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Effects of different tillage system on arbuscular mycorrhizal fungal propagules and physical properties in a Mediterranean agroecosystem in central Chile

Gustavo Curaqueo [a], José Miguel Barea [b], Edmundo Acevedo [c], Rosa Rubio [a], Pablo Cornejo [a], Fernando Borie [a,*]

[a] Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera, P.O. Box 54-D, Temuco, Chile
[b] Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas (CSIC), 18008 Granada, Spain
[c] Universidad de Chile, Facultad de Ciencias Agronómicas, Casilla 1004, Santiago, Chile

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi improve soil quality by increasing soil structure stability through the glomalin (glomalin related soil protein, GRSP) production, but diverse tillage systems can differentially affect AM activity and the consequential GRSP content in soil. The aim of this study was to evaluate the effect of no-tillage (NT) and conventional tillage (CT) on AM fungal propagules (spore density, total and active fungal hyphae), GRSP content, and its relationship with some physical–chemical soil properties in a Mollisol from Central Chile. For this study, two plots managed for 6 and 10 years under NT (NT6 and NT10), were compared with another plot maintained under CT management. In all cases a continuous spring wheat (*Triticum turgidum* L.)–maize (*Zea mays* L.) rotation was established. The number of mycorrhizal propagules, total soil carbon (T-C) and GRSP content in NT6 was higher compared to CT and NT10. This trend was also observed for water stable aggregates (WSA) and water drop penetration time. Significant relationships were found between total mycelium and GRSP ($r = 0.58$, $p < 0.05$), GRSP and WSA ($r = 0.66$, $p < 0.01$) and between GRSP and T-C ($r = 0.60$, $p < 0.05$), suggesting an active role of AM fungi and GRSP on soil aggregation, particularly under NT6 management. In addition, the long-term NT management (NT10) produced a decrease in the parameters here assayed which suggest the application of one moderate plowing when parameters such as T-C and/or GRSP show a decrease in long-term programs of reduced or NT management applied in medium/heavy soils.

1. Introduction

Arbuscular mycorrhizal (AM) fungi are plant symbionts widely distributed in terrestrial ecosystems and in a vast diversity of climate and soil-types (Smith and Read, 2008), which establish associations with the majority of plant species, including most of the agricultural plants (Jeffries et al., 2003). This kind of fungi plays an important role in many microbiological and ecological processes, influencing soil fertility, cycling of nutrients, soil organic matter (Finlay, 2008), soil aggregation (Wright et al., 2007), as well as influencing plant health and nutrition (Jeffries et al., 2003). The AM fungi are also known to confer increased resistance to pathogens to their host plant (Barea et al., 2005) and other environmental stresses, including an improvement of water relations (Ruiz-Lozano et al., 2008).

Establishment of the AM symbiosis regulates carbon fluxes between biosphere and atmosphere by different mechanisms (Zhu and Miller, 2003). One of them is the production of glomalin, a proteinaceous compound (Wright and Upadhyaya, 1998), which presents high recalcitrance and is accumulated in the soil profile in variable concentrations (Driver et al., 2005). Glomalin has been operationally defined and extracted from soil as glomalin-related soil protein (GRSP) and associated with soil aggregate stability (Rillig, 2004) and soil C accumulation (Lovelock et al., 2004b). The GRSP contributes to reduce significantly soil organic matter degradation through the protection of labile compounds inside soil aggregates because of its role on soil particle aggregation, thus enhancing carbon sequestration in soil ecosystems (Rillig, 2004).

Soil management, specifically the use of different tillage systems, affects not only soil properties and microbial characteristics, but also AM fungal activity (Sieverding, 1991), community structure (Jansa et al., 2003) and glomalin production (Wright et al., 2007). In fact, plowing disrupts soil aggregates by the destruction of mycelium network (Álvaro-Fuentes et al., 2008b), compacts the subsoil, and decreases soil organic matter (SOM) content, cation exchange capacity (CEC), and microbial and faunal

* Corresponding author. Tel.: +56 45 325430; fax: +56 45 325440.
E-mail addresses: gcura@ufro.cl (G. Curaqueo), josemiguel.barea@eez.csic.es (J.M. Barea), eacevedo@u.uchile.cl (E. Acevedo), rrubio@ufo.cl (R. Rubio), pcornejo@ufro.cl (P. Cornejo), fborie@ufro.cl (F. Borie).

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activities (Castillo et al., 2006). Furthermore, when root and fungal mycelium networks are disrupted the stability of soil aggregates decreases, then favoring leaching, nutrient losses and soil erosion (Borie et al., 2008; Martínez et al., 2008). In contrast, some soil managements such as low-till agriculture returning organic residues to soil, and diversifying cropping systems, have a positive effect on soil characteristics (Chesworth, 2008). Thus, the study of the role of AM symbiosis and GRSP on soil C dynamics and its contribution to C sequestration in the different agricultural systems is required when is intended to improve agroecosystem sustainability. Accordingly, the aim of this study was to evaluate the effect of no-tillage and conventional tillage management on AM fungal propagules, GRSP content and to relate them with physical soil characteristics in a Mollisol from Central Chile.

