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Tillage and N-fertilizer influences on selected organic carbon fractions in a North Dakota silty clay soil

Rakesh Awale *, Amitava Chatterjee, David Franzen

Department of Soil Science, North Dakota State University, Fargo, ND 58108-6050, United States

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ABSTRACT

Physical, chemical, and biological fractions of SOC pools, such as coarse particulate organic matter C (CPOM-C), permanganate oxidizable C ($KMnO_4$-C), microbial biomass carbon (MBC), and mineralizable C (Cmin) respond to changes in management practices and provide sensitive indication of changes in the SOC dynamics than commonly reported total soil C alone. We hypothesized that tillage and N-fertilizer managements induced changes in SOC at the surface 0–15 cm soil would predominantly be reflected by parallel changes in different fractions of SOC. Three field experiments (Expt1: 2008–2011, Expt2: 2005–2011, Expt3: 2005–2011) were conducted in a Fargo (Typic Epiaquerts)-Ryan (Typic Natraquerts) silty clay complex in Fargo, North Dakota, USA. Our objectives were (i) to evaluate the effects of tillage (conventional till [CT], strip till [ST] and no-till [NT] and different N-fertilizer managements on SOC, CPOM-C, $KMnO_4$-C, MBC, and Cmin and (ii) to determine any relationships among these C fractions within corn (*Zea mays*)-sugarbeet (*Beta vulgaris*)-soybean (*Glycine max*) rotation. Compared with CT, ST and NT had significantly higher SOC concentration by 3.8 and 2.7%, SOC stock by 7.2% and 9.2%, CPOM-C by 22 and 25%, and $KMnO_4$-C by 4.8 and 4.1%, respectively in Expt2 and had significantly higher SOC concentration by 3.9 and 6.6%, SOC stock by 11.9 and 8.7%, and CPOM-C by 33 and 45%, respectively in Expt3. The $KMnO_4$-C and 30 d cumulative Cmin were greater under ST than CT by 3.3 and 23%, respectively in Expt3. The amounts of Cmin were consistently higher under ST and NT than CT throughout the incubation period except at 7 d, in Expt3. Across the study, CPOM-C was 16.3–22.1%, MBC was 3.4–4.5%, cumulative Cmin was 0.7–1.4%, and $KMnO_4$-C was 1.6–1.7% of the total SOC. Significant correlations were observed among SOC, CPOM-C and Cmin in all the experiments. CPOM-C was the most sensitive fraction to tillage changes. Tillage influences on SOC fractions followed the order: physical (CPOM-C) > biological (cumulative Cmin) > chemical ($KMnO_4$-C), however, the sequence change with soil type and crop rotation requires further investigation.

1. Introduction

Increasing concern about global climate change, driven by rising atmospheric concentration of greenhouse gases, particularly CO_2, have enhanced the interest in soil C sequestration as a strategy to offset anthropogenic CO_2 emissions (Lenka and Lal, 2013). Globally, agricultural lands have the potential to sequester approximately 5500–6000 Mg CO_2-eq. yr^{-1} by 2030 (Smith et al., 2008). Strategies for increasing the SOC pool is needed not only to mitigate CO_2 emissions but also to improve soil quality and economic crop production (Kahlon et al., 2013).

In the Northern Great Plains (NGP), conservation tillage practices (e.g. no-till [NT], strip till [ST]) – under continuous cropping system with adequate nitrogen (N) fertility – have been proposed to prevent the loss of SOC (Cihacek and Ulmer, 1998; Halvorson et al., 2002). For instance, Campbell et al. (1996) reported 14.5% higher SOC content with NT than CT and 19.2% higher SOC with NT than minimum tillage within Canadian Prairie Province cropping systems in the NGP. Similarly, Halvorson et al. (2002) documented that the storage of SOC within 0–7.6 cm and 7.6–15.2 cm soil depths, after 12 years of imposing tillage treatments, were significantly higher with NT than conventional tillage (CT) by 13% and 11%, respectively in silt loam soils in North Dakota (ND). According to Tan et al. (2007), conversion of CT to NT practice could result in a reduction of 104 kg C ha^{-1} yr^{-1} release from the croplands of western ND.

Usually, NT and ST practices leave most of the crop residues on undisturbed surface whereas CT incorporates residues into soil thereby increases soil-residue contact, favoring rapid decomposition of soil organic matter through oxidation (Campbell et al., 1996). According to Mikha and Rice (2004), NT greatly enhances C accumulation within soil aggregates and increased tillage intensity in many conventional tillage systems; such as plowing, chisel plowing and multiple tillage trips prior to seeding disrupts soil

* Corresponding author. Tel.: +1 701 936 1417.
E-mail addresses: rakesh.awale@ndsu.edu, awalerakesh@gmail.com (R. Awale).

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aggregates and expose aggregate protected C to microbial attack. Moreover, tillage can greatly modify edaphic factors and thereby influences the rate of C mineralization (Huggins et al., 2007; Curtin et al., 2012). Tillage can also increase the effect of drying-rewetting and freezing-thawing cycles, which increases the susceptibility of aggregates to physical disintegration (Lee et al., 2009).

Previous studies have pointed out that SOC increases in reduced as compared with intensive tillage systems (Kahlon et al., 2013). In some studies, however, tillage effects on SOC have been limited or absent (Liebig et al., 2004; Cookson et al., 2008). The magnitude and direction of tillage induced changes are soil and site specific (Chatterjee and Lal, 2009). And, because of high background C content, the effect of tillage practices on SOC contents may not be detectable early (Geisseler and Horwath, 2009). Therefore, a variety of physical, chemical, and biological fractions of SOC such as coarse particulate organic matter carbon (CPOM-C), potassium permanganate oxidizable carbon ($KMnO_4$-C), microbial biomass carbon (MBC), and mineralizable carbon (Cmin) have recently received more attention due to their sensitivity to management practices compared with total SOC (Weil et al., 2003; Liebig et al., 2004; Dou et al., 2008). These fractions change rapidly with time and can provide an early assessment of SOC changes induced by management practices such as tillage, cropping system, and N fertilization (Chen et al., 2009).

