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TILLAGE EFFECTS ON SOIL ORGANIC CARBON, MICROBIAL BIOMASS CARBON AND BETA-GLUCOSIDASE ENZYME ACTIVITY IN A TYPIC HAPLOXERERT SOIL

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Abstract

Biological indicators allow to define early changes in soil environment due to the impacts in land management over time. This study investigated the effects of long-term (2006-2015) two conventional (CT-1 and CT-2), three reduced (RT-1, RT-2 and RT-3) and a no-till (NT) on soil organic carbon (SOC), microbial biomass carbon (MBC) and beta-glucosidase enzyme activity (BGA) in eastern Mediterranean region, Turkey. Experimental design was randomized complete block with three replications. Results indicated that SOC, MBC and BGA significantly differed among tillage treatments. Non-disturbed soils under NT had nearly 75% higher SOC (8.80 gkg^{-1}), 359% higher BGA (207.66 mg PNP kg^{-1}h^{-1}) and 68% higher MBC (185.9 mg C kg^{-1} soil) compared with highly disturbed soils under CT-1 system. The BGA and MBC concentrations under NT were also significantly higher than the three RT treatments. In contrast to BGA and MBC, the SOC contents under RT systems did not differ from that of NT treatment. Higher concentrations of BGA and MBC under NT and partially under RT compared to CT treatments were most likely related to the increased organic matter contents and non-disturbed environmental conditions.

***Key words**: soil organic carbon, microbial biomass carbon, beta-glucosidase enzyme activity, conventional tillage, conservation tillage.*

INTRODUCTION

Conventional tillage using mouldboard ploughing, subsequent disking and floating for seed bed preparation has been frequently used tillage practice by farmers in many parts of the Turkey. Even in state farms, as in Ceylanpınar that is the largest (163,000 ha) state owned and managed farm in Turkey, conventional practices have been widely used. Farmers prefer this practice for appropriate seed bed preparation by breaking down crop residues along with weeds, incorporating the crushed biomass into the soil, increasing seed-soil contact by breaking larger aggregates or soil clods and providing warmer and drier seedbed for early spring planting (Soane et al., 2012; Dozier et al., 2017).

Despite the short-term benefits of conventional tillage for seed bed preparation, aggressive physical disturbance induces decomposition of soil organic matter (SOM) by creating appropriate soil environment for microorganisms, and subsequently reduces soil fertility and quality (Álvaro-Fuentes et al., 2013; Abdullah, 2014). Alteration in SOM status of soils results in substantial changes in soil microbial biomass and enzyme activities, both are highly correlated with soil organic carbon (SOC) content of soils (Acosta-Martínez et al., 2003; Melero et al., 2008). Therefore, soil microbial properties such as microbial biomass, as a primary source of soil enzymes and pool of SOC and activities of soil enzymes are considered important and sensitive indicators to understand and compare the effects of soil disturbance resulting from tillage practices on soil quality (Álvaro-Fuentes et al., 2013; Kabiri et al., 2016). Conservative tillage systems, reduced or no-till, minimize the soil disturbance and results in improvement of soil quality by increasing SOC with a higher microbial activity, nutrient and water supply and water stable aggregates (Lal, 2015). Minimizing incorporation of crop residue reduces rate of mineralization of organic matter by physically protecting organic matter from microbial decomposition (Tripathi et al., 2014). Experiments on investigating the effects of conservative tillage on microbial properties of soils have been studied elsewhere in many countries of the world, but very few long-term studies were available reporting the effects of tillage practices on microbial properties in Turkey. Thus, this study was conducted to investigate the responses of SOC, microbial biomass C and activity of β-glucosidase enzyme to long-term (nine years) two conventional, three reduced and no-till practices under Mediterranean climate in southern Turkey.