2. Materials and methods

2.1. Study area and test soil

The study was carried out in an agroecosystem located at Antumapu Experimental Station, Universidad de Chile (33°40′S, 70°38′W; 608 m above sea level). The climate of the agroecosystem is temperate, Mediterranean-semiarid with dry summers and cold winters. The maximum mean temperature is 28.7 °C (January); the minimum mean temperature is 3.4 °C (July) and the mean annual rainfall is 330 mm (Santibañez and Uribe, 1990). The soil is a thermic Entic Haploxeroll (sandy clay alluvial soil with coarse loamy over sandy, skeletal, mixed) with a flat level (0.5–2%). A field assay comparing conventional tillage (CT) and no-till (NT) managements were established in 192 m^2 plots (40 m × 4.8 m), varied in the time at which the managements were implemented: (i) one site was cropped under CT for 10 years; (ii) another one was maintained under NT for 10 years and (iii) the last one was under NT for 6 years. Each treatment considered three field replications plots. In all cases a durum spring wheat (*Triticum turgidum* L., var durum)–maize (*Zea mays* L., var México cl) rotation was established. The study reported here was carried out after of maize phase in the crop rotation. Maize was planted at a rate of 110,000 plants ha^{-1} at a distance of 70 cm between rows with a NT drill (Semeato SHM 11/13, Brazil) in both tillage treatments (CT and NT). The soil was fertilized with urea and triple super phosphate. 250 kg N ha^{-1} and 52 kg P ha^{-1} applied at sowing and 250 kg N $ha^{-1}$of urea were broadcasted at eight-leaf growth stage. The weeds were controlled with 114 g of imazapic + imazapyr (BASF, Germany) + 200 mL ha^{-1} of fat alcohol polyalkoxylate phosphate (BASF, Germany) applied at post-emergency. The amount of maize residues on top of soil prior to wheat sowing was estimated in 16 Mg ha^{-1} for all treatments. In the CT treatment, the maize residues before to wheat sowing were mechanically shredded and buried using a moldboard plow to a depth of 20 cm. Later, the soil was disked twice, with a disk harrow before wheat sowing. In the case of NT treatment, maize residues were shredded and left on the top of soil. Soil samples consisted of 10 sub-samples, collected at two depths (0–2 and 2–5 cm depth) at 60 days after maize harvesting. Samples were homogenized and transported in plastic bags to the laboratory and stored at 4 °C until their analysis.

2.2. Soil measurements

Soil pH was determined in a 2/5 (w/v) soil:water mixture Available P was extracted with a 0.5 M $NaHCO_3$ solution at pH 8.5 and quantified according to Olsen and Sommers method (1982). Total P was determined according to Dick and Tabatabai (1977). Total C, N and S were determined by dry combustion in a C, H, N, S Analyzer VARIO/EL (Elementar Analysensysteme GmbH, Deutschland).

Soil bulk density (ρ_b) was determined by the cylinder method (Blake and Harte, 1986), using a 5-cm diameter cylinder. Using the data obtained from the bulk density, the total soil porosity (f) was calculated in relation to a real density of 2.65 g cm^{-3}. The water stable soil aggregates (WSA) were measured using the procedure of Kemper and Rosenau (1986). Briefly, air-dried soil sieved (1 mm) was placed in a 0.250 mm sieve and immersed in an aluminum pan with distilled water for 3 min with a stroke length of 1.3 cm and a frequency of 35 cycles min^{-1}. Then, the aluminum pan containing the soil derived from the sieving was dried at 105 °C until water evaporated. The soil retained in the sieve was immersed again in a sodium hexametaphospate solution (2 g L^{-1}) for 15 min and 35 cycles min^{-1} and the aluminum pan was also dried. After drying, aggregate fractions (from water and hexametaphospate) were weighed to obtain WSA percentage.

The particle size distribution and mean weight diameter (MWD) were determined according to the methodology of Kemper and Rosenau (1986). The particle size distribution was carried out by dry sieving in a six sieves set (6.68, 4.75, 2.00, 1.18, 0.250, and 0.106 mm openings) using a vibratory sieve shaker at 1000 rpm for 10 min. After sieving, the MWD was calculated as:

$$\mathrm{MWD} = \sum_{i=1}^{n} \bar{X}_i W_i \quad (1)$$

where MWD is the mean weight diameter (mm), X_i is the arithmetic mean diameter of aggregates in the $i+1$ and i sieving opening (mm); W_i is the proportion of weight [weight of aggregates in the i size fraction (g)/total soil weight (g)] of the aggregates; and n is the number of size fractions (in this case 5). Additionally, dry aggregates were grouped in macroaggregates ($2 \leq 0.250$ mm) and microaggregates ($\leq$0.250 mm) according to the classification of Oades and Waters (1991) for determining the total-GRSP and EE-GRSP content in each aggregates fraction.

Water holding capacity (WHC) was measured according to the methodology described by Zagal et al. (2003) with minor modifications. Briefly, 20 g of non-sieved soil sample was placed in a funnel with an absorbent membrane. The soil samples were saturated with distilled water until the excess water is drawn away by gravity. Once equilibrium is reached, WHC is calculated based on the weight of the water held in the sample vs. weight of dried sample.