Previous works conducted – to evaluate the relative sensitivity of different SOC fractions to provide indications on management induced changes on SOC dynamics – have yielded contrasting results (Dou et al., 2008; Chen et al., 2009). For example, Dou et al. (2008) reported that that MBC was 5–8%, Cmin was 2%, CPOM-C was 14–31%, hydrolyzable C was 53–71%, and DOC was 1–2% of SOC in silty clay loam soil. Chen et al. (2009) documented that $KMnO_4$-C and CPOM-C were the most sensitive SOC fractions measured, with respectively about 4 and 2 times more responsive to differences in tillage practices than SOC in Loess Plateau of Northern China. Likewise, Aziz et al. (2013) showed that soil biological quality indices (MBC, basal respiration) were more consistent, sensitive, and early indicators of soil quality as compared with soil physical (porosity, aggregates stability, sand free CPOM-C) and chemical (SOC, $KMnO_4$-C, total organic N) quality indicators in response to tillage management in Ohio, suggesting microbial life is a key indicator of SQ that regulates physical and chemical properties in soil. On the other hand, Zagal et al. (2009) documented that soil MBC, mineralizable C and N, and dehydrogenase activity but CPOM-C, were responsive to crop managements for silty-volcanic alluvium in Chile. Sequeira and Alley (2011) found that SOC, CPOM-C, CPOM-N, IL soil N test, and $KMnO_4$-C but free light fraction C were not different between tillage gradients after 3 years in silt loam soil in VA. Similarly, based on the fact that $KMnO_4$ also reacts with the stable fraction of total C, the reliability of this method as an index of soil labile carbon has been questioned recently (Tirol-Padre and Ladha, 2004). In sharp contrast, several researchers have documented that the $KMnO_4$-C fraction is a sensitive indicator to management (Mirsky et al., 2008; Culman et al., 2012).

Soil organic C fractions are interrelated properties of soil (Haynes, 2005). Sensitivity of these fractions may vary with site, climate, and management practices. The complex nature of soil represents a multitude of soil processes and functions (Gregorich and Beare, 2008). Therefore, measurement of a suite of SOC fractions and elucidation of the interactive relationships among different SOC fractions would perhaps more reflect tillage and N management induced changes in soil quality (Strosser, 2010). Relatively few studies have emphasized on the combined effects of tillage and N-fertilizer management on the physical, chemical, and biochemical fractions of SOC in silty clay soils in the NGP in the U.S. Knowledge about the changes in SOC and its fractions under different tillage practices coupled with N managements is necessary to assess the feasibility of adoption of conservation practices for sustaining productivity and protecting the environment. The objectives of this study were to (i) determine the effect of three tillage practices (ST, NT, and CT) and N-fertilizer managements on SOC, MBC, CPOM-C, $KMnO_4$-C, and Cmin (ii) test the relationships among the SOC fractions, within corn–sugarbeet–soybean rotation in an ND silty clay soil. Overall, we hypothesized that management induced changes in SOC at the surface 0–15 cm soil would predominantly be reflected by parallel changes in different physical, chemical and biological fractions of SOC.

2. Materials and methods

2.1. Description of experimental sites and treatments

The research was based on three conservation tillage experiments conducted under a corn–soybean–sugarbeet rotation at the North Dakota State University Agricultural Experimental Station farm in Fargo, ND (46°53′ N, 96°48′ W) on a Fargo-Ryan silty clay soil complex. The soils are classified as fine, smectitic, frigid Typic Epiaquerts (Fargo) and fine, smectitic, frigid Typic Natraquerts (Ryan) with 0–1% slope (Soil Survey Staff, 2013). The average annual temperature and precipitation for this region are 6.2 °C and 610 mm respectively. The primary crop rotation within this region includes corn–sugarbeet–soybean cropping sequence (Olson, 2013). In 2011, the experiments comprised three crops (corn, sugarbeet, and soybean) grown under strip-till (ST), no-till (NT), and conventional-till (CT) management practices. Experiment details are presented in Table 1. Experiment 2 (Expt2) and experiment 3 (Expt3) were both established in 2005. Prior to the

Table 1
Experimental details.

	Experiment 1	Experiment 2	Experiment 3
Year established	2008	2005	2005
Crop planted in 2011	Corn	Sugarbeet	Soybean
Crop rotation	C-SB-S-C	S-C-SB-S-C-SB	C-SB-S-C-SB-S
Experimental design	Split-Plot	Split-Plot	RCBD
	(a) Main plot: N management 1. 168 kg N ha^{-1} at preplant (full early) 2. 168 kg N ha^{-1} at V5/V6 leaf stage (full late) (b) Sub plot: tillage management 1. Strip-till (ST) 2. No-till (NT) 3. Conventional till (CT)	(a) Main plot: N management 1. 112 kg N ha^{-1} at preplant (full early) 2. 112 kg N ha^{-1} at V6 leaf stage (full late) 3. 56 kg N ha^{-1} at preplant and 56 kg N ha^{-1} at V6 leaf stage (half early/half late) (b) Sub plot: tillage management 1. Strip-till (ST) 2. No-till (NT) 3. Conventional till (CT)	Tillage management: 1. Strip-till (ST) 2. No-till (NT) 3. Conventional till (CT)

C: corn; SB: sugarbeet; S: soybean; RCBD: randomized complete block design.

establishment of these experiments, the areas were under continuous pinto beans (*Phaseolus vulgaris*) cropping system. The two experiments were separated by a 9.7 m wide turn-row. In 2008, experiment 1 (Expt1) was established by extending the area – previously under fallow with no history of agricultural disturbances – to the west section of Expt2 and Expt3. Expt1 was separated from the other two by a 0.6 m wide border. Each plot under the experiments measured 7.6 m long by 3.4 m wide. Row spacing was 55 cm, resulting in 6 rows per plot area for all crops.