MATERIALS AND METHODS

Study Site and Experimental Details

The experiment was conducted at the Agricultural Research Station of the Cukurova University (37°00′54″ N, 35°21′27″ E; 32 m altitude) with Mediterranean climate in Adana, Turkey. The average annual precipitation is 639 mm, and temperature is 19.2°C. The experimental plots were established in 2006 on a clayey soil classified as smectitic, active, mesic Typic Haploxererts. The initial soil properties in the surface layer (0-30 cm) were 50% clay, 32% silt and 18% sand, pH (saturation paste) is 7.82, electrical conductivity (saturation paste) is 0.15 dS m^{-1}, calcium carbonate is 244 g kg^{-1} (Çelik, 2011).

The plots were 12 m width and 40 m length (480 m^2) with 4 m buffer between each plot. In this study, six tillage systems in rotation of winter wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.) – grain maize (*Zea mays* L.) were applied for nine years.

In all tillage methods, the harvest residues on soil surface were chopped prior to tillage operations except CT-2. The tillage treatments were:

1) Conventional tillage with residue incorporated (CT-1): In CT-1, soil was tilled to 30-33 cm depth using a moldboard plow before winter wheat followed by two passes of disc harrow at 13-15 cm and 2 passes of float. For the second crop, soil was tilled with a heavy tandem disc harrow (HTD) to a depth of 18 to 20 cm, followed by 2 passes of disc harrow to 13-15 cm depth and 2 passes of float.

2) Conventional tillage with residue burned (CT-2); In CT-2, crop residues were burned after each harvest differed from CT-1 and also chisel plow instead of HTD to the depth of 35 to 38 cm was used in second crop.

3) Reduced tillage with heavy tandem disc harrow (RT-1); In RT-1, soil was tilled with a HTD to a depth of 18-20 cm (2 passes) and followed by 2 passes of float before wheat planting. For the second crop, rotary tiller (RoT) was used to 13-15 cm depth and 2 passes of float.

4) Reduced tillage with rotary tiller (RT-2); In RT-2, RoT was used at 13-15 cm depth and followed by 2 passes of float before first and second crop planting.

5) Reduced tillage with heavy tandem disc harrow followed by no tillage for the second crop (RT-3); In RT-3, soil was tilled with a HTD to 18-20 cm depth and followed by 2 passes of float before wheat. A non-selective herbicide (500 g ha^{-1} Glyphosate) was applied for weed management, and NT planter was used for planting of second crop soybean or corn.

6) No-tillage, direct planting (NT); In NT, crop residue on soil surface were chopped as in all other treatments except CT-2, a non-selective herbicide (500 g ha^{-1} Glyphosate) was applied for weed management, and NT planter was used for planting in both the first and the second crop.

Chemical fertilizer application rate was the same regardless of tillage method: 170-180 kg N ha^{-1} and 55-60 kg P_2O_5 ha^{-1} for wheat, 250-265 kg N ha^{-1} and 60-65 kg P_2O_5 ha^{-1} for corn and 120-130 kg N ha^{-1} and 40-45 kg P_2O_5 ha^{-1}

for soybean based on soil analysis. Commercially available corn and soybean cultivars at seeding rates of 8.4 and 23.6 plants per m^2 were planted in the third week of June and harvested in the second week of October.

Soil Samplings and Laboratory Analyses

Disturbed and undisturbed soil samples at the 0-10 cm depth were taken after second crop harvest of corn in 15th of December, 2015.

The activity of β-glucosidase enzyme was determined following the method based on the colorimetric estimation of the p-nitrophenol (Tabatabai, 1982). In this method, 1 g of soil was incubated for 1 h at 37°C with a buffered p-nitrophenyl-β-D-glucopyranoside solution (pH 6.0) and toluene. The p-nitrophenol formed by the hydrolysis of the p-nitro-phenyl-β-D-glucopyranoside at 37°C for 1 h was determined by measuring the yellow filtrate colorimetrically after color development of the soil suspension with 1 mL 0.5 mol L^{-1} $CaCl_2$ and 4 mL of tris (hydroxymethyl) aminomethane buffer (pH=12). The β-glucosidase activity was expressed as micrograms of p-nitrophenol released per gram dry soil per hour.