The water drop penetration time (WDPT) was used to determine the hydrophobicity of soil (Dekker and Ritsema, 1994). Bulk soil samples were placed in a Petri dish and conditioned in a climate chamber (25 °C and 50% relative air humidity) for at least 1 week. Prior to testing, samples were deferred 2 days in the lab room to allow them to equilibrate with the ambient air humidity and temperature. Then, three drops of distilled water (6 mm diameter) were placed on soil surface samples recording the time for when complete penetration was observed.

2.3. Fungal measurements

Arbuscular mycorrhizal spores were separated from soils by wet sieving and decanting (Sieverding, 1991), transferred to Petri plates and counted under stereoscopic microscope at 30–50× (Gerdemann and Nicholson, 1963). Total hyphal length was measured according to Rubio et al. (2003). Briefly, 3 g of soil sample were mixed with a solution of glycerol:HCl:water (12:1:7 v/v), shaken for 30 min at 80 °C and the suspension filtered through 250 and 38 μm sieves. The material retained in the 38 μm sieve was re-suspended in 100 mL dH_2O, shaken for 1 min and allowed

Table 1
Chemical properties in a Mollisol under different tillage systems.

Treatment	Depth (cm)	pH	Total-P (mg kg^{-1})	Available-P (mg kg^{-1})	C (g kg^{-1})	N (g kg^{-1})	S (g kg^{-1})	C/N
CT[a]	0–2	7.5 (0.05) a	1084 (41) a	7.7 (1.7) a	17.0 (0.48) b	1.4 (0.04) b	1.5 (0.27)	12 a
NT6[b]		7.4 (0.06) a	1459 (50) a	29.4 (2.4) a	26.1 (2.49) a	2.3 (0.24) a	1.5 (0.06)	11 a
NT10[c]		7.4 (0.13) a	1221 (15) a	17.5 (3.6) a	23.1 (1.72) ab	1.9 (0.16) ab	1.2 (0.05)	12 a
CT	2–5	7.7 (0.05) a	1201 (51) a	2.5 (0.3) c	17.3 (0.93) a	1.4 (0.07) a	1.4 (0.17)	12 a
NT6		7.5 (0.06) ab	1153 (41) a	10.3 (0.6) b	23.4 (2.26) a	2.0 (0.25) a	1.3 (0.15)	12 a
NT10		7.4 (0.04) b	1290 (45) a	15.5 (1.7) a	19.4 (0.95) a	1.6 (0.10) a	1.2 (0.14)	12 a

In a column, different letters in the same depth indicate significant differences among tillage systems according to the Tukey test ($P \leq 0.05$). Standard error is presented in parentheses.
[a] CT: Conventional tillage.
[b] NT6: No tillage for 6 years.
[c] NT10: No tillage for 10 years.

to stand for 30 s. Three mL of suspension were transferred to a membrane filter (0.45 μm pore size, 47 mm diameter, grid-line interval 3 mm), stained with trypan blue solution (0.05% w/v) and the hyphae were quantified under stereoscopic microscope at 100× (Giovannetti and Mosse, 1980). To determine the metabolically active hyphae, an aliquot from the hyphal suspension was stained by flooding the filters with a solution containing equal volume of iodonitrotetrazolium salt solution (1 mg mL^{-1}) and 0.2 M tris buffer solution at pH 7.4 (Kabir et al., 1998). The filter was incubated for 2 h at room temperature and measured as above by the grid-line intersect method.

The GRSP fractions were obtained according to Wright and Upadhyaya (1998), with minor modifications. The easily extractable (EE) GRSP was taken, from 1 g of soil in 8 mL of citrate buffer (20 mM, pH 7.0) and autoclaving at 121 °C for 30 min. Total-GRSP was extracted from 1 g of soil in 8 mL of 50 mM citrate buffer at pH 8.0 and autoclaving for 1 h at 121 °C, repeating this procedure several times on the same sample until the typical reddish-brown color of GRSP disappeared from the supernatant. Both fractions were centrifuged at 8000 × *g* for 15 min and filtered through Whatman No. 1 filter. The content of protein in both fractions was determined by Bradford protein assay (Bio Rad Protein Assay; Bio Rad Labs) with bovine serum albumin as standard (Wright et al., 1999).

2.4. Experimental design and statistical analysis

The experimental design was completely randomized, with three tillage systems, two soil depths, and three replicates in each combination. The data were statistically analyzed using one way ANOVA and the means were compared using the Tukey test ($P \leq 0.05$). Correlation analysis was performed using Pearson coefficient to evidence the linear relationship among the studied variables. The obtained data sets were subjected to principal component analysis (PCA) and the obtained correlation among the different variables and the obtained principal components (PC) were analyzed using the Pearson correlation coefficient. Later, a non-hierarchical cluster analysis was used, applying the complete linkage clustering as agglomerative method for grouping the different experimental units. The statistical analyses were performed with the SPSS software v. 14.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Soil properties

Significant differences in total C and N contents were found between NT6 and CT at 0–2 cm deep, whereas NT10 and CT showed no significant differences (Table 1). In this sense, total soil C was increased in an average of 44% with the use of NT6 treatment compared with CT. In contrast, total-P and S content were not affected by the tillage treatments; thus, the available-P was higher in NT10 treatment at 2–5 cm deep followed by NT6 and CT. The pH values presented only small differences at 2–5 cm deep being, in general, similar in all assayed treatments and depths. The C/N ratios were similar in all tillage systems and soil depths.

