TILLAGE EFFECT ON SOIL ORGANIC MATTER, MYCORRHIZAL HYPHAE AND AGGREGATES IN A MEDITERRANEAN AGROECOSYSTEM

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) and their product glomalin (GRSP) play a decisive role in the soil aggregation, affecting the carbon (C) dynamics in agroecosystems. Tillage affects the AMF activity and GRSP content, influencing the stability and the soil C forms as well. The aim of this study was to compare the effect of no tillage (NT) and conventional tillage (CT) on: i) arbuscular mycorrhizal hyphal length and GRSP content; ii) the nature of soil organic matter by means of physical fractionation (free particulate organic matter [fPOM]; occluded particulate organic matter [oPOM] and mineral-associated soil organic matter [Mineral]), as well as chemical fractionation (fulvic acid, humic acid and humin), and iii) the relationships between AMF parameters, soil carbon and water stable aggregates (WSA) in a Mollisol of Central Chile managed for 6 years under NT and CT using a wheat-corn rotation. Higher values in the AMF hyphal length, GRSP and WSA in NT compared with CT were observed. Significant relationships were found between GRSP and WSA ($r = 0.66$, $p < 0.01$) and total mycelium and GRSP ($r = 0.58$, $p< 0.05$). The total carbon increased 44% under NT compared with CT. The chemical fractionation showed percentage greater than 95% for humim in both treatments. Physical fractionation indicates that the higher part of the SOC (89.4 - 95.1%) was associated with the mineral fraction.

Keywords: Agroecosystem, Glomalin Related Soil Protein, Mollisol, Organic Matter Fractions, Soil Aggregates.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts fungi, which have a wide distribution in the terrestrial ecosystems and in a vast diversity of climate and soil-types, forming symbiotic associations with the majority of plants (Fitter *et al.*, 2000; Jeffries *et al.*, 2003).

In an ecosystem context, the AMF activity affects the carbon dynamics by different mechanisms (Zhu and Miller, 2003), among them, the protection of organic matter into soil aggregates by means of the AMF mycelium and the production of glomalin. This compound has been operationally defined and extracted from soil as glomalin-related soil protein GRSP (Rillig, 2004) of a proteic nature (Gadkar *et al*., 2006; Wright and Upadhyaya, 1998) and highly recalcitrance compound (Driver *et al*., 2005). Previous studies have linked the GRSP content with the aggregate stability (Rillig, 2004; Wright *et al*., 2007) and soil C accumulation (Lovelock *et al*., 2004; Treseder and Turner, 2007). Soil management, in special, the tillage systems, affects all soil properties, including AMF activity, diversity (Alguacil *et al*., 2008; Borie *et al*., 2008; Cornejo *et al*., 2009; Jansa *et al*., 2003; Sieverding, 1991), and glomalin production (Wright *et al*., 2007; Wright *et al*., 1999), being important factors controlling organic C storage in soils. They may also change the relative importance of different mechanisms of soil organic matter (SOM) stabilization (John *et al*., 2005). On the other hand, no-tillage agriculture, which returns organic residues to soil, can produce a positive effect on soil characteristics. The aims of this study were to evaluate the effect of non tillage (NT) and conventional tillage (CT) on: i) AMF hyphal length and GRSP content. ii) the SOM nature by means of physical and chemical fractionation and iii) the relationships between AMF parameters, soil carbon and water stable aggregates. Thus, the study of AMF role and glomalin is important for evidencing soil C dynamics and its contribution to the stabilization of soil C in the agricultural systems with the goal of improving the sustainability of agroecosystems.

MATERIALS AND METHODS

Site description

The experiment was carried out in a Mediterranean agroecosystem placed in the Antumapu Experimental Station of the Universidad de Chile (33º40`S, 70º38`W). The used soil was a Mollisol (thermic Entic Haploxerolls) with a little slope (0.5 - 2%). The climate of the agroecosystem is temperate, Mediterranean-semiarid with dry summers and cold winters. The maximum mean temperature is 28.7ºC (January), the minimum mean temperature is 3.4ºC (July) and the mean annual rainfall is 330 mm (Santibañez and Uribe, 1990). In this site, a durum spring wheat (*Triticum turgidum* L. var durum)-corn (*Zea mays* L.) rotation experiment under 6 years of no tillage (NT), and conventional tillage (CT) was performed.