Expt1 consisted of corn–sugarbeet–soybean–corn cropping sequence. Within the rotation (one crop only each year), NT, ST and CT were arranged in a randomized complete block design with 6 replications. In 2011, the experimental design was changed to a split plot design with two N-fertilizer management as the main plot and three tillage practices (ST, NT, and CT) as the sub plot with 3 replications. The N-fertilizer treatments consisted of the application of 168 kg N ha^{-1} at preplant in the form of ammonium nitrate and 168 kg N ha^{-1} at 5/6 leaf stage in the form of urea ammonium nitrate (UAN) streamed between the rows. Following the harvest of soybeans in the fall of 2010, the ST treatment was imposed, which consisted of a fall 0.4 cm wide shank operated at a 15 cm depth, and residue managers to bare the soil in a 17.5 cm strip directly over each shank path. No tillage operations were performed in the NT plots. Residue managers were used on the planter to sweep residue out seed furrow path, but were not aggressive enough to disturb any soil. The CT treatment consisted of chisel plowing to a depth of 15 cm in fall followed by spring field cultivation to a 7.5 cm soil depth at planting. The required rate of ammonium nitrate granules was applied as preplant and UAN was dribbled between the corn rows. Corn variety Pioneer P8581R (RR2) was seeded at 84,000 seeds ha^{-1}. Pre- and post-applications of Roundup Max (1.6 L ha^{-1}) were performed to control weeds. Corn was harvested manually, and were dried and shelled for yield measurement.

Expt2 consisted of soybean–corn–sugarbeet–soybean–corn–sugarbeet cropping sequence. In 2011, sugarbeets were grown in the plots previously under corn cultivation. Before 2011, the plots were managed as a randomized complete block with three tillage treatments – NT, ST and CT – with 12 replications. In 2011, the experimental design was changed to a split plot randomized complete block design with 4 replications. The main plot consisted of N-fertilizer managements: (1) 112 kg N ha^{-1} at preplant in the form of NH_4NO_3 (full early), (2) 112 kg N ha^{-1} at 6 leaf stage in the form of UAN (full late), and (3) 56 kg N ha^{-1} at preplant in the form of NH_4NO_3 and 56 kg N ha^{-1} at 6 leaf stage in the form of UAN (half early/half late); and the sub plot treatments include three tillage practices (ST, NT, and CT). Nitrogen fertilizer treatments and tillage practices were employed as described above. Sugarbeet variety Crystal 658 Roundup Ready (treated with Poncho Beta tachigeran 45) was planted at a 3.1 cm depth with a rate of 156,500 plants ha^{-1}. Sugarbeet crops were harvested using a two-row machine harvester.

Expt3 contained corn–sugarbeet–soybean–corn–sugarbeet–soybean cropping sequence. In 2011, soybean crops were grown under three tillage treatments (ST, NT, and CT) arranged in a randomized complete block design with 12 replications. The tillage treatments were employed as described above. Peterson Farms Seed 1108RRSTS were planted at a 3.8 cm depth using a rate of 320,000 seeds ha^{-1}. Prior to planting, Roundup Max (1.6 L ha^{-1}) was applied to burn down weeds and again applied twice as post emergence weed control. Soybeans were harvested using a Hege plot combine harvester with a 1.2 m swath width.

2.2. Soil sampling and analyses

Following the harvest of all the crops in the fall of 2011, soil cores of 3.6 cm diameter from the top 0–15 cm depth were collected from the experimental plots using a soil probe. A sample was taken at the center of each plot in between the (harvested) crop rows. The surface litter was removed before the samples were taken. The field moist soils were transferred to the laboratory in Ziploc bags and weighed. A portion of moist soil was oven dried at 105 °C for 24 hours to determine gravimetric soil water content, which were used to compute bulk density (Blake and Hartge, 1986). Bulk densities were used to convert concentrations of C to a mass basis. For all other physical and biochemical assays, soil samples were air-dried, finely ground in a mechanical grinder and passed through 2 mm sieve. The basic soil properties are shown in Table 2. Soil pH and electric conductivity (EC) were measured electrometrically using an Oakton, PC 700 pH Bench Meter (Oakton Instr., IL, USA) of 1:2.5 soil: water extracts (Thomas, 1996). Soil particle size distribution was determined by hydrometer method as outlined by Elliott et al. (1999), which consisted of dispersing 40 g of air-dried soil with 100 mL of 5% sodium hexametaphosphate solution, taking hydrometer readings at 4.5 h and 8 h to compute clay and silt fractions, and finally weighing the sand fraction after sieving the dispersed soil suspension through 53 μm screen. Total KCl extractable-N (NH_4^+–N + NO_3^-–N) was measured according to Maynard et al. (2008). Briefly, 5 g of air-dried soil was extracted with 25 mL of 2 M KCl after shaking the mixture for 30 min in a reciprocal shaker. The soil suspension was filtered through a Whatman no. 2 filter paper and the extract was then analyzed for inorganic N (NH_4^+–N and NO_3^-–N) contents using an automated TL2800 Ammonia Analyzer (Timberline Instruments, Boulder, CO). A portion of air-dried soil was passed through 0.5 mm sieve to obtain a fine and homogeneous powder, which were analyzed for total soil organic carbon by the dry combustion method (Cihacek and Jacobson, 2007) at 1000 °C using a CA-100 Primacs^{SC} TOC analyzer (Skalar Analytical, Norcross, GA). Prior to C analysis, a Fizz test performed, in all the soil samples, using a 6 N HCl confirmed an absence of inorganic C in all of the soil samples (Halvorson et al., 1999).

Table 2
Soil characteristics in the surface 0–15 cm depth at the experimental sites.

Experiment	pH	EC	Bulk density	KCl extractable-N ($NH_4^+ + NO_3^-$)	Particle size distribution		
					Sand	Silt	Clay
		$ds\,m^{-1}$	$g\,cm^{-3}$	$\mu g\,g^{-1}$	$g\,kg^{-1}$		
Expt1	7.58	2.62	1.17	21.1	26	458	516
Expt2	7.66	1.10	1.16	14.1	21	467	513
Expt3	7.96	1.73	1.12	18.9	23	477	500

Microbial biomass C was analyzed by fumigation-extraction method (Vance et al., 1987). Duplicate set of 10 g of each dry soil were incubated for 7 d at 25 °C after adding sufficient de-ionized water to bring the soils at 50% of water holding capacity (WHC). After 7 d, the first set of soil was then fumigated with ethanol free chloroform for 24 h, while the other set was not. Both the fumigated and un-fumigated soils were mixed with 50 mL of 0.5 M K_2SO_4, shaken on a reciprocal shaker (200 strokes per min) for 1 h, filtered through Whatman no. 2 filter paper, and finally the extracts were analyzed for dissolved organic carbon (DOC) using a Shimadzu TOC-VCPH/CPN Analyzer (Shimadzu Corp., Kyoto, Japan). The C content of the microbial biomass was calculated by dividing the difference in DOC values of the fumigated and un-fumigated soil samples by a correction factor (K_c) of 0.45 (Alvarez et al., 1995).

