smith & Read, 2008). According to our experiments, all treatments with *R. irregularis* increased the plant nutrients (N and P) and also the plant biomass. Therefore, the improved plant growth appears to be a product of AM (Smith & Read, 2008; Borie *et al.*, 2010) mineralization of the organic matter contained in the organic residue, resulting in the direct

deposit of nutrients into the soil (Antolin *et al*., 2005) with positive effects by the saprophytic fungi (Arriagada *et al*., 2009a).

The limitation in crop production in acid soils is mainly due to high concentrations of toxic elements, such as aluminium (Al) or manganese (Mn) and by phosphorus deficiency (Kochian *et al*., 2004). The increase in pH values in the treatments with organic residues may be due to the contribution of these residues in the exchangeable bases and their ability to form complexes with Al^{3+} (Bulluck *et al*., 2002; Valarini *et al*., 2009). The increase in pH could enhance mycorrhizal colonization (Coughlan *et al*., 2000) and crop nutrition in acid soils and may have contributed to the beneficial effect of *T. harzianum* on the dry weight of tomato (Tang & Yu, 1999). However, although both saprobe fungi increased soil pH, there was no beneficial effect of *C. rigida* or *T. harzianum* on plant growth. It is known that the metabolical capacity of both saprobe fungi is different and the role of these fungi on degradation of organic residues is carried out by different metabolical systems in which the production of lacasse by *C. rigida* is the main enzymatic way of organic residues degradation (Saparrat *et al*., 2014), whereas the production of cell wall degrading enzymes, such as cellulase and hemicellulase, by *T. harzianum* is implicates in the degradation of biomass (Horta *et al*., 2014). This may explain the different effects of these fungi on the stimulation of biomass accumulation of mycorrhizal plants in the presence of organic residues.

The enhancement of acid phosphatase, FDA and dehydrogenase activities by organic residues indicates beneficial effects of organic matter, carbon, nitrogen, phosphorus and other physicochemical characteristics of the amendment (Ros *et al*., 2006). However, organic residues only increased soil β-glucosidase when it was incubated with the saprobe fungi *C. rigida* or *T. harzianum*, but the enhancement of these soil enzymatic activities decreased when the time of incubation of the organic residue with the saprobe fungi increased. Fresh organic amendments provide easily degradable and metabolizable materials via the enzymatic systems of soil microorganisms, resulting in greater soil enzyme activity, whereas incubation of organic amendments stabilizes the residues generating more resistant compounds (Bastida *et al*., 2008). Thus, during the prolonged period of incubation with the saprobe fungi, the nutrients in the substrate may have been used by these fungi resulting in the decrease of available nutrients for native soil microorganisms.

Conclusions

The combination of sewage sludge with wheat straw applied to the soil and the inoculation with *S. lycopersicum* with *R. irregularis* improved plant biomass. The highest shoot dry mass was achieved when the organic residue was incubated for 24 h with *T. harzianum* and added to mycorrhizal plants. However, *C. rigida* inhibited the positive interaction between the AM fungus and the organic residue on plant growth. N and P contents of soil as well as several soil enzymatic activities were increased with addition of the organic residues, contributing positively to promote plant growth when it was inoculated with AM fungi. These results show the potential of these residues and microorganisms to improve chemical and biochemical properties of soils and enhance the growth of tomato.

Acknowledgements

This work was supported by FONDECYT Project 1130662; CONICYT Doctoral Fellowship 24080106 and Dirección de Investigación, Universidad de La Frontera DIUFRO. We would like to also thank Claudio Lagos, Javiera Soto and Hector Herrera for their technical assistance.

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