Soil sampling and analysis

Soil samples from rotation experiment were collected from plots of 192 m^2 (40 x 4.8 m) one month before wheat sowing (May) at 2006-2007 season. Each soil sample was composed by 10 sub-samples obtained at 0-5 cm soil depth. The soil samples were homogenized and transported in plastic bags to the laboratory and stored at 4ºC until the implementation of the different determinations.

Mycorrhizal determination

The extraction of hyphae (total and active) was carried out according to Rubio *et al*., (2003), and quantified using the method of grid-line intersection (Giovannetti and Mosse, 1980). The glomalin fractions were obtained according to the method of Wright and Upadhyaya (1998). The easily extractable GRSP (EE-GRSP) was extracted from 1 g of soil in 8 mL of citrate buffer (20 mM and pH 7.0) and autoclaving at 121ºC for 30 min. Total GRSP (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 121ºC, repeating this procedure several times on the same sample until the reddish-brown color typical of GRSP disappeared from the supernatant. Both fractions were centrifuged at 8000 g for 15 min and filtered through Whatman filter Nº 1. The content of protein was determined by Bradford protein assay (Bio Rad Protein Assay; Bio Rad Labs) with bovine serum albumin as standard (Wright *et al.*, 1999). After the extraction, one fraction of the GRSP was precipitated by slow addition of 2 M HCl up to pH 2.5, centrifuged at 8000 g for 20 min, redissolved in 0.5 M NaOH, dialyzed against deionized H_2O using a dialysis membrane 6000-8000 Da (Spectra/Por, Spectrum Labs. Inc) and freeze-dried in a Freeze dryer Alpha 1-2 (CRHIST, Inc.).

Soil aggregation and fractionation

The water aggregate stability (WAS) was measured using the procedure of Kemper and Rosenau, (1986). Additionally, dry aggregates were grouped in macroaggregates (2≤0.250 mm) and microaggregates (≤0.250 mm) according to Oades and Waters (1991) and Tisdall, (1994) classification for determining the total-GRSP content in each aggregate fractions.

The physical fractionation of SOM was performed by the method of density fractions (John et al., 2005) obtaining free particulate organic matter <1.6 g cm^{-3} (fPOM); occluded particulate organic matter 1.6 to 2.0 g cm^{-3} (oPOM) and mineral-associated soil organic matter >2 g cm^{-3} (Mineral). The chemical fractionation was carried out following the method proposed by Swift (1996), obtaining humin (Hum), humic acid (HA) and fulvic acid (FA) fractions. Total C from bulk soil, C associated to glomalin (GRSP-C), C from the different SOM chemical fractions (Hum, HA and FA) and physical fraction (fPOM, oPOM and mineral) was determined using a C, H, N, S analyzer (VARIO/EL).

The experimental design was completely randomized, with two tillage systems and three replicates in each case. The data were statistically analyzed using the t-student test ($P \leq 0.05$). Correlation analysis using Pearson coefficient (r) was performed to evidence some linear relationships among the studied variables. The statistical analyses were performed using SPSS software, version 14.0 (Visauta, 2007).

RESULTS AND DISCUSSION

The arbuscular mycorrhizal parameters such as total and active AM hyphae, GRSP and EE-GRSP contents are shown in Figure 1. Total and active AM hyphal lengths were not significantly affected by the different tillage systems used; nevertheless, an increase in both, total and active hyphae length were observed in NT system when compared with CT (Figure 1A). Total hyphal length ranged between 3.65 and 4.98 m g^{-1} for CT and NT treatment, while, the active hyphae fraction ranged from 0.59 to 0.91 m g^{-1} in CT and NT, respectively. The active AM hyphae represented a 16.2% and 18.3% of total hyphae for CT and NT, suggesting a higher activity of AMF under less intensive treatment, such as NT in relation to CT.