3.2. Arbuscular mycorrhizal parameters

No significant differences were observed in AM fungi spore density at either two assayed soil depths or tillage systems (Table 2). In the same way, total and active AM hyphae were not significantly affected by the different tillage systems used (Table 2), but the highest values in both cases (spores and hyphae)

Table 2
Densities of arbuscular mycorrhizal propagules and glomalin related soil protein (GRSP) fractions in a Mollisol under three tillage regimes.

Treatment	Depth (cm)	AM spore (No. 100 cm^{-3})	Total AM hyphae (m g^{-1})	Active AM hyphae (m g^{-1})	Hyphae activity %	GRSP[a] (mg g^{-1})	EE-GRSP[b] (mg g^{-1})
CT[c]	0–2	381 (67) a	4.02 (0.72) a	0.81 (0.34) a	20.15 a	4.13 (0.69) b	1.90 (0.34) a
NT6[d]		484 (112) a	5.96 (3.52) a	0.91 (0.26) a	15.27 a	8.98 (1.33) a	3.25 (0.17) a
NT10[e]		272 (70) a	3.74 (0.97) a	0.81 (0.26) a	21.66 a	4.93 (0.61) b	2.45 (0.08) a
CT	2–5	517 (271) a	3.27 (0.40) a	0.78 (0.12) a	23.85 a	3.79 (0.56) a	1.45 (0.21) b
NT6		531 (118) a	3.99 (1.53) a	1.30 (0.35) a	32.58 a	7.33 (0.83) a	2.64 (0.13) a
NT10		258 (26) a	1.87 (0.24) a	0.52 (0.21) a	27.81 a	5.03 (1.41) a	1.65 (0.07) ab

In a column, different letters in the same depth indicate significant differences among tillage systems according to the Tukey test ($P \leq 0.05$). Standard error is presented in parentheses.
[a] GRSP: Glomalin related soil protein.
[b] EE-GRSP: Easily extractable glomalin related soil protein.
[c] CT: Conventional tillage.
[d] NT6: No tillage for 6 years.
[e] NT10: No tillage for 10 years.

Table 3
Some physical parameters analyzed in a Mollisol under three tillage regimes.

Treatment	Depth (cm)	ρ_b[a] (g cm^{-3})	f[b] (%)	MWD[c] (mm)	WHC[d] (%)	WDPT[e] (s)	WSA[f] (%)
CT[g]	0–2	1.40 (0.04) a	47.2 (1.5) a	4.42 (0.13) a	45.4 (2.5) a	0.42 (0.03) b	31.33 (3.94) b
NT6[h]		1.33 (0.05) a	49.6 (2.1) a	5.05 (0.33) a	49.7 (3.0) a	0.58 (0.04) ab	57.13 (7.22) a
NT10[i]		1.38 (0.14) a	47.9 (5.3) a	4.51 (0.05) a	42.8 (2.7) a	0.68 (0.04) a	49.29 (6.72) ab
CT	2–5	1.43 (0.14) a	45.9 (5.3) a	4.52 (0.21) b	43.5 (2.8) a	0.42 (0.01) b	33.01 (4.08) b
NT6		1.39 (0.06) a	47.6 (2.1) a	5.12 (0.04) a	47.2 (7.2) a	0.63 (0.02) a	60.96 (4.42) a
NT10		1.40 (0.09) a	47.1 (3.4) a	4.70 (0.20) ab	41.7 (4.8) a	0.58 (0.02) a	57.07 (6.56) a

In a column, different letters in the same depth indicate significant differences among tillage systems according to the Tukey test ($P \leq 0.05$). Standard error is presented in parentheses.
[a] ρ_b: Bulk density.
[b] f: Porosity.
[c] MWD: Mean weight diameter.
[d] WHC: Water holding capacity.
[e] WDPT: Water drop penetration time.
[f] WSA: Water stable aggregates.
[g] CT: Conventional tillage.
[h] NT6: No tillage for 6 years.
[i] NT10: No tillage for 10 years.

were observed in NT6 treatment. Thus, total hyphae presented a range from 1.87 to 5.96 m g^{-1}, where the lowest value corresponded to NT10 system at 2–5 cm deep soil, and the highest value was obtained under NT6 at 0–2 cm deep soil. The active mycelia represented between 15.3% and 32.6% of total hyphae, being higher at 2–5 cm deep than at 0–2 cm, although there was a decrease in the total AM hyphae with soil depth.