Permanganate oxidizable C was measured as described by Weil et al. (2003). Briefly, 5 g of air-dried soils were weighed into 50 mL polypropylene conical centrifuge tubes, to which 2 mL of 0.2 M $KMnO_4$ and 18 mL of deionized water were added. The tubes were vigorously shaken for 2 min on a reciprocal shaker (200 strokes per min) and allowed to settle for 10 min. After 10 min, 0.5 mL of the

supernatant from the upper 1 cm of the suspension was transferred into another 50 mL centrifuge tube and mixed with 49.5 mL of deionized water. Finally, the diluted solution was measured for its absorbance in a Spectronic 20D+ spectrophotometer (Thermo Fisher Scientific Inc., Madison, WI), set at 550 nm. The values for $KMnO_4$-C were determined using the following equation:

$$KMnO_4 - C(\text{mg kg}^{-1}\text{ soil}) = [0.02\ \text{mol L}^{-1} - (a + b \times \text{absorbance})] \times (9000\ \text{mg C mol}^{-1}) \times \left(\frac{0.02\ \text{L solution}}{0.005\ \text{kg soil}}\right)$$

where, 0.02 mol L^{-1} is the initial solution concentration, a is the intercept and b is the slope of the standard curve, 9000 is mg C oxidized by 1 mol of MnO_4 changing from Mn^{7+} to Mn^{4+}, 0.02 L is the volume of $KMnO_4$ solution reacted, and 0.005 is the kg of soil used.

Coarse particulate organic matter C was determined by following the procedure of Sollins et al. (1999). Ten grams of air-dried soils (<2 mm) were mixed with 30 mL of 5 g L^{-1} sodium hexametaphosphate solution and shaken in a reciprocal shaker for 18 h. After shaking, the dispersed materials were then passed through a 53 μm sieve by rinsing several times with deionized water. The retained material (sand and POM) on the sieve was dried in an oven at 105 °C for 24 h and weighed. The mass of sand-free POM was determined by mass differences after burning the dried material at 550 °C for 4 h in a Thermolyne Benchtop Muffle Furnace (Thermo Fisher Scientific Inc., Beverly, MA). The POM fraction (i.e. POM of size ranging 53–2000 μm) measured represented the CPOM-C in our study.

Mineralized C was estimated using a short-term incubation method (Anderson, 1982). Briefly, 100 g of air-dried soils were weighed into 1 L mason jars and de-ionized water was added to bring the soils at 50% of WHC. The soils were then incubated in the presence of 20 mL of 0.5 M NaOH (in a vial) at 25 °C for 30 d. Water content of soils were regularly monitored by weighing the jars containing soils and required amount of de-ionized water was added, if necessary. The vials containing NaOH were removed at 7 d, 14 d, 21 d, and 30 d, which were then measured for the evolved CO_2–C by titration using 0.5 M HCl. The jars were replaced with vials containing fresh NaOH at each removal day until 21 d. The cumulative C mineralization within 30 d was then calculated from the summation of the measured C mineralized at the respective days.

2.3. Data analysis

Analysis of variance for all parameters (bulk density, KCl extractable-N, SOC, MBC, $KMnO_4$, CPOM-C, and Cmin) was calculated by SAS PROC MIXED procedure using Split Plot Design with nitrogen fertilizer management and tillage as main factors in Expt1 and Expt2, and using a Randomized Complete Block Design with tillage as the only factor in Expt3. Means of main effects were compared using Fisher's least significant difference (LSD) at alpha level = 0.05. Pearson correlation coefficients were used to evaluate the relationships among the parameters at $p < 0.05$.

3. Results and discussion

Management induced changes in SOC and SOC fractions were largely limited to tillage practices, which primarily were observed in Expt2 and Expt3, while Expt1 was irresponsive to tillage (Table 3). In Expt2, SOC concentration, SOC stock, CPOM-C, and $KMnO_4$-C were significantly affected by tillage, while N-fertilizer management significantly influenced soil MBC. Similar trend was observed for Expt3, where tillage affected all the measured SOC parameters, except MBC. Tillage induced changes in SOC and SOC fractions might not be apparent in short term studies (≤5 years) as compared with longer-term tillage effect studies (Geisseler and Horwath, 2009). Similarly, management practices (e.g. tillage and crop rotation) employed under similar soils – for different durations (number of years) – can have a profound influence on soil quality indicators (Liebig et al., 2004). In the present study, both Expt2 and Expt3 were initiated in 2005; hence tillage managements in these experiments have been imposed since 6 years compared with only 4 years in Expt1, which was established in 2008. Therefore, the lack of response of tillage to the SOC parameters in Expt1 might be due to the short duration (4 years) of time since treatments were imposed. And, different trends observed with tillage treatments among the experiments could possibly be attributed to the differences in the number of years since the treatments were imposed in these experiments.

3.1. Soil organic carbon concentration and stock

Tillage significantly influenced SOC concentrations in Expt2 and Expt3 (Table 3, Fig. 1A). Both in Expt2 and Expt3, the ST and NT treatments, although not different from each other, were significantly higher in SOC concentrations than CT. In Expt1, generally higher SOC concentrations were found in ST and NT treatments as compared with CT, although the differences were not significant. These results suggest that tillage induced changes in SOC are apparent after 6 years of imposing the treatments, with

Table 3
The ANOVA table of the analysis of soil organic carbon (SOC) concentration and stock, microbial biomass carbon (MBC), permanganate oxidizable carbon ($KMnO_4$-C), coarse particulate organic matter carbon (CPOM-C), and 30-d cumulative mineralizable C (Cmin) in the three experiments (Expt1, Expt2 and Expt3).