There are several studies in Ultisols and Andisols from Southern Chile where the same trend was observed (Borie *et al.*, 2006; Borie *et al.*, 2000; Castillo *et al.*, 2006; Cornejo *et al.*, 2009). In this sense, there are several studies showing that the use of conservation tillage systems, as no-tillage or minimum tillage produce positive effects on AMF propagules, including spore number, colonized root and hyphal length (Alguacil *et al*., 2008; Cornejo *et al*., 2009). In contrast, intensive land use and conventional tillage have a negative impact on AMF hyphae (Kabir *et al*., 1998). In soils under no-tillage systems, the hyphal network remains intact; thus, the density of active hyphae is greater than under CT soils (Cornejo *et al*., 2009; Kabir, 2005; Kabir *et al*., 1997)

The contents of both GRSP fraction measured (GRSP and EE-GRSP) differed significantly between the evaluated treatments (Figure 1B). No-tillage system increased the GRSP content compared with conventional tillage in both fractions (GRSP=8.16 mg g^{-1}; EE-GRSP=2.03 mg g^{-1} under NT and GRSP=3.96 mg g^{-1}; EE-GRSP = 1.16 mg g^{-1} under CT). The GRSP values obtained in this study were higher than those reported by Wright *et al*., (2007) in an Ultisol in the Mid-Atlantic area of the USA managed under chisel-tillage. Besides, these contents were higher than those reported in two Ultisols of Southern Chile under no-tillage, reduced tillage and conventional tillage with and without stubble burning (Borie *et al*., 2006). The different results may be attributed to no soil disturbance in NT system, improving the amount and the activity of AMF hyphae in relation to CT (Cornejo *et al*., 2009; Kabir *et al*., 1997), and, consequently, the levels of glomalin (Kabir, 2005).

Total soil C content was higher in NT than CT showing an increase of 44% under NT compared with CT (Table 1). This increase in the C content under conservation tillage systems compared with conventional tillage agrees with other previous studies in this soil (Acevedo and Martínez, 2003; Martínez *et al*., 2008).

Physical fractionation of organic matter showed that mineral fraction was the most important fraction in the two assayed tillage systems, with values of 91.5% for NT and 93.8% for CT. The oPOM fraction ranged between 4.4% under CT to 7.4% under NT. Finally, the fPOM fraction (fraction < 1.6 g cm^{-3}) presented lower values compared with the oPOM and mineral fractions, which obtained values from 1.1% in NT to 1.8% in CT. The values for the different obtained fractions are in accordance with John *et al*., (2005). In both treatments, the bulk of the SOC was associated with the heavy mineral fraction (>2 g cm^{-3}) with a 95.0% for CT and 89.4% for NT, while occluded particulate organic matter (oPOM fraction: 1.6 to 2.0 g cm^{-3}) showed values between 4.4% under CT and 10.3% under NT. Free particulate organic matter (fPOM) has a labile nature relation to oPOM fraction (Christensen, 2001; Franzluebbers *et al*., 2000; Jones and Donnelly, 2004), in which it can be protected against microbial attack (Bossuyt *et al*., 2002; Grünewald *et al*., 2006). Chemical fractionation of soil organic matter showed similar results in both NT and CT systems, presenting the highest C proportion in the humin fraction (CT=95.5% and NT=96.4%) and closer values for humic and fulvic acids (range between 0.4% to 0.6%). The sum of all chemical fractions (Hum, HA and FA) is considered the stable-C form in the soil. The humin fraction in this study was higher compared with studies carried out in Andisols and Ultisols from Chile (Heredia *et al*., 2007), in which the humin fraction ranged between 61.4% to 81.56%. Humic and fulvic acid was lower that those reported for the same authors.

The GRSP concentration among macro and microaggregates showed an increase in GRSP concomitant with the increase in aggregate size for both treatments. In both aggregate fractions (macro and micro aggregates), significant differences in the GRSP content were found between CT