The amount of soil microbial biomass carbon (MBC, mg C kg^{-1} soil) was determined using the substrate induced respiration (SIR) method (Anderson and Domsch, 1978). In the SIR method, 5 g of moist soil was weighted into small jars, 1 ml glucose was added (0.5% w/w) and waited for 2 hours. After two hours, 2.5 ml of 0.05 M NaOH within small tubes were placed into the jars as an alkali trap. The jars were tightly closed and inserted into the incubator for 4 to 6 hours at 25°C. The same operations were repeated without soil as controls. After the incubation, the NaOH was removed, and 5 ml 0.5 M $BaCl_2$ was added to precipitate the absorbed CO_2 as insoluble carbonate, and the supernatant was titrated with phenolphthalein indicator against 0.05 M HCl to calculate CO_2 released from soil (mg C kg^{-1} soil), against corresponding controls.

Bulk density was determined using soil cores (length 5.1 cm, diameter 5.0 cm) collected from three depths. The soil samples of known volumes were weighed, oven dried at 105ºC for 24 h to a constant weight and weighed to calculate bulk density (Blake and Hartge, 1986).

Soil organic carbon (SOC) was calculated through the dividing soil organic matter content by the Van Bemmelen coefficient of 1.724 organic matter is equal to 58% of carbon (Nelson and Sommers, 1982).

Organic C stock of soils under each of tillage system was calculated on an equal mass basis references to 0-10 cm depth using the organic C concentrations and soil bulk densities of each sampled plot (Lal et al., 1998; Mishra et al., 2010).

$$Cs = OC\ x\ Bd\ x\ D\ x\ A$$

where: Cs is the organic C stock (kg ha^{-1}); OC is the soil organic carbon (g kg^{-1}); Bd is the soil bulk density (Mg m^{-3}); D is the thickness of soil horizon (m); A is the area (ha: 10^4 m^2).

Microbial quotient calculated as the ratio of MBC to SOC (Insam et al. 1989; Anderson and Domsch, 1989).

Statistical Analyses

Kolmogorov-Smirnov test was used to control the distribution of data for normality. The data had normal distribution and no need to use any kind of transformation to normalize the data. The effects of tillage systems and the differences between tillage systems were assessed by analysis of variance (ANOVA) test. Differences among treatments were evaluated by DUNCAN test ($P<0.05$). The statistical analyses were performed using IBM SPSS statistical package (version 21.0, SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSIONS

Soil Organic Carbon

Tillage effect on soil organic carbon (SOC) was statistically significant ($P<0.01$) (Figure 1). The SOC increased with the decrease in tillage, and the increase in SOC concentration among tillage systems was in this order: CT-1 > CT-2 > RT-1 > RT-2 > RT-3 > NT. The SOC concentration of soils was ranged from 8.80±0.48 g C kg^{-1} soil (CT-1) to 15.40±0.93 g C kg^{-1} soil (NT) among six tillage treatments. The NT had 75% and 58% higher SOC concentrations than CT-1 and CT-2 treatments.

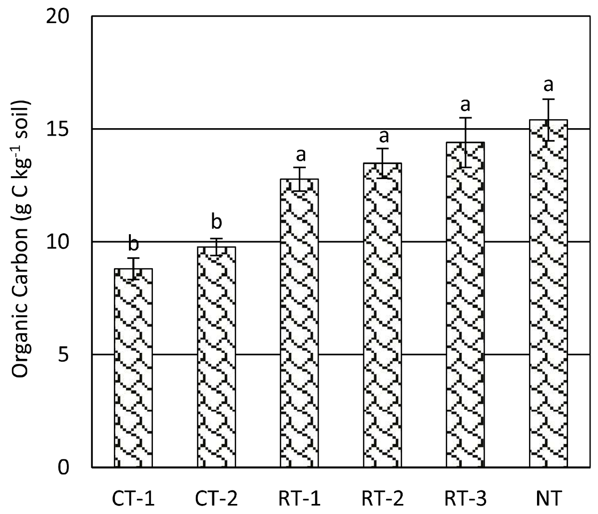

Figure 1. Soil organic carbon concentration (g C kg^{-1} soil) of soils under different tillage systems. Different letters on each bar indicate significant differences among tillage treatments at $p<0.05$