Source	SOC concentration	SOC stock	MBC	$KMnO_4$-C	CPOM-C	Cumulative Cmin
Expt1						
N fertilizer (N)	NS	NS	NS	NS	NS	NS
Tillage (T)	NS	NS	NS	NS	NS	NS
N × T	NS	NS	NS	NS	NS	NS
Expt2						
N fertilizer (N)	NS	NS	[a]	NS	NS	NS
Tillage (T)	[a]	[a]	NS	[a]	[a]	NS
N x T	NS	NS	NS	NS	NS	NS
Expt3						
Tillage	[a]	[a]	NS	[a]	[a]	[a]

NS represents not significant.
[a] Significant at the 0.05 level.

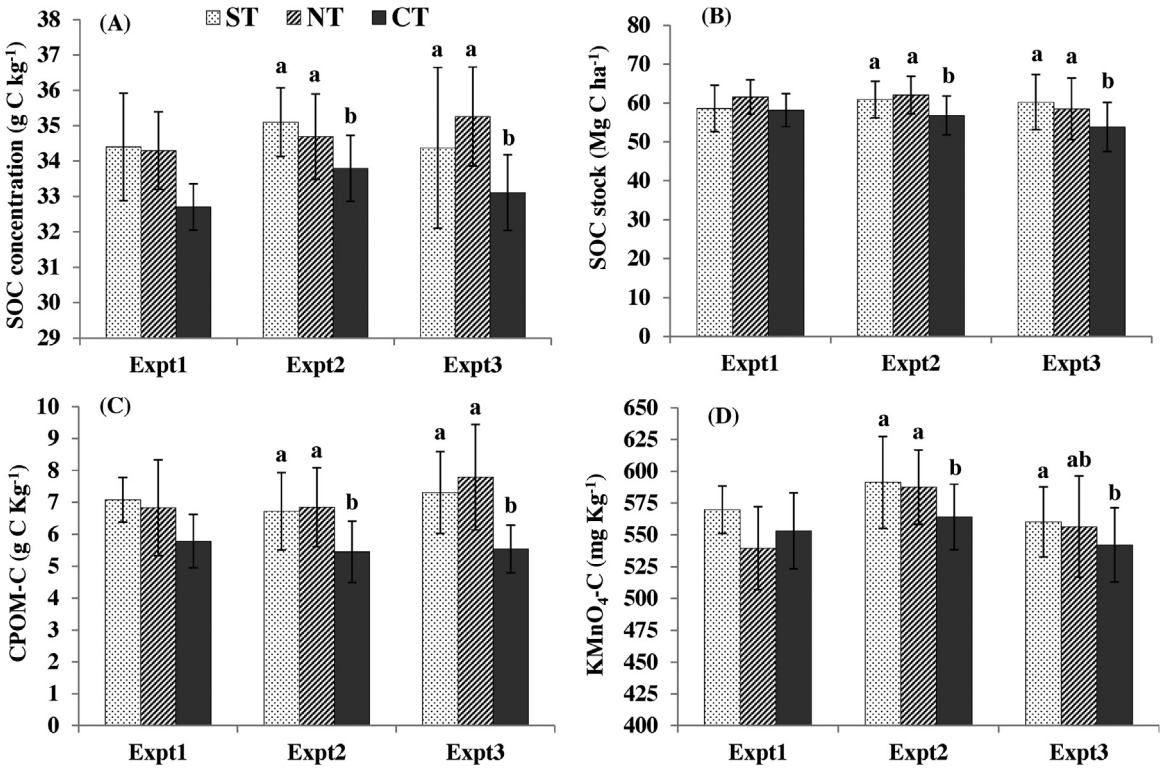

Fig. 1. Effect of tillage practices (strip-till [ST], no-till [NT], and conventional till [CT]) on (A) SOC concentration (g kg^{-1}), (B) SOC stock (Mg ha^{-1}), (C) CPOM-C (g kg^{-1}), and (D) $KMnO_4$-C (mg kg^{-1}) under three experiments within 0–15 cm soil depth. Bars represent standard deviations (n = 6, Expt1; n = 12, Expt2; n = 12, Expt3). Different lowercase letters within an experiment indicate significant differences at 0.05 significance level.

up to 3.9 and 6.6% SOC levels enriched, respectively under ST and NT as compared with CT. Several studies have reported higher SOC concentrations under conservation tillage practices than CT due to disruption of soil aggregates and increased soil respiration as a result of the release of protected SOC in the latter (Campbell et al., 1996; Six et al., 2000; Mikha and Rice, 2004; Sainju et al., 2008; Lewis et al., 2011; Kahlon et al., 2013). Comparatively lower SOC with CT than ST and NT could also be attributed to higher soil temperature favoring rapid C mineralization – resulted from increased soil microbial activities – under CT (Alvarez et al., 1995; Dou et al., 2008). However, in Expt1, tillage managements were imposed since 2008. And, the lack of pronounce differences in SOC concentrations in response to tillage in this experiment might be due to the short duration (4 years) of time since treatments were imposed (Geisseler and Horwath, 2009). The magnitude of change in SOC levels under ST and NT compared with CT in the current study, however, is relatively lower than that reported for clay soils in the NGP by Campbell et al. (1996), who documented about 14.5% increment in SOC under NT as compared with CT after 11 years. The differences in variable SOC concentrations between these two studies support the previous findings that the potential to conserve SOC varies widely due to complex interactions among climate, soil type, crop rotation, duration and management factors (Sainju et al., 2008; Chatterjee and Lal, 2009).

The SOC stock followed a similar trend as SOC concentration. Soil bulk density was not influenced by tillage practices in any of the experiments (Table 4). Therefore, treatment effects on SOC stock resulted mainly from differences of SOC concentration. Tillage significantly influenced SOC stock in Expt2 and Expt3, but not in Expt1 (Table 3, Fig. 1B). The stock of SOC in the 0–15 cm soil depth was significantly higher under ST and NT by 7.2 and 9.2% than CT, respectively in Expt2 and was significantly higher under ST and NT by 11.9 and 8.7% than CT, respectively in Expt3. The amounts of SOC, however, were similar under ST and NT in both Expt2 and Expt3. Generally, higher SOC stocks were observed in ST and NT as compared with CT, although the differences were not significant statistically in Expt1. Our results are in agreement with a study conducted in silt loam soil in North Dakota by Halvorson et al. (2002) who reported that CT increases crop residue-soil contact and also creates a more oxidative soil environment resulting in more rapid decomposition of SOC, relative to minimum and no-till practices. Neither N management nor tillage × N management interaction had a significant effect on both SOC concentration and SOC stock in Expt1 and Expt2 (Table 3).

