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Full Length Research Paper

Effects of various tillage systems on soil CO_2-C fluxes and on bacteria and fungi populations in *Zea mays*

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The objective of this study is to determine the effects of strip tillage and full-width tillage treatments on soil carbon (IV) oxide-carbon (CO_2-C) fluxes, bacterial and fungal populations in growing period of *Zea mays*. A row-crop rotary hoe with C type blades was used to create three strip widths by changing the connection of blades of the rotary hoe on the flanges. Strip widths were 22.5 (T30), 30 (T40) and 37.5 cm (T50). The full-width tillage practice (moldboard plow + disc harrow + leveler) gave 100% surface soil disturbance (T100) and was included in the experiment to make comparisons with the strip tillage system. During the growth of the *Zea mays*, periodic measurements of CO_2-C fluxes, bacterial and fungal populations were made. Statistically significant differences in CO_2-C fluxes, microbial populations and soil bulk density were observed between the different tillage systems. Highest CO_2-C fluxes and bacteria populations were observed with the full-width T100 treatment and the lowest values were observed in the T30 treatment during flowering and harvesting periods. Increasing tillage intensity increased soil CO_2-C fluxes and bacteria population, but decreased fungi population and soil bulk density.

Key words: Carbon IV oxide-carbon flux, strip tillage, strip width.

INTRODUCTION

Atmospheric CO_2 concentrations in March 2011 were approximately 392 parts per million (ppm), (Mantua et al., 2010) higher than any level in the past 650.000 years (Pike et al., 2010) and 41% higher than the pre-industrial value (278 ppm) (Mantua et al., 2010). Current CO_2 concentrations are about 3.4% higher than the 2005 concentration as reported by the IPCC's Fourth Assessment Report (AR4: 379 ± 0.65 ppm) (Chang and Jones, 2010). From 2000 to 2004, the actual emissions trajectory was close to that of the high-emissions of A1F1 scenario (Mantua et al., 2010). The potential consequences on climate change are severe and numerous. Thus, the reduction of CO_2 emissions and storage of emitted carbon is being looked into at an international level, especially, within the context of the Kyoto protocol (Sanchez et al., 2002). The important role of emissions from soils in the carbon cycle has only been clearly recognized for nearly a decade. Soil respiration on a global scale is 77×10^{15} g C year^{-1}, which is approximately, 10 times the contribution of industrial CO_2 emissions (Schlesinger, 1997; Schlesinger and Andrews, 2000; Sanchez et al., 2002). Due to the large order of magnitude, small changes in soil CO_2 flux across large areas can produce a great effect on CO_2 atmospheric concentrations. Only 14% of the world terrestrial area is cultivated (Lal, 2004).

Soil tillage allows a rapid and uniform seed emergence, deep penetration of the roots, good soil drainage, weed control and seedbed preparation (Altikat and Celik, 2011). Intensive tillage often involves using the moldboard plough to invert the soil followed by secondary tillage tools to break up and homogenize soil clods. One effect of such intensive tillage is to increase compactness, which decreases space between pores, thereby, changing the pathway for CO_2 diffusion (Sanchez et al., 2002). Tillage accelerates soil CO_2

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emission by improving soil aeration, disaggregating soil, increasing the contact between soil and crop residue, and speeding organic C decomposition (Bilen et al., 2010). Tillage often increases short-term CO_2 flux from the soil due to a rapid physical release of CO_2 trapped in the soil air spaces. Prior et al. (2000) found that the amount of CO_2 released into the atmosphere differed with different tillage systems and the amount lost was related to the amount of soil disturbance. In the weeks following tillage, keeping crop residues on the soil surface reduces CO_2 flux rates as compared to incorporation.

Conservation tillage practices have been widely adopted for agronomic crop production. Benefits of conservation tillage have been discussed in previous publications, including reduced equipment and labor costs, reduced soil erosion, improvements in soil quality, and in some situations, increased yields. Strip tillage is a form of conservation tillage that involves cultivation of narrow bands, or strips in the row area, separated by band of undisturbed soil. Strip tillage has the potential advantages of providing a suitable seedbed for vegetable crop establishment while leaving surface residue in the inter-row area to reduce soil erosion. Equipment for strip tillage has usually consisted of modified rototiller (rotary strip tiller) or a sub-soiling shank and fluted coulter system (shank/coulter system) (Albiero et al., 2011a, b; Celik and Altikat, 2010). Strip tillage has the potential advantage of providing a suitable seedbed for various row crops establishment while leaving surface residues in the inter-row area to reduce soil erosion (Albiero et al., 2011a, b; Celik and Altikat, 2010). Strip tillage is hypothesized to decrease the amount of CO_2 loss relative to plowing. Only a relatively small amount of CO_2 was detected immediately after strip tillage and this amount was related to the volume of soil disturbed (Reicosky, 1999).