Higher contents of total and EE GRSP were found under NT6 and NT10 at both soil depths compared with CT (Table 2). Total GRSP ranged from 3.8 to 8.9 mg g^{-1}, whereas EE-GRSP ranged between 1.5 and 3.3 mg g^{-1}. The GRSP contents presented significant differences at 0–2 cm deep soil whereas at 2–5 cm no differences were found. In contrast, EE-GRSP contents showed no significant differences at 0–2 cm deep, but statistical differences were founded only between NT6 and CT at 2–5 cm deep.

3.3. Physical parameters

Tillage regimes produced no differences on ρ_b and porosity for all evaluated treatments and soil depths (Table 3). In general, ρ_b increased with depth although higher porosity values were observed under NT6 and NT10 compared with CT at both soil depths. The f for all treatments and depths ranged from 45.9% under CT to 49.6% under NT6; nevertheless, statistical differences were not found. The MWD of soil aggregates was greater in NT6 compared with NT10 and CT, being significant at 2–5 cm deep. The aggregates were preferentially distributed in the ≥2.0 mm diameter class for all treatments (Fig. 1).

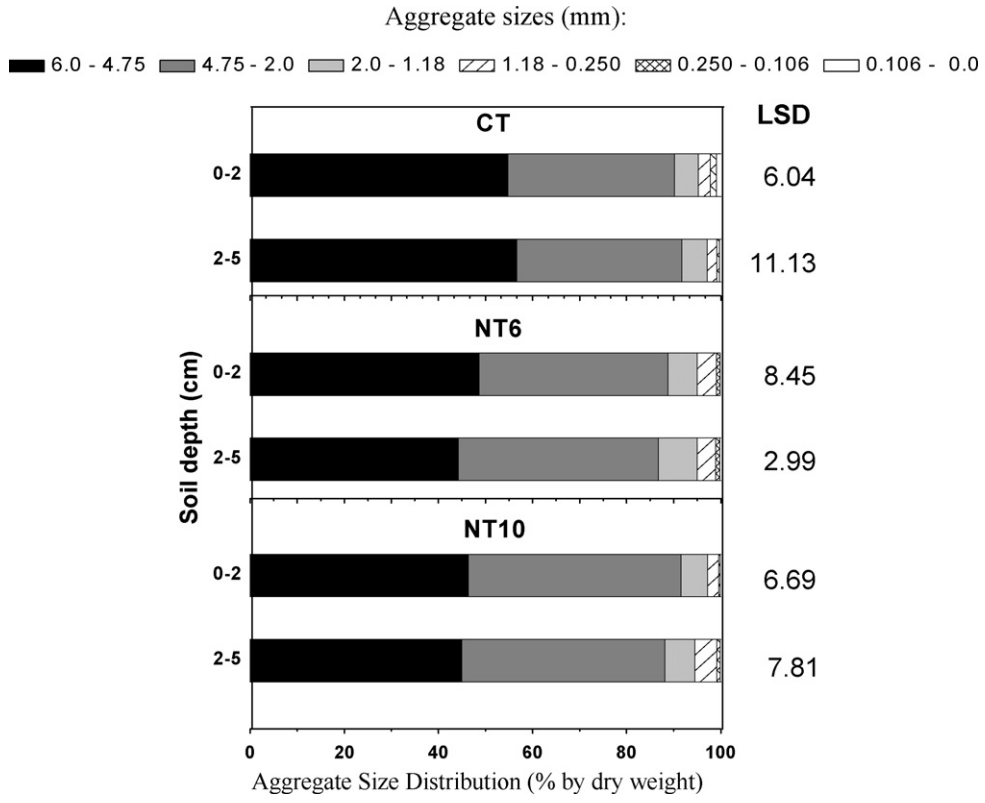

Fig. 1. Aggregate size distribution in a Mollisol under three tillage regimes. LSD values indicate differences among aggregate size fractions in each tillage system and soil deep.

Table 4
Concentration (mg g^{-1}) of glomalin related soil protein fractions (EE-GRSP, GRSP) in soil macro and micro aggregates derived from a Mollisol under different tillages treatment.

Treatment	Depth (cm)	Macroaggregates		Microaggregates	
		EE-GRSP[a]	GRSP[b]	EE-GRSP[a]	GRSP[b]
CT[c]	0–2	1.19 (0.13) b	3.73 (0.23) b	0.86 (0.07) a	3.25 (0.08) b
NT6[d]		1.94 (0.12) a	8.78 (0.68) a	1.36 (0.19) a	7.62 (0.47) a
NT10[e]		1.77 (0.07) ab	4.55 (0.30) b	1.27 (0.15) a	3.91 (0.13) b
CT[c]	2–5	1.04 (0.17) b	3.54 (0.16) b	0.73 (0.07) b	2.99 (0.23) b
NT6[d]		1.77 (0.12) a	7.15 (0.40) a	1.23 (0.16) a	6.69 (0.18) a
NT10[e]		1.25 (0.04) b	4.41 (0.31) b	0.78 (0.07) b	3.86 (0.40) b

In a column, different letters in the same depth and GRSP fractions indicate significant differences according to the Tukey test ($P \leq 0.05$). Standard error is presented in parentheses.
[a] EE-GRSP: Easily extractable glomalin related soil protein.
[b] GRSP: Glomalin related soil protein.
[c] CT: Conventional tillage.
[d] NT6: No tillage for 6 years.
[e] NT10: No tillage for 10 years.