3.2. Soil organic carbon fractions

3.2.1. Coarse particulate organic carbon

The CPOM-C responded similarly as SOC to tillage (Table 3, Fig. 1C). Significant effects of N management and tillage × N management, however, were not observed in both Expt1 and Expt2 (Table 3). As with SOC, tillage significantly influenced CPOM-C at 0–15 cm soil depth in Expt2 and Expt3; however, there were no differences among the tillage treatments for CPOM-C in Expt1 (Fig. 1C). The CPOM-C was 22 and 25% greater under ST and NT, respectively than under CT in Expt2 and was 33 and 45% greater under ST and NT, respectively than under CT in Expt3. Strip tillage and NT were equivalent in their CPOM-C contents in both Expt2 and Expt3. Generally, higher amounts of CPOM-C were evident with reduced tillage practices (7.1 g kg^{-1} and 6.8 g kg^{-1} respectively for ST and NT) than CT (5.8 g kg^{-1}) in Expt1. The differences observed in the CPOM-C contents are in agreement with most studies comparing the effect of conservation tillage (ST and NT), with minimal soil disturbances and CT, with greater intensity of tillage (Dou et al., 2008; Chatterjee and Lal, 2009; Chen et al., 2009). According to Chatterjee and Lal (2009), crop residues are left on the soil surface under NT and ST practices whereas residues are incorporated in the soil during tillage under CT, thereby favoring

Table 4
Effect of tillage (strip-till [ST], no-till [NT], and conventional till [CT]) on soil bulk density at 0–15 cm depth in three experiments.

Experiments	ST	NT	CT	LSD ($\alpha = 0.05$)
	Bulk density ($g\,cm^{-3}$)			
Expt1	1.14 ± 0.13[a]	1.20 ± 0.08	1.19 ± 0.09	0.11
Expt2	1.16 ± 0.09	1.19 ± 0.09	1.12 ± 0.13	0.08
Expt3	1.17 ± 0.10	1.11 ± 0.09	1.08 ± 0.12	0.08

Different lowercase letters within a row indicate differences at 0.05 significance level.
[a] ± Values are standard deviations from means (Expt1: $n = 6$; Expt2: $n = 12$; Expt3: $n = 12$).

increased mineralization of CPOM-C by soil microbes under the latter. Similarly, tillage induces the disruption of soil aggregates. And, increased exposure of soil aggregate protected CPOM-C to microbial decomposition following the collapse of aggregates by increased tillage intensity (CT) as compared with reduced tillage (ST and NT) may have contributed to the low levels of CPOM-C under CT treatment (Cambardella and Elliott, 1992; Mikha and Rice, 2004).

3.2.2. Microbial biomass carbon

The effect of tillage and tillage × N management were not significant for soil MBC in any of the experiments (Table 3). However, when averaged across the N fertilizer managements, soil MBC ranged from 1.17 g kg^{-1} under CT to 1.49 g kg^{-1} under ST. These values represent about 3.4–4.5% of the SOC within this study, which is quite comparable to 3.4% as reported by Liebig et al. (2004) for North Dakota soils. Similar to our results, Carpenter-Boggs et al. (2003) did not find any difference in soil microbial biomass at 0–15 cm surface soils managed under contrasting tillage managements (NT vs. CT) at 11 sites in South Dakota. In contrast, several studies have reported that NT management increased MBC in the surface soils (Doran, 1987; Dou et al., 2008; Balota et al., 2003; Alvarez et al., 1995). Lack of soil disturbance under NT provides steady source of organic C substrates for soil microorganisms, which enhances their activity and accounts for higher soil MBC as compared with CT – where a temporary flush of microbial activity with tillage events results in large losses of C as CO_2 (Balota et al., 2003). However, the differences in MBC reported in these studies include the comparison between no-till and moldboard plow treatments and tilled field in the present study were mainly chisel plowed. Therefore, different tillage intensity (mainly chisel plowed) imposed in our current study was not as great as in other studies that have compared no-till and moldboard plow treatments in revealing significant differences in MBC. Also, most of these studies reporting significant differences in soil MBC across tillage gradients are concentrated mainly up to 5–7.5 cm surface soils, with little or no difference in MBC below these depths (or within whole 0–15 cm soil profile) (Jacinthe and Lal, 2005; Dou et al., 2008; Alvarez et al., 1995). Tillage has shown to stratify both the availability of SOC and distribution of microbial biomass (Doran, 1987; Cookson et al., 2008). In the present study, MBC was evaluated for the entire 0–15 cm depth. Hence, the lack of sensitivity of tillage to MBC within this study may be attributed to the difference in depth of soil being evaluated (0–15 cm), as opposed to most studies that evaluated MBC only up to 5–7.5 cm soil depth. Another explanation to the insignificant effects in MBC in response to tillage in our current study could probably due to the fact that soil microbial biomass probably being more responsive to short-term environmental conditions (Carpenter-Boggs et al., 2003; Cookson et al., 2008).

On the other hand, N fertilizer management significantly influenced soil MBC in Expt2 (Table 3, Fig. 2). When MBC levels were averaged across the tillage treatments in Expt2, full early N treatment produced similar soil MBC as full late N treatment (Fig. 2); however, both full early and full late N treatments produced significantly higher soil MBC than half early/half late N fertilizer treatment by 47 and 49%, respectively. Soil microbial biomass has been generally thought to be limited by energy substrates rather than mineral nutrients. However, studies have demonstrated that soil microbial growth can be constrained by N availability (Wang and Bakken, 1997a,b; Kaye and Hart, 1997). Nitrogen is a nutrient required by both crops and soil microbial biomass. Application of N fertilizers to field crops in split doses can improve the synchrony between plant N demand and soil N availability, which thereby increases plant N uptake (Gehl et al., 2005). And, efficient uptake of N by plants can reduce soil available N and exacerbate N constraints on soil microbial biomass (Hu et al., 2001). Similarly, Wang and Bakken (1997b) documented that soil microbial biomass was significantly lower in the soil planted with barley than in the soil without any barley plants because of N-depletion created by plant root uptake of available N. In the present study, half early/half late fertilizer-N treatment generally resulted in lower soil available N than when full fertilizer-N were either applied early at preplant or late in the growing season, although not different significantly among each other (Table 5). Hence, significant reduction in soil MBC under half early/half late N fertilizer treatment in the present study could be attributed to N limitations on soil MBC because of enhanced plant N uptake.