Bilen et al. (2010) determined the effects of strip tillage and full-width tillage treatments on soil carbon (IV) oxide-carbon (CO_2-C) fluxes, bacterial and fungal populations in growing period of sunflower (*Helianthus annus*). According to the results, highest CO_2-C fluxes, bacteria populations and total porosity were observed in the full-width application and the lowest values were observed in the strip tillage (30 cm strip width) treatment during flowering and harvesting periods. Increasing tillage intensity increased soil CO_2-C fluxes and bacteria population, but decreased fungi population and soil bulk density.

Bauer et al. (2005) examined various soil tillage methods on CO_2 flux, to determine if chemical or physical properties after 25 years of conventional or conservation tillage correlated with flux rates. According to obtained results, flux rates in conservation tillage averaged 0.84 g CO_2 m^2h^{-1} in summer 2003, 0.36 g CO_2 m^2h^{-1} in fall 2003, 0.46 g CO_2 m^2h^{-1} in spring 2004, and 0.86 g CO_2 m^2h^{-1} in summer 2004. Flux rates from conventional tillage were greater for most measurement times. Dao (1998) determined soil CO_2 flux following wheat in the 11th year of a tillage study and found the cumulative CO_2 evolved from soil in a 2-month period was much higher for moldboard plowing than for no-tillage.

Soil micro-organisms and the processes that they govern are essential for long-term sustainability of agricultural systems (Wardle, 1999) and a major component in soil formation and nutrient cycling. Changes in soil chemical and physical conditions will influence microbial activity and population structure. On a primary basis, the natural environment provides a long-term and seasonal fluctuation in temperature, moisture, and plant growth to which micro-organisms must respond. Agriculture further alters the soil environment from its natural state, either amplifying or reducing seasonal cycles and influencing how the soil responds to climatic factors. Changes in soil physical and chemical properties that occur as a result, and subsequent changes in microbial community composition, will in turn influence soil processes (Schimel, 1995). When the crop production system is changed to less intensive tillage system, the microbial biomass and the biologically active C and N pools respond rapidly and the changes are more easily measured than changes in total C and N. No-tillage changes both the profile distribution of biological activity and the biological community itself, with fungi becoming more dominant under no-tillage (Six et al., 2006). Fungal dominating soil communities may enhance C storage and slow soil organic matter turn-over due to both the fungal alteration of soil physical properties and to fungal physiology. Ecosystems with soils dominated by fungi thus, reduce CO_2-C flux and sequester more C than systems with lower fungal abundance (Six et al., 2006).

Tillage leads to the development of soil microbial communities dominated by aerobic micro-organisms with high metabolic rates, typically bacteria, whereas, under conservation practices, plant residues left at or near the soil surface encourage fungal growth and the temporary immobilization of nutrients (Pankhurst et al., 2002). An increase in the proportion of microbial biomass attributable to fungi was found in reduced tillage (RT) (Frey et al., 1999) and no-tillage (Beare et al., 1997) systems.

Soil organic matter is a resource for soil biota and there is a strong relationship between the abundance of soil organisms and the content of organic matter (Wardle et al., 2001; Nakamoto and Tsukamoto, 2006). Many soil organisms receive benefits because of a reduction in soil disturbance and an increase in surface crop residues. The purpose of this study is to evaluate the effects of different widths strip tillage system and full-width inversion tillage system on soil bulk density, CO_2-C fluxes and on bacterial and fungal populations in *Zea mays.*

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the research farm of Ataturk

Table 1. Monthly rainfall, mean temperature and average relative humidity for 2006.