Table 5
Correlation matrix of some selected variables studied in a Mollisol managed under conventional tillage and no-tillage.

Variables	pH	EE-GRSP	GRSP	P Olsen	T-C	Total hyphae	WDPT	GRSP-macro	GRSP-micro	EE-GRSP-macro	EE-GRSP-micro
EE-GRSP[a]	−0.45ns										
GRSP[b]	−0.25ns	0.47*									
P Olsen	−0.62**	0.50*	0.65**	0.19ns	0.37ns	0.31ns	0.40ns	0.96**	0.79**	0.72**	0.43ns
T-C[c]	−0.30ns	0.60**	0.60*	0.59**	0.61**	0.25ns	0.43ns	0.78**	0.61**	0.60**	0.78**
Total hyphae	−0.11ns	0.28ns	0.58*	0.51*	0.59*	0.25ns	0.65**	0.65**	0.64**	0.87**	
WDPT[d]	−0.37ns	0.66**	0.48*	0.44ns	0.67**	0.26ns	0.51*	0.56*	0.76**		
GRSP-macro	−0.20ns	0.56*	0.81**	0.44ns	0.67**	0.36ns	0.74**	0.75**			
GRSP-micro	−0.14ns	0.62**	0.81**	0.45ns	0.31**	0.33ns	0.76**				
EE-GRSP-macro	−0.22ns	0.82**	0.61*	0.41ns	0.60**	0.61**					
EE-GRSP-micro	−0.28ns	0.49*	0.57**	0.54**	0.81**						
WSA[e]	−0.36ns	0.62**	0.66**	0.71**							
PC1[f]	−0.36ns	0.79**	0.77**								

Pearson correlation coefficients (r) were calculated from three replicates of each sampling situation ($n = 18$).
Significance conventions: ns = not significant.
[a] EE-GRSP: Easily extractable glomalin related soil protein.
[b] GRSP: Glomalin related soil protein.
[c] T-C: Soil total carbon.
[d] WDPT: Water drop penetration time.
[e] WSA: Water stables aggregates.
[f] PC1: Principal component 1.
* $p \leq 0.05$.
** $p \leq 0.01$.

Water holding capacity ranged from 41.7% to 49.7% under NT10 at 2–5 cm depth and NT6 at 0–2 cm depth, respectively. In addition, water repellency, expressed as WDPT, presented values lower than 1 s in all cases, although significant differences in WDPT were observed between NT systems compared with CT. All treatments have presented WSA values higher than 30%, ranging from 31.3% in CT at 0–2 cm deep to 60.9% in NT6 at 2–5 cm deep (Table 3). In general, an increase in WSA was observed at 2–5 cm deep under NT treatments, showing statistical differences between NT6 and CT; thus, the aggregates fraction ≥4.75 mm was higher in CT, while the microaggregate fraction (≤0.250 mm) was predominant in NT treatments.

The EE-GRSP and GRSP content into the macroaggregate fraction (2 ≤ 0.250 mm) showed higher values under NT6 compared with NT10 and CT in both assayed soil depths (Table 4). In the microaggregates, was observed a decrease in both EE-GRSP and GRSP contents compared with the macroaggregates; thus, the values for EE-GRSP ranged from 0.73 to 1.36 mg g^{-1} in microaggregates in CT and NT6, showing significant differences between NT6 and CT at 2–5 cm soil deep. The total GRSP content was higher in macroaggregates (ranged from 3.54 to 8.78 mg g^{-1}) compared to microaggregates (2.99–7.62 mg g^{-1}), where NT6 showed significant higher values than CT and NT10.

3.4. Relationship among variables studied and multivariate analysis

Several direct relationships among different parameters were found (Table 5). Strong direct correlations were found between GRSP (total and easily extractable) and total C ($r = 0.60$, $p < 0.05$; $r = 0.60$, $p < 0.01$, respectively), GRSP and total hyphae ($r = 0.58$, $p < 0.05$), GRSP and WSA ($r = 0.66$, $p < 0.01$) and total C and WSA ($r = 0.60$, $p < 0.01$). The GRSP contents in macro and microaggregates also showed positive relationships with WSA. Principal components analysis showed that PC1 (35.7%) and PC2 (24.3%) explained 60% of the experimental variance (Fig. 2) and PC1 showed a high correlation with all the parameters studied, except with pH (Table 5). Three different groups were obtained by non-hierarchical cluster analysis. The CT and NT6 treatment formed homogeneous groups with great distance between them, while NT10 showed an intermediate position between the groups previously mentioned.