Furthermore, improving soil N availability via split fertilizer-N application usually increases the aboveground litter production and thereby increases C substrate as litter becomes incorporated into the soil (McAndrew and Malhi, 1992). These are reflected by higher SOC concentration with half early/half late fertilizer-N treatment than full early N and full late N treatments in the current study (Table 5). And, given the availability of N in soil is improved by half early/half late N treatment for plant uptake – with less N available to soil microbial biomass, a higher C substrate result in disproportionate supplies of C and N, which could further

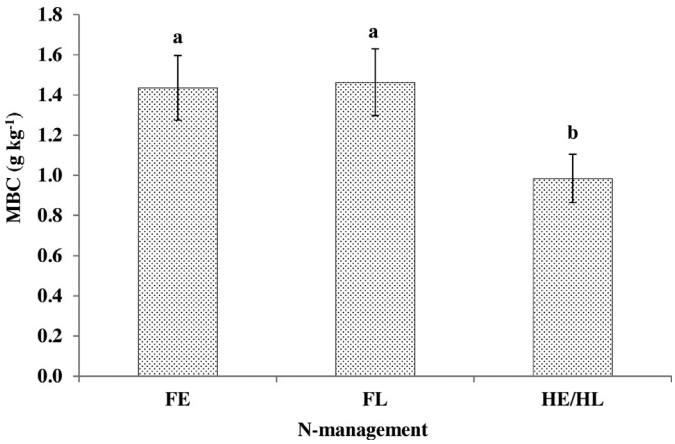

Fig. 2. Effect of N-management (FE: Full N-applied early; FL: Full N-applied late; HE/HL: Half N-applied early/Half N-applied late) on soil microbial biomass carbon (MBC, g kg^{-1}) within 0–15 cm depth across tillage in Experiment 2. Bars represent standard errors ($n = 12$). Different lowercase letters indicate differences at 0.05 significance level.

Table 5
Effect of N-management (FE: Full N-applied early; FL: Full N-applied late; HE/HL: Half N-applied early/Half N-applied late) on SOC concentration (g kg^{-1}) and mean KCl extractable-N ($NH_4^+ + NO_3^-$) ($\mu g\, g^{-1}$) within 0–15 cm depth across tillage in experiments1 and 2.

N-management	SOC concentration		KCl extractable-N	
	Expt1	Expt2	Expt1	Expt2
	$g\, kg^{-1}$		$\mu g\, g^{-1}$	
FE	33.6 ± 0.3[a]	34.2 ± 0.3	21.0 ± 1.9	14.3 ± 0.5
FL	33.8 ± 0.4	34.4 ± 0.3	21.4 ± 2.8	14.5 ± 0.6
HE/HL	–	35.0 ± 0.4	–	13.5 ± 0.5
LSD ($\alpha = 0.05$)	0.3	0.7	6.7	1.3

Different lowercase letters within a column indicate differences at 0.05 significance level.
[a] ± Values are standard errors of means (Expt1: $n = 9$; Expt2: $n = 12$).

aggravate N limitations on soil microbial biomass and influence their growth (Green et al., 1995; Wang and Bakken, 1997a). Wang and Bakken (1997b) reported that the depressing effect of plant uptake of N on soil microbial biomass was much pronounced in N poor barley straw layer suggesting that microbial growth in soil can be limited by nutrient supply rather than C availability. In our study, half early/half late N treatment had higher C substrate with lower available N concentration than the full N treatments. This disproportionate supply of C and N substrates with half early/half late fertilizer-N treatment could possibly have induced a more N stress on soil microbial biomass and hence is responsible for the observed reduction in soil MBC than full N late and early treatments. However, differences observed in soil N availabilities (0.2 g kg^{-1}) and SOC concentrations (0.2 g kg^{-1}) between full N early and full N late treatments in Expt2 were very small, which resulted in similar soil MBC between these treatments. This is supported by a similar trend observed in Expt1, where no difference in soil MBC between full N early and full N late treatments was observed due to very small differences in SOC concentrations and soil N availabilities (Table 5). Overall, these results suggest that N availability rather than C availability is more critical for microbial growth and biomass production, which can in turn influence SOC dynamics in silty clay soils.

3.2.3. Mineralized carbon

The evolution of mineralized C was significantly affected by tillage in Expt3 (Table 3, Fig. 3C). In this experiment, Cmin were consistently higher under ST and NT than CT throughout the 30 d incubation period except at d 7, where all the tillage treatments evolved similar CO_2–C. Here, as for the 14 d period, while the amounts of CO_2–C produced under NT was not significantly different than ST and CT; Cmin was 27% higher under ST than CT. Soil CO_2–C mineralized at d 21 and 30 showed similar trends. The amounts of Cmin under ST and NT were significantly higher than CT by 33 and 30% respectively at 21 d and were 34 and 28% higher than CT respectively at 30 d. Mineralized C were not different between ST and NT at both 21 and 30 d. The cumulative amount of Cmin at the end of 30 d under NT (433 mg kg^{-1}) was not different than ST and CT treatments, however, ST significantly evolved 23% higher cumulative CO_2–C as compared with CT (Fig. 3D). Our results are in accordance with earlier studies, which reported greater Cmin under reduced tillage practices than CT (Carpenter-Boggs et al., 2003; Mikha and Rice, 2004; Dou et al., 2008). Reduced tillage practices allow C to build-up in the plow layer by enhancing soil aggregation and reducing oxidation (Carpenter-Boggs et al., 2003). In contrast, frequent tillage under CT increases the loss of aggregate protected C through microbial decomposition (Mikha and Rice, 2004). Higher Cmin under NT and ST could be attributed to higher availability of C substrates for decomposition by microbial biomass (Chen et al., 2009). This is also supported by the significant correlations of Cmin with SOC and CPOM-C observed in Expt3 (Table 6), indicating that both SOC and CPOM-C serves as substrates for microbial respiration.