Month	Monthly rainfall (mm)	Mean temperature (°C)	Average relative humidity (%)
January	17.8	-11.2	81.6
February	10.9	-5.6	77.0
March	13.4	1.2	73.5
April	77.4	7.2	74.4
May	41.6	11.4	67.3
June	19.2	18.4	56.7
July	20.7	20.3	62.5
August	3.5	22.6	50.9
September	29.2	14.1	60.2
October	90.1	8.6	76.0
November	25.3	-0.1	70.9
December	8.3	-9.8	75.4
Average	29.8	6.4	68.9

University (39° 54^{l} N and 41°13^{l}E, altitude 1883 m), Erzurum, Turkey. The soil on the experimental site was classified as an Ustorthents according to the USDA soil taxonomy (Soil Survey Staff, 1999). Some climatic data related to the experimental area are given in Table 1. The initial crop was winter wheat and was harvested together with the wheat from the experimental area at the end of growing period. Stubble height was left around 12 cm. Soil tillage and *Zea mays* planting was performed after wheat harvest during the third week of May, 2006.

Experimental design

Plots were arranged in a randomized complete block design with three replications. Each treatment plot was 3 by 30 m in size and plots were separated by buffer areas that were one-half the size of the treatment plots. A strip tillage (ST) system with three different strip widths (22.5 cm, 30% soil surface disturbance (T30), 30 cm, 40% soil surface disturbance (T40), and 37.5 cm, 50% soil surface disturbance (T50) was compared with a full-width inversion tillage system with 100% soil surface disturbance (T100). The three strip widths were achieved by changing the connection of blades of the rotary hoe on the flanges. Tilled zones of 30, 40 and 50% of the field area were achieved by the use of two, three or four flanges with four blades on each, respectively (Figure 1). The rotary hoe was operated at constant rotor rotational speed of 370 rpm. Tillage in the full-width inversion system (T100) involved the use of a moldboard plow followed by a disk harrow and a soil leveler. The tillage depth was kept constant at 12 cm for the strip tillage systems and 20 cm for the full-width inversion tillage system. The tractor operating speed was kept constant at 1.5 m s^{-1} by using a DJRVS II speed radar and a DJCMS100 monitor made by Dickey-John (5200 DICKEY-John Road Auburn, IL).

Soil tillage was performed on 16th May, 2006. After the seedbed preparation, all plots were sown using a four row pneumatic planter, commonly used in *Zea mays* planting in Turkey, with 70 cm inter-row spacing. Urea fertilizer (50 kg ha^{-1}N) and triple super phosphate (70 P_2O_5 kg ha^{-1}) was applied at planting. Weed control was accomplished by hand hoeing two times per month.

Soil sampling and laboratory analysis techniques

Plots were sampled for soil at three different maize growing periods that was included at planting (June, 20th), flowering (August, 10th) and at dough maturity (September, 12th). Depth of sampling was 20 cm and samples were removed from the field using a 5 cm diameter borer. Separately, on May, 10th before maize sampling, the soil was sampled and this sample was used as an untreated control. Three soil samples were collected from Ap horizon of each plot in each three period every day during the week. Collected soil samples were dried at 105°C for 24 h and sieved through a 2-mm mesh opening.

Soil organic C was determined by the Smith-Weldon method (Tiessen and Moir, 1993), $CaCO_3$ content was determined using a Schleibler calcimeter (Tee et al., 1993), total N by the micro Kjeldahl method (McGill and Figueiredo, 1993), soil pH by using a glass electrode pH meter (1: 2.5, soil: water), exchangeable cations and cation exchange capacity were determined by atomic absorption spectrophotometer after the method of Handershot et al. (1993), available P by the Na_2CO_3 extraction method (Olsen and Sommers, 1982), field water capacity by the tensiometer method (Topp et al., 1993), soil texture by the Bouyoucos hydrometer method (Shieldrick and Wang, 1993), and electrical conductivity (EC) by using an EC meter according to the method of Janzen (1993). Measured chemical and physical properties of the experimental site soil (depth 0 to 20 cm) are shown in Table 2.