The increase in SOC concentration with minimal or no soil disturbance implies considerable C sequestration potential of soils under RT and NT systems. The final total SOC stock after 9 years under NT treatment (21127 kg C ha^{-1}) was 77 and 61% higher than conventionally tilled CT-1 (11952 kg C ha^{-1}) and CT-2 (13103 kg C ha^{-1}) treatments. The difference in C stock among NT and RT treatments was lower compared to CT, and the differences among NT and RT treatments were not significant (Figure 2). Soil carbon may reach to an equilibrium under NT within 15-20 years or 20-25 years (West and Post, 2002) depending on genetic such as climate, soil type and amount of initial SOC (Schneider, 2007), and dynamic factors i.e., crop rotation and other management options. Thus, our soils can still sequester great amount of C till reaching to equilibrium. Similar to our results on C sequestration, Martinez et al. (2013) stated that after nine years of NT implementation, soil C concentration under NT was 4980 kg C ha^{-1} higher than CT. Karlen et al. (2013) also reported significantly lower SOC concentration under 31 years of conventional tillage using moldboard plough in a corn/soybean rotation and 26 years of conventional tillage in continuous corn cultivation compared to NT. Higher SOC accumulation in surface soils under NT compared to CT systems was ascribed to slow decomposition of crop residue in the compacted surface layer (Martinez et al., 2013), and physical protection of soil organic C within soil aggregates (Souza et al., 2014).

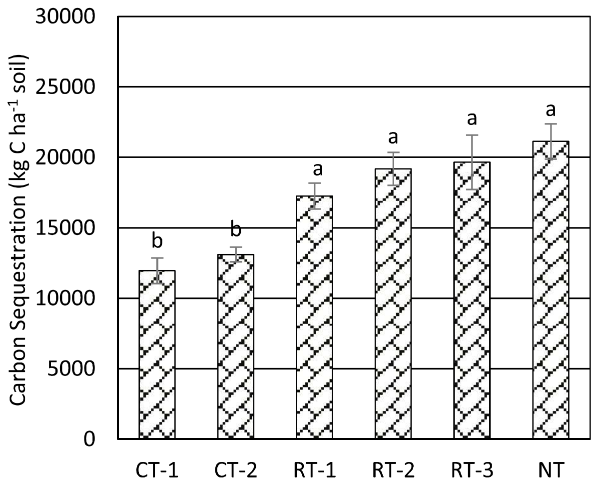

Figure 2. Carbon sequestration (kg C ha^{-1} soil) for nine years under different tillage systems. Different letters on each bar indicate significant differences among tillage treatments at $p<0.05$

Microbial Biomass Carbon

Microbial biomass is defined as the living organic matter fraction of soils and an important source as an energy and nutrient reservoir (Souza et al., 2014). Similar to SOC, the microbial biomass carbon (MBC) concentrations of soils were statistically different ($P<0.01$) among tillage systems (Figure 3). The MBC concentration of soils presented the same behavior as that observed for SOC which increased with the decrease in soil disturbance. The MBC was the highest under NT (312.3 ± 10.6 mg C kg^{-1} soil) due to better soil conditions for microorganisms, while it was the lowest under CT-2 (178.2 ± 12.5 mg C kg^{-1} soil) where crop residues were burnt after each harvest of crop in rotation.

Higher MBC under NT management implies better soil biological quality under non-disturbed soil environment (Doran and Parkin, 1994). After nine years, the MBC concentrations under RT-1 (247.0 ± 18.4 mg C kg^{-1} soil) and RT-2 (249.9 ± 12.4 mg C kg^{-1} soil) were significantly higher compared to CT-1 and CT-2, however they were significantly lower compared to NT. In other studies, similar increases in MBC with the decrease in soil tillage intensity and frequency have been reported by others. For example, Martin-Lammerding et al. (2015), in a study conducted under the semi-arid region of central Spain, obtained a very high value of MBC under NT system compared to RT and CT systems. In a 6 years old tillage experiment, the MBC concentrations under CT using moldboard and disc plows were found 25 and 43% lower than less disturbed soils using chisel and rotary plows under RT system (Kabiri et al., 2016). The increased MBC under NT and RT in comparison to CT emphasizes the improved environmental conditions due to the minimal disturbance and lower decomposition of organic matter and the importance of conservative tillage practices in Mediterranean climate on enhancing microbial efficiency of soils. In contrast to the reports on significant effects of tillage on MBC, Mbuthia et al. (2015) found no significant influence of long term (31 years) tillage on MBC under continuous cotton at West Tennessee Research and Education Center in Jackson, TN, USA. However, they reported significant alterations in the microbial community structure composition by tillage. This contradictory result was ascribed with a combination influence of soil type (sandy texture) and the low biomass produced under cotton compared to other crops.