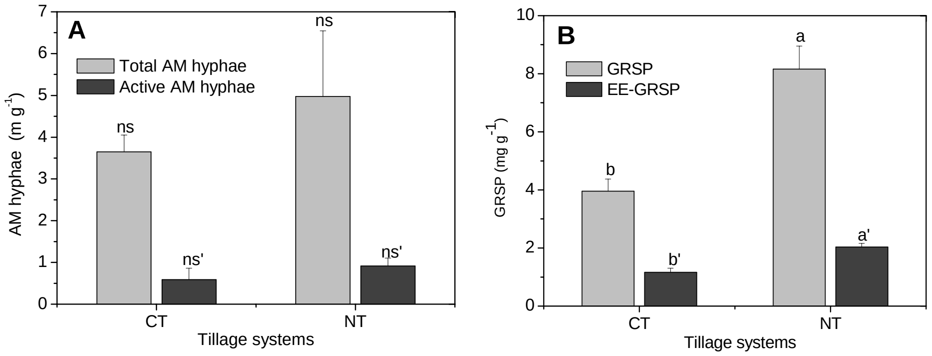

Figure 1. Arbuscular mycorrhizal parameters in a Mollisol of Central Chile managed six years under conventional tillage (CT) and no-tillage (NT) systems. **A)** Total and active AM hyphae. **B)** Glomalin fractions, GRSP: total glomalin; EE-GRSP: easily extractable glomalin. Different letters indicate significant differences among tillage systems according to the t-student test (P≤0.05). Errors bars indicate the standard error (SE).

Table 1. Effect of conventional and no-tillage on total C, physical and chemical fractionation and GRSP content in macroaggregates and microaggregates in a Mollisol of Central Chile.

Tillage Treatment	Total C	fPOM	oPOM	Mineral	Hum	HA	FA	Macroaggregate-GRSP	Microaggregate-GRSP
	($g\ kg^{-1}$)	*(% of dry weigth)*			*(% of dry weigth)*			*($mg\ g^{-1}$)*	*($mg\ g^{-1}$)*
CT	17.1b	1.80	4.35	93.84	95.5	0.6	0.5	3.75b	3.09b
NT	24.8a	1.12	7.38	91.50	96.4	0.5	0.4	7.96a	7.16a

Different letters indicate significant differences among tillage systems according to The t-student test (P≤0.05). CT: Conventional tillage; NT No-tillage; fPOM: Free particulate organic matter; oPOM: Occluded particulate organic matter; Hum: Humin; HA: Humic acid; FA: Fulvic acid.

and NT systems, where higher GRSP content was found in NT system in relation to CT. The GRSP in macroaggregates was 3.75 to 7.96 mg g^{-1} and 3.09 to 7.16 mg g^{-1} in microaggregates. This trend agrees well with Wright *et al*., (2007).

Water stable soil aggregates presented higher values under NT (59%) than CT treatment (32%) (Figure 2A); these results are in accordance with previous studies (Castro Filho *et al*., 2002; Martínez *et al*., 2008; Pikul *et al*., 2009). There are various evidences in relation to the fact that the CT management produces a decrease in the soil aggregation due to mechanical effects or by destruction of the network of AMF fungal mycelium (Alvaro-Fuentes *et al*., 2008; Six *et al*., 1999; Wright and Upadhyaya, 1998).

Carbon balance is shown in Figure 3, where the data for total C, humin C (Hum-C), humic acid C (HA-C), fulvic acid C (FA-C), C associated to GRSP (GRSP-C), free particulate organic matter C (fPOM-C), particulate organic matter C (POM-C) and mineral C (Mineral-C) are presented. In both tillage systems, the

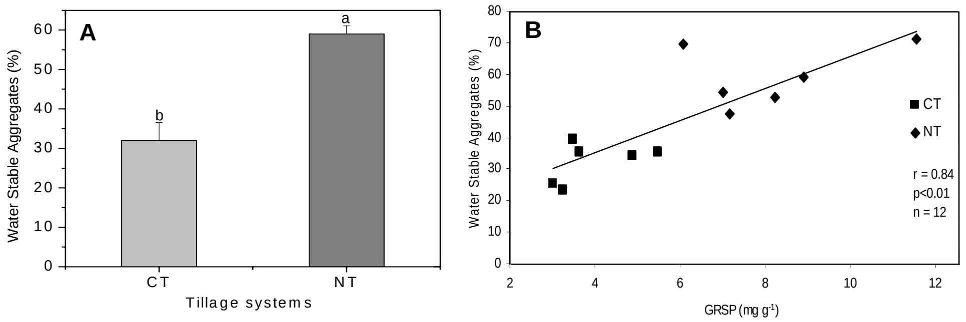

Figure 2. A) Effect of six years of conventional tillage (CT) and no-tillage (NT) systems on water stable aggregates (WSA) in a Mollisol from Central Chile. **B)** Correlation between GRSP contents and % of WSA in the agroecosystem studied. Different letters indicate significant differences among tillage systems according to the t-student test (P≤0.05). Errors bars indicate the standard error (SE).