Microbial population analysis

Determination of viable microbial bacteria and fungi counts were carried out at four different growing periods (Initial, Sowing, Flowering and Harvesting) of *Zea ma*ys. Gentle tapping separated the rhizosphere soil, adhering to the root, and this recovered soil that was air-dried at room temperature (25°C). Culturable bacteria (colony forming units, CFU) were enumerated by the spread soil dilution plate method on full strength nutrient Soil Extract Agar (SEA) (Ogram and Feng, 1997). For fungi, dextrose-peptone agar (DPA) was used. To inhibit bacterial growth during fungi measurements 30 mg L^{-1} streptomycin were added to the SEA. To inhibit fungal growth during bacterial measurements, 30 mg L^{-1} rose bengal was added to the DPA (Alef, 1995).

For the soil dilution plate method, each 10 g soil sample was homogenized in 100 ml phosphate-buffered saline (PBS, 0.15 M potassium phosphate, 0.85% NaCl, pH 7.0). The sample was centrifuged for 7 min at 250 × g and the supernatant decanted into a sterile flask. The pellet was resuspended and washed twice by centrifugation in sterile PBS. All fractions were pooled in a sterile flask and serially diluted (10^6 to 10^7) in PBS (McDermott, 1997). For

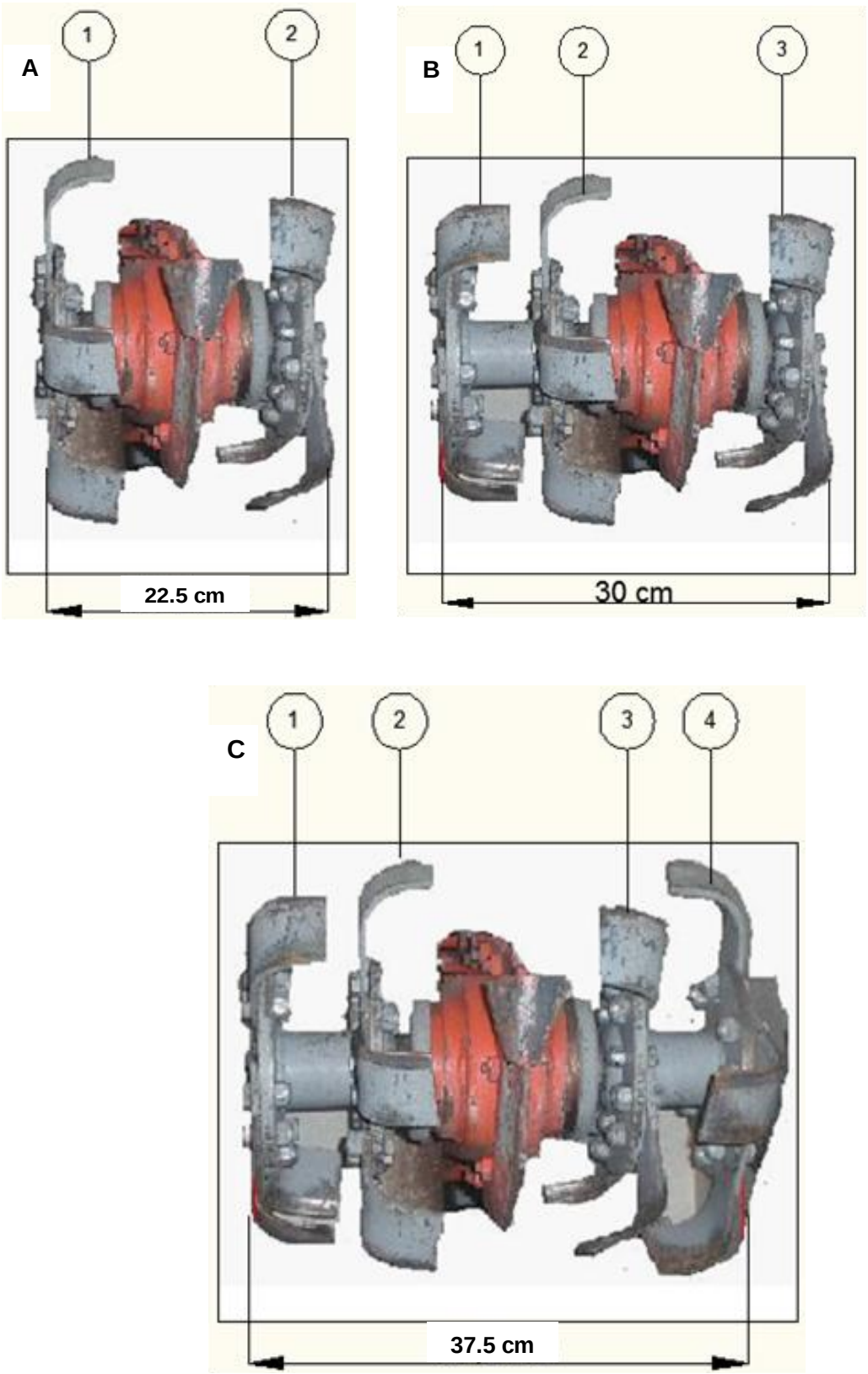

Figure 1. Application of tillage strip widths by modification of rotary hoe; (A) T30 = Strip width of 22.5 cm, 30% soil surface disturbance. two flanges with four blades; (B) T40 = Strip width of 30 cm, 40% soil surface disturbance, three flanges with four blades; (C) T50 = Strip width of 37.5 cm, 50% soil surface disturbance, four flanges with four blades.

Table 2. Some initial chemical, physical and microbiological properties of experiment site.

Soil properties	Value	Soil properties	Value
		Cation exchange capacity, cmol kg^{-1}	**35.0**
pH (1: 2.5)	7.16	Electrical conductivity, dS m^{-1}	0.65 × 10^{3}
Organic matter g kg^{-1}	2.29	Salt %	0.016
Lime ($CaCO_3$) g kg^{-1}	1.11	Field capacity at 1/3 atm. g kg^{-1}	26.7
Total N g kg^{-1}	0.14	Wilting capacity at 15 atm. g kg^{-1}	15.4
Plant Available P mg kg^{-1}	4.71		
Exchangeable cations cmol kg^{-1} soil		Soil texture content: Sand %	32.3
Ca	22.49	Silt %	44.1
Mg	7.27	Clay %	23.6
K	2.61	Bulk density. Mg m^{-3}	1.51
Na	0.29	Porosity vol. %	42.25