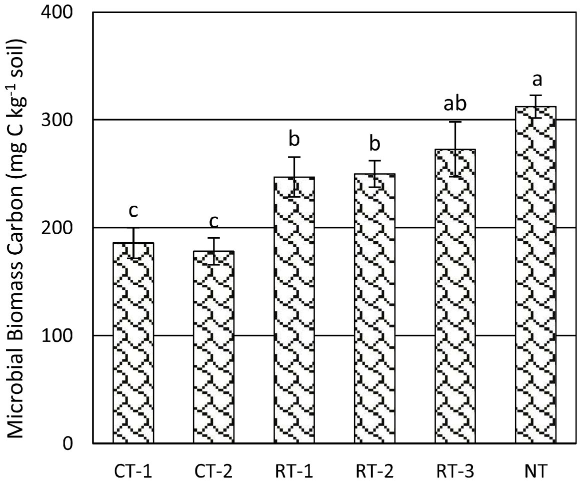

Figure 3. Microbial biomass carbon concentration (mg C kg^{-1} soil) of soils under different tillage systems. Different letters on each bar indicate significant differences among tillage treatments at $p<0.05$

The ratio of MBC to SOC is defined as microbial quotient and widely used for an early indicator of microbial activity due to alteration in soil environment (Sparling, 1992; Martin-Lammerding et al., 2015; Deng et al., 2016). Although both SOC and MBC were significantly different among tillage systems, MBC/SOC ratio slightly different among tillage systems and the difference was not significant (P=0.554). Previous studies reported that MBC represented 2 to 4% of TOC (Moreira and Siqueira, 2006) and 1.13 to 1.43% of TOC (Souza et al., 2014). The MBC concentrations in this experiment were corresponded to 1.82 (CT-2) to 2.11% (CT-1) of SOC obtained.

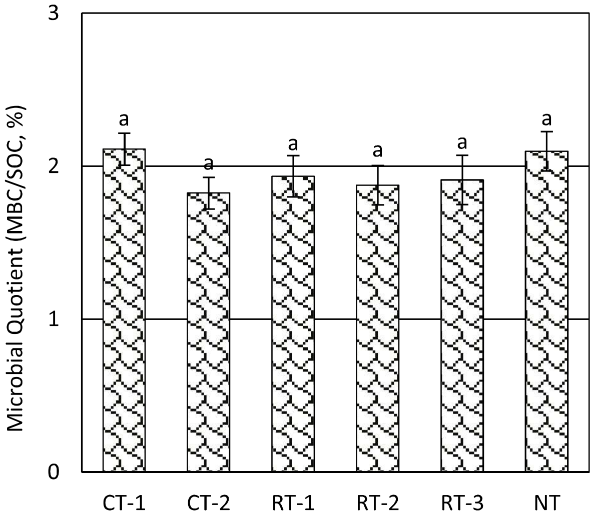

Figure 4. Microbial efficiency rate of soils under different tillage systems. Different letters on each bar indicate significant differences among tillage treatments at $p<0.05$