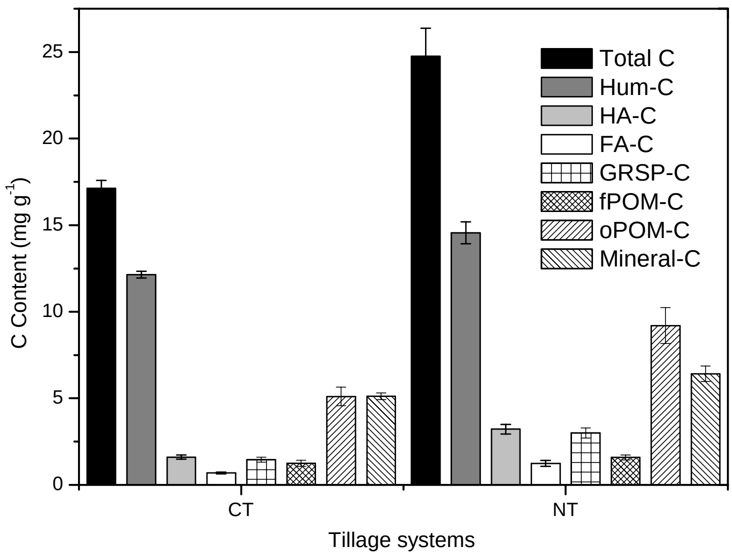

Figure 3. Carbon content from different pools in a Mollisol of Central Chile managed six years under conventional tillage (CT) and no-tillage (NT). Errors bars indicate the standard error (SE). Hum-C: C associated to humin fraction; HA-C: C associated to humic acid fraction; FA-C: C associated to fulvic acid fraction; GRSP-C: C associated to GRSP; fPOM-C: C associated to free particulate organic matter fraction; oPOM: C associated to occluded particulate organic matter fraction; Mineral-C: C associated to mineral fraction.

Hum-C was the most important fraction that contributed to C pool, ranging from 12.15 to 14.56 g kg^{-1} of C, representing 70.9% and 58.8% of total C of soil in NT and CT, respectively. GRSP-C obtained a concentration of 1.46 g kg^{-1} in CT, while in NT the concentration was 3.0 g kg^{-1} with an increase of 100%.

The sum of chemical C fractions plus GRSP-C represents almost 93% of total soil C in CT, while in NT, the same fractions reached about 88.9%. The GRSP-C presented similar values with the Hum-C and ranged between 8.5% under CT and 12.1% in NT. The fractions derived from the physical fractionation presented lower values (67.0% in CT and 69.5% in NT) compared with the chemical fractions. The oPOM-C and Mineral-C fractions presented similar contribution to C pool in CT, while in NT system, the contribution of oPOM-C was higher than the Mineral-C.

The effect of tillage systems on the C level in each fraction showed that all the C concentrations were increased under NT compared with CT. For the chemical fractionation, total-C presented an increase of 44.6%, Hum-C a 19.84% and FA-C fraction increased 100%. The increase in the GRSP-C pool was 105.5%. The pools derived from physical fractionation obtained an increase of 27.2% for fPOM-C, 80.0% for oPOM-C and 25.4% for Mineral-C under NT compared with CT.

Significant relationships among different parameters were found. The direct relationship found between GRSP and WSA ($r = 0.66$, $p < 0.01$) (Figure 2B), which have been previously documented (Rillig *et al.,* 2002), is notable. Other significant relationships were found between GRSP and total C ($r = 0.60$, $p<0.05$ for both GRSP fractions), GRSP and total hyphae ($r = 0.58$, $p < 0.05$), total C and WSA ($r = 0.60$, $p < 0.01$) and the inverse correlation between pH and the other variables studied. The GRSP contents in macro and microaggregates also showed positive relationships with WSA.

CONCLUSIONS

Our results show clear evidence that tillage treatment affected soil organic matter (in quality and quantity), mycorrhizal parameters and soil aggregation. Thus, positive effects of NT in all evaluated parameters compared with CT treatment were observed. In this sense, soils under NT systems promote the C accumulation in more stable physical and chemical forms. Additionally, the obtained results suggest an active role of AMF and GRSP on soil aggregation and its concomitant contribution to the stability of organic matter in the temperate agroecosystem here studied.

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