Microelements mg kg^{-1}		Microbiological properties	
Fe	5.97	Number of bacteria CFU* g^{-1} soil	2.55 × 10^{7}
Cu	1.79	Number of fungi CFU g^{-1} soil	2.45 × 10^{5}
Zn	1.17	Total C respired as CO_2 mg m^{-2} h^{-1} (CO_2-C)	3.2 (0.27 Mg C ha^{-1} y^{-1})
Mn	9.52		

*CFU; Colony-forming units.

bacteria, 0.1 ml of each dilution of the series was placed onto a Petri dish with SEA. For fungi, 0.1 ml of each dilution was placed onto a Petri dish with DPA. Three replicate dishes were made for each dilution. The agar plates were aerobically incubated at 30°C for 7 days to obtain bacterial counts and at 25°C for 7 days for fungi counts. After the incubation period, the CFU of the bacteria and fungi developed on the respective agar plates were enumerated using an automated colony counter. The averaged colony forming units (CFU) per gram of oven-dried soil was calculated for each soil sample (Canbolat et al., 2006; Madigon and Martingo, 2006) and results are reported in Table 2.

Basal respiration and CO_2-C analysis

Basal respiration (BR), as a measure of soil biological activity, was determined by using *in vitro* static incubation of unamended field moist soil (Islam and Weil, 2000). About 20 g ODE of field-moist soil adjusted at 70% water-filled porosity (WFP) was taken in 25 ml glass beakers. Each soil sample was placed in a 1 L mason jar along with a glass vial containing 10 mL of distilled deionized water to maintain humidity and a plastic vial containing 10 mL of 0.5 M NaOH to trap CO_2 evolved from the incubated soil. The mason jars were sealed airtight and incubated in the dark at 25 ± 1°C for 20 days. The CO_2 evolved over time was absorbed in the 0.5 M NaOH followed by precipitation as $BaCO_3$ by the addition of excess 1M $BaCl_2$. The remaining NaOH in each vial was then titrated to the phenolphthalein endpoint with a standardized 0.5 M HCl solution (Table 2). The BR rate was calculated as:

BR rates (mg CO_2/kg soil) = (CO_2soil - CO_2air)/20 days

Statistical analysis

Analysis of variance (ANOVA) was used to evaluate the significance of each treatment on soil properties and CO_2 fluxes and on bacteria and fungi populations. Comparison of means was performed, when the F-test for treatment was significant at the 5% level, using Duncan's Multiple Range Tests.