β-glucosidase Enzyme Activity

β-glucosidase enzyme activity for each tillage practice are represented in Figure 5. The β-glucosidase enzyme activity, one of the important indicators of soil quality (Stott et al., 2010), is sensitive to any change in the management practices that may impact the amount of organic matter (Ekenler and Tabatabai, 2003). Therefore, the decrease in tillage intensity resulted in a significant increase in β-glucosidase enzyme activity being 359 and 365% higher from CT-1 and CT-2 compared to NT, respectively. The activity of β-glucosidase which ranged from 44.86 mg PNP kg^{-1} h^{-1} (CT-2) to 207.66 mg PNP kg^{-1} h^{-1} (NT). The increase in β-glucosidase enzyme activity among tillage systems was in this order: CT-2 > CT-1 > RT-1 > RT-2 > RT-3 > NT (Figure 5). The reduction in tillage intensity resulted in higher MBC which favored the β-glucosidase enzyme activity due to increased availability of substrate and reduction of soil disturbance (Sinsabaugh et al., 2008). Although activities of β-glucosidase were higher under RT (113.03, 121.72 and 134.15 mg PNP kg^{-1} h^{-1} for RT-1, RT-2 and RT-3, respectively) systems compared to CT systems, they were significantly lower than that of NT treatment. Our results are in accordance with those reported by Martin-Lammerding et al. (2015) who indicated that β-glucosidase activity was at the highest level under NT followed by RT, with CT having the lowest level of β-glucosidase activity. Similarly, higher MBC concentration coupled with higher activity of β-glucosidase enzyme under NT in comparison to CT was also reported by Mendes et al. (2003) for a Brazilian Cerrados.

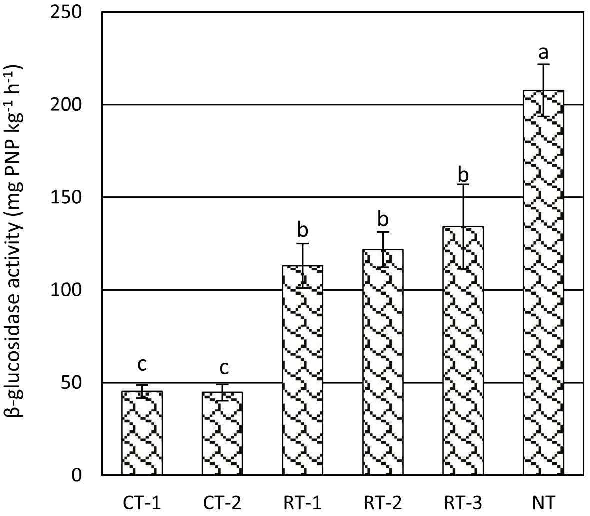

Figure 5. β-glucosidase activity (mg PNP kg^{-1} h^{-1}) of soils under different tillage systems. Different letters on each bar indicate significant differences among tillage treatments at $p<0.05$

Pearson correlation was used to evaluate the relationships between SOC, MBC and β-glucosidase activity (Table 1). The significant positive correlations between β-glucosidase activity and SOC (r=0.43) and MBC (r=0.80) revealed that β-glucosidase activity is closely related to SOC and provides information on alterations in SOC concentration of soils. Significant correlations between enzyme activities and SOC and MBC were associated to enhancing the stabilization and activity of microorganisms and protection of extracellular enzymes such as β-glucosidase under conservative tillage systems (Gajda et al., 2013).

Table 1. Pearson's correlation coefficients for soil organic carbon, microbial biomass carbon and β-glucosidase enzyme activity

	SOC	MBC	BGA
SOC	1.00		
MBC	0.37*	1.00	
GEA	0.43**	0.80**	1.00

SOC: Soil organic carbon, MBC: Microbial biomass carbon, BGA: β-glucosidase enzyme activity.
* Correlation is significant at $p<0.05$, ** Correlation is significant at $p<0.01$

CONCLUSIONS

Nine years of conservative tillage practices, reduced and no-till improved soil organic carbon sequestration potential, β-glucosidase activity and resulted in significant SOC accumulations in soil surface compared to conventional tillage practices which have been widely used by the farmers in eastern Mediterranean region of Turkey. The amount of C sequestered was decreased by the increase in tillage intensity. The no-till system led to approximately 10 Mg C ha^{-1} higher soil C sequestration compared to conventional tillage practices.

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