4. Discussion

Under the assayed conditions no tillage management, specially NT6 promoted the production of a higher density of AM fungal propagules with respect to the conventional tillage practice (CT),

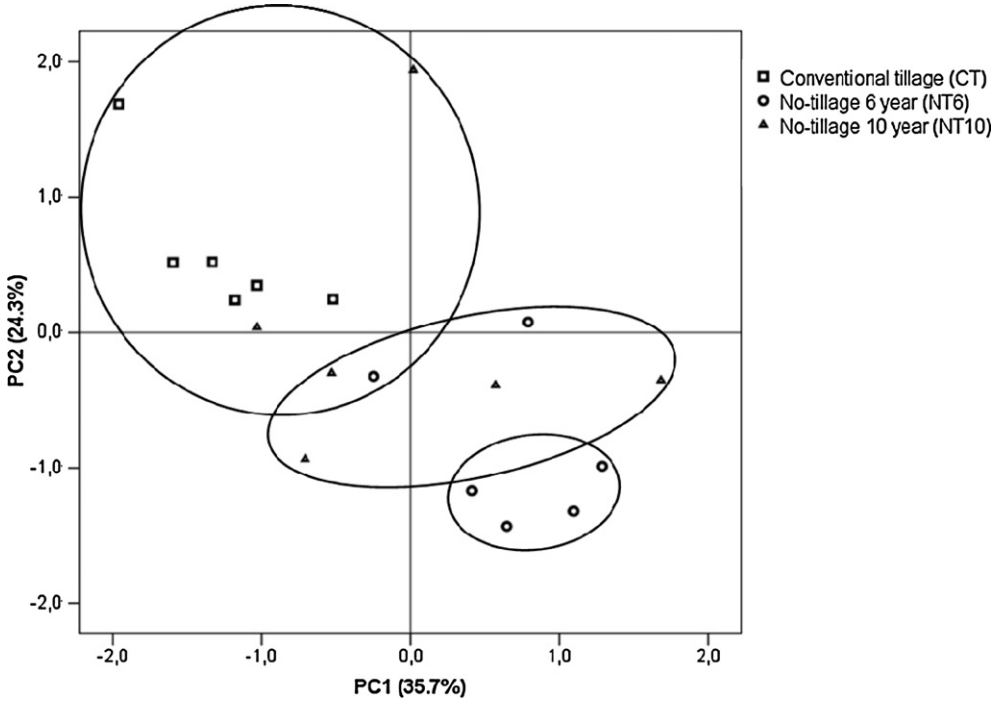

Fig. 2. PCA scores for tillage treatments in a Mediterranean agroecosystem in central Chile. Three replicates of each sampling situation. Percentage values in parenthesis indicate the variation explained by each PC. The circles comprise individuals of similar characteristics according to the cluster analysis, and should be understood as a visual aid for the discrimination of groups.

which is in accordance with previous reports for other type of soils from Central and Southern Chile (Borie et al., 2000, 2006; Castillo et al., 2006; Cornejo et al., 2009; Curaqueo et al., 2010). The lower density of both AM fungi spore and fungal hyphae in CT treatment could be explained by the strong influence of the soil disturbation generated by plowing (Galvez et al., 2001; Kabir, 2005). In this sense, soil plowing reduces the ability of AM fungi to colonize roots, breaking down their hyphal network (Alguacil et al., 2008). On the opposite, in soils under NT, the hyphal network remains intact; and consequently the density of active hyphae is usually greater than soil managed under CT (Kabir, 2005; Cornejo et al., 2009).

On the other hand, GRSP levels measured in NT6 showed values twofold higher than CT, which could represent the active role of AM fungi on glomalin production and accumulating in higher quantities under NT management (Lovelock et al., 2004a). In addition, the contribution of GRSP to total soil C was a 8.9% for CT, while in NT6 the GRSP represented 12.8% of total soil C. Furthermore, the GRSP contents here found were higher than others previously reported in two Ultisols from Southern Chile under NT, reduced tillage and CT with and without stubble burning (Borie et al., 2006) and under CT and NT (Morales et al., 2005). In general, GRSP content decreases with soil depth, and should be related to a lower AM hyphal growth (Lovelock et al., 2004a). Additionally, the great amount of AM mycelium under NT may lead to a more abundant production of glomalin in comparison with CT management. On the contrary, the CT management and the associated disruption of the hyphal network would likely lead to a reduction of glomalin production (Borie et al., 2000) and reduced aggregate stability (Kabir, 2005), as can be observed in the no existing relationship obtained (see Table 5).

Bulk density was not affected by the different applied tillage systems, which agrees with observation by Martínez et al. (2008) and Roscoe and Buurman (2003). In this sense, contrasting effects of soil management in ρ_b are common. In general, it appears obvious that a long-term period under NT management produces an increase of ρ_b, especially under NT10, which could be explained by compacting processes derived from the use of planting machinery (Botta et al., 2009). These process generate a reduction in soil porosity that may lead to a more limited O_2 supply for heterotrophic microbial decomposition (Álvaro-Fuentes et al., 2008a), explaining also the accumulation of a high SOM contents. Tillage is the most widely studied management practice that affects soil hydraulic properties and processes, including WHC (Strudley et al., 2008); thus, higher ρ_b reduced soil porosity and increase WHC values changing the ratio of WHC: air capacity (Husnjak et al., 2002).