RESULTS AND DISCUSSION

CO_2 - C Fluxes

According to obtained results in the research, the highest CO_2-C fluxes from soils in sowing, flowering and harvesting periods were observed at T100 system (3.78, 4.73, 5.55 mg C m^{2}h^{-1}, respectively). The minimum CO_2-C fluxes were observed at the T30 system during the all measurement periods (3.77, 4.40, 4.99 mg C m^{2}h^{-1}). The CO_2-C fluxes generally increased with increasing strip widths. Among the strip widths, the highest fluxes were observed for the T50 systems and the lowest for the T30 systems (Table 3). The CO_2-C fluxes from the T30, T40 and T50 treatments were statistically different in the flowering and harvesting periods, but similar effect was not observed at the sowing period. The highest CO_2-C fluxes were obtained at the harvesting period and the minimum fluxes were obtained at the sowing period. At the harvesting period, plant root growth, soil biomass and soil microbial activity had increased and these increases contribute to increased CO_2-C fluxes.

Carbon dioxide fluxes from soil are due to the decomposition of organic material by micro-organisms and root respiration. Tillage increased the rate of organic C decomposition. Data of the stability of the soil organic carbon in the soil are shown in the parenthesis as Mg C

Table 3. Effect of soil tillage intensity on soil bulk density. and CO_2-C fluxes at sowing, flowering, and harvesting periods of *Zea mays.*

Tillage system	Sowing period		Flowering period		Harvesting period	
	Bd	CO_2-C fluxes	Bd	CO_2-C fluxes	Bd	CO_2-C fluxes
T30	1.19[a]	3.77 (0.326)[ns]	1.21[a]	4.40 (0.380†)[c]*	1.23[a]	4.99 (0.431)[c]
T40	1.20[a]	3.71 (0.321)[ns]	1.20[a]	4.42 (0.382)[c]	1.22[a]	5.27 (0.455)[b]
T50	1.19[a]	3.74 (0.323)[ns]	1.22[a]	4.62 (0.399)[b]	1.22[a]	5.36 (0.463)[b]
T100	1.13[b]	3.78 (0.327)[ns]	1.18[b]	4.73 (0.408)[a]	1.20[b]	5.55 (0.480)[a]
Average	1.18[A]	3.75 (0.324)[C]	1.20[A]	4.54 (0.393)[B]	1.22[A]	5.29 (0.457)[A]
P	0.001	0.345	0.001	0.001	0.001	0.001
SEM	0.256	1.233	0.564	0.114	0.254	0.118

*Means denoted with the same letter are not statistically significant ($p < 0.01$); Bd. bulk density (Mg m^{-3}); CO_2-C fluxes (mg m^{-2} h^{-1}); †Variables in parentheses Mg C ha^{-1} y^{-1} are indicator of the stability of the soil organic carbon in the soil; SEM: Standard errors of means; P: Level of significance; Ns: Non significance.

ha^{-1} y^{-1} in Table 3. These measurements showed that carbon is more stable in the strip-tilled than in the T 100 plots.

Average soil bulk density which was affected by tillage treatments and plant growing periods were found to be significantly different. The lowest soil bulk density was observed in T100 treatment at the sowing, flowering and harvesting period of plant growth (Table 3). Fluxes of CO_2-C from agricultural soils are the result of complex interaction between climate and several biological, chemical and physical soil properties (Oorts et al., 2007). Tillage systems may affect all these soil properties and therefore, influence the release of CO_2-C (Robertson et al., 2000). Increased surface roughness and larger voids produced by soil disturbance increase soil CO_2 - C flux (Li et al., 2010).

Soil tillage is among the important factors affecting soil physical properties (Khurshid et al., 2006). The tillage treatments affected the soil physical properties, especially, when similar tillage system has been practiced for a longer period (Mielke and Wilhelm, 1998). Structure of the Ap horizon is largely influenced by soil tillage system and the implements used for tillage (Husnjak et al., 2002). All tillage tools reduced the bulk density and raised porosity to the depth of tillage (Ferreras et al., 2000). In our study, we observed that strip tillage and a full-width inversion tillage system effected soil bulk density.

Bacterial and fungal population

The effects of soil tillage systems on average bacterial numbers were statistically significant ($p < 0.01$) in the flowering and harvesting periods but similar results were not obtained at the sowing period. The highest bacterial numbers were observed for the T100 system at flowering and harvesting periods with 9.18 and 9.29 CFU g^{-1} dry soil, respectively. Among the strip tillage systems, the T30 plots supported the lowest bacterial numbers (9.01 and 9.12 CFU g^{-1} dry soil) and the T50 plots supported the highest bacterial numbers (9.08 and 9.19 CFU g^{-1} dry soil) at flowering and harvesting periods, respectively (Figure 2). The effects of strip tillage on average fungi population were statistically significant only at the flowering period. Among the strip tillage systems, the highest fungi populations were observed in T30 system at flowering period (9.06 CFU g^{-1} dry soil). However, among the soil tillage systems, the highest fungi numbers were observed in T100 system at flowering and harvesting periods with 9.32 and 9.42 CFU g^{-1} dry soil, respectively (Figure 3).

In the research, the T100 tillage system produced the highest fungi and bacterial populations when compared to the three strip tillage treatments. Although, microbial activity is strongly dependent on plant, soil and climatic conditions, soil tillage intensity had a definite effect on microbial activity. Among the sampling periods, the harvesting period supported higher bacterial and fungi populations when compared to the other two periods. At both flowering and harvesting periods, the highest bacterial populations were observed for the T100 system and minimum values were observed for the T30 system. Tillage decreases soil organic matter (Gebhart et al., 1994), and when the crop production and tillage systems change, the microbial biomass and the biologically active C and N also rapidly change (Six et al., 2006). Tillage causes immediate changes in microbial community structure, but little concomitant change in total microbial biomass (Jackson et al., 2003). The cumulative effect of tillage and many cropping rotations caused 30 to 50% decrease in soil C that is an undesirable change in soil physical, chemical and biological properties (Reicosky and Archer, 2007). Similar results were obtained from this study.

Conclusions

In this study, we examined the effects of different widths

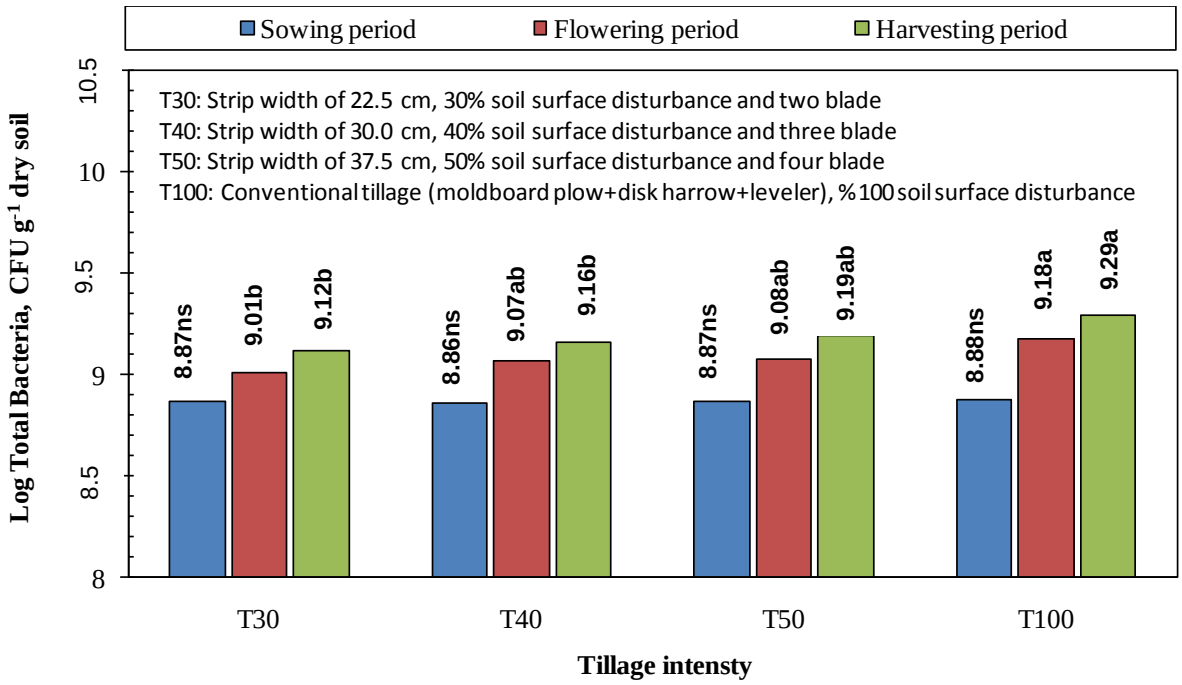

Figure 2. Effect of soil tillage intensity on soil bacteria populations at sowing, flowering and harvesting periods.