Porosity and WHC showed similar values in both soil depths, and were closely related to ρ_b, as previously reported in the same soil (Martínez et al., 2008) and in a Cambisol from China (Tangyuan et al., 2009). In general, it is widely accepted that conservation practices such as NT increase f due to the contribution of organic carbon (Lugato et al., 2009). The collapse of larger unstable aggregates characterized by a minor MWD, and the associated increase in the smaller aggregate sizes is likely to contribute to a lower f and greater ρ_b in the CT systems, compared with NT systems (So et al., 2009).

Mean weight diameter presented similar values to those reported by Martínez et al. (2008), and these values are related to the SOM content in this soil ($r = 0.71$, $p < 0.01$; data not showed), which affects soil particles aggregation (Bronick and Lal, 2005) and distribution. The profits of NT on soil aggregation occurred in the top few cm of soil, as it was expected (Pinheiro et al., 2004). In addition, the higher values in the aggregate size $\geq$2.0 mm found in this study for the NT management could be related to a minor soil water content under tillage systems, as observed by Barzegar et al. (2004).

In the aggregate hierarchy model proposed by Tisdall and Oades (1982), it was suggested that macroaggregates (>250 mm) are formed by binding stable microaggregates (20–250 mm) by temporary (fungal hyphae and roots) and transient linking agents (microbial- and plant-derived polysaccharides). Additionally, Tisdall (1994) indicated the important role of AMF hyphae in the stabilization of macroaggregates. The results obtained in this

study support such role of the AM fungal mycelium, since the hyphal density was higher in NT6 treatment.

The WDPT values observed indicate water uptake or non-water repellence in any treatment (Dekker and Ritsema, 1994). Chenu et al. (2000) found that SOM associated with clay minerals increased hydrophobicity, resulting in resistance of soil aggregates to slaking. Wright and Upadhyaya (1998) suggested a link between the physical presence of glomalin and infiltration rates; therefore, the GRSP would reduce the soil water infiltration rate, thus reducing the effects of slaking. In this sense, we found a positive correlation between EE-GRSP and WDPT, while the correlation between the total GRSP fraction and WDPT was not significant. Nevertheless, Feeney et al. (2004), by means of a soil water sorptivity and repellency test, determined that the presence and amount of GRSP had no effect on soil hydrophobicity.

The NT6 and NT10 treatments presented a higher WSA compared with CT, which corroborate previous studies in order to establish WSA as a parameter highly dependent of tillage system (Borie et al., 2000; Pikul et al., 2009; Curaqueo et al., 2010). There are multiple evidences in relation to a decrease in soil aggregates by the CT management due to the mechanical effects or by destruction of AMF fungal mycelium (Wright and Upadhyaya, 1998; Álvaro-Fuentes et al., 2008b). Another important mechanism involved in the soil aggregation process is the presence of glomalin (Rillig et al., 2002; Nichols and Wright, 2005; Wright et al., 2007). In addition, EE-GRSP and total GRSP contents among macro and microaggregates in the different tillage treatments assayed presented a higher proportion under NT6 compared with the other treatments. In the same way, both glomalin fractions contents were higher in macroaggregates than in microaggregates, presumably contributing to bind microaggregates into the macroaggregates (Wright et al., 2007).

Positive correlations between both glomalin fractions and WSA were found (see Table 5), as it was also found in other studies (Rillig et al., 2002; Cornejo et al., 2008; Curaqueo et al., 2010). Our results, especially considering PCA (Fig. 2), suggest a high level of relationship of soil conditions and tillage treatments, evidencing the formation of three dissimilar groups, in complete concordance with the different treatments assayed. In this sense, these formed groups reveal an improvement in almost all parameters in NT respect to CT. However, the extension by 10 years under NT produced a global reduction in the beneficial effect observed in NT6, similar to those found in CT. In this sense, our results suggest that the soil compactation produced for machinery (Table 3) may be related to a decrease in the values of desirable soil characteristics after extended periods under NT, and could justify the intercalation of a moderate plowing in long-term programs of reduced or no-tillage in medium/heavy soil as the here used. Based on the results here obtained, and the high direct correlations obtained between PC1 and total C (0.81, $p < 0.001$) and the diverse GRSP fractions (in general close to 0.8), these parameters could be used as indicators of the time needed to include a soft soil decompactation step in a long-term NT programs in agricultural soils intended to annual crops. The inclusion of a crop with strong roots in the rotation system could be feasible for overcoming the compactation using such crop as biological plow.

5. Conclusions

Results showed strong effects of the diverse tillage/management systems on mycorrhizal propagules, glomalin contents and soil physical properties. The positive relationship between GRSP contents, total hyphae and water stable aggregates corroborated the beneficial role of AM fungi and GRSP on soil aggregation, increasing this relationship under non disturbing systems. Although the no tillage systems presented beneficial effects in all evaluated variables compared with conventional tillage, a decrease in the majority of these parameters was observed when long-term no tillage system is applied. Thus, further studies are needed for avoiding the strong compactation in long-term no tillage programs for redistributing SOM and nutrients, and improving overall soil physical properties. In this sense, some global parameters as total carbon could be useful to establish the adequate time for plowing in medium/heavy soils intended to annual crops.

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