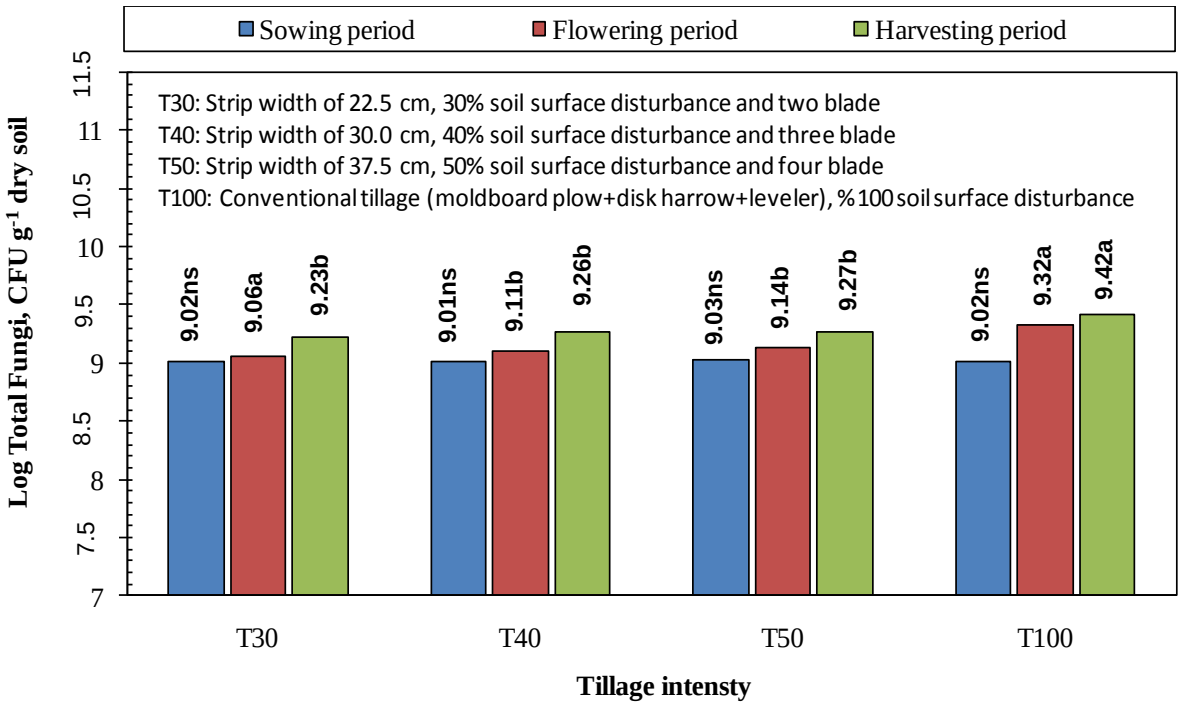

Figure 3. Effect of soil tillage intensity on soil fungal populations at sowing, flowering and harvesting periods.

strip tillage system and full-width inversion tillage system on soil bulk density, CO_2-C fluxes, bacterial and fungal populations in *Zea mays*. According to obtained results, the T100 system exhibited large CO_2-C flux from soil. The increase in CO_2-C flux was related to tillage intensity with the smallest flux associated with the T30 treatment and the largest flux associated with the full-width inversion tillage (T100). This is because increasing tillage intensity

disaggregates soil and improves soil aeration.

Tillage treatments affected the soil physical and biochemical properties. Increasing tillage intensity reduced the soil bulk density to the depth of tillage. The full-width inversion T100 tillage system showed the highest population levels of bacteria and fungi, and the T30 treatment showed the lowest levels. In consequence, to preserve soil C and soil microbial populations, reduced tillage systems, such as the strip tillage systems evaluated in this study, can effectively decrease CO_2 emission.

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