The effects of tillage, N fertilizer, and tillage × N fertilizer interaction were not significant for the cumulative Cmin at the end of 30 d as well as the amounts of Cmin throughout the 30 d incubation period in both Expt1 and Expt2 (Table 3, Fig. 3A, B, and D). But, the trends showed that the cumulative Cmin in 30 d and the amounts of Cmin at 7, 14, 21, and 30 d were generally greater with ST and NT in Expt1, and greater with ST in Expt2. The amounts of cumulative Cmin ranged from 227 to 244 mg kg^{-1} in Expt1 and ranged from 430 to 481 mg kg^{-1} in Expt2. As earlier investigations, this study confirms that Cmin during 30 day can provide an assessment of SOC changes induced by tillage practices.

3.2.4. Permanganate oxidizable carbon

Similar to SOC and CPOM-C, tillage significantly influenced $KMnO_4$-C in Expt2 and Expt3 (Table 3, Fig. 1D). Across the tillage treatments, $KMnO_4$-C levels ranged from 553 to 570 mg kg^{-1} for Expt1, 564 to 592 mg kg^{-1} for Expt2, and 542 to 560 mg kg^{-1} for Expt3, which is quite similar to the range of 462–600 mg kg^{-1}, reported by Weil et al. (2003) for North Dakota soils. In Expt2, the $KMnO_4$-C was significantly higher under ST and NT as compared with CT, while ST and NT were similar. In Expt3, ST produced significantly higher $KMnO_4$-C than CT; however, both ST and CT were not different than NT statistically. In Expt1, although the effect of tillage practices was not significant, $KMnO_4$-C fractions were higher with ST than NT and CT. The results obtained in the present study are in agreement with earlier investigations reporting higher levels of $KMnO_4$-C under conservation tillage practices (Weil et al., 2003; Lewis et al., 2011; Chen et al., 2009). The $KMnO_4$-C method includes digestion or oxidation of SOC (Blair et al., 1995), which (oxidation) is enhanced by the exposure of physically protected soil aggregates (Chen et al., 2009). According to Lewis et al. (2011), increasing tillage intensity could reduce $KMnO_4$-C levels in soils as a result of destruction of soil macroaggregates and elevated respiration. Lower amount of $KMnO_4$-C, hence is likely under CT due to increased soil disturbances subjecting aggregated protected SOC fraction to rapid decomposition via oxidation. Our results suggest that $KMnO_4$-C fraction is sensitive to tillage management practices.

3.3. Relationships between soil organic carbon and its fractions

Pearson correlations among SOC, MBC, CPOM-C, $KMnO_4$-C, and cumulative Cmin within each experiment across the tillage treatments are shown in Table 6. Soil organic C was significantly and positively correlated with MBC, CPOM-C and Cmin in Expt1; and CPOM-C and Cmin in both Expt2 and Expt3. Likewise, Cmin was significantly correlated with CPOM-C in all three experiments and with MBC in Expt1. The CPOM-C is considered to include decomposing organic matter, consistent with the theoretical characteristics of SOC pools of intermediate lability (Gregorich

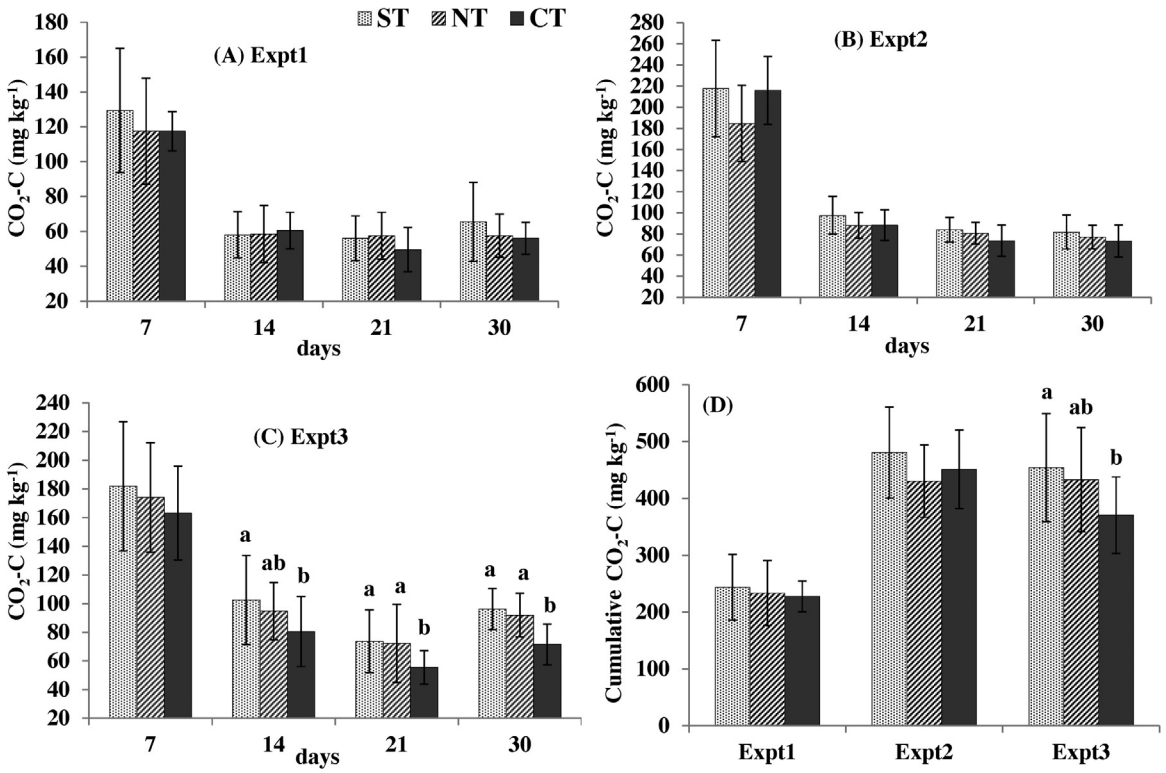

Fig. 3. Soil C mineralized (CO_2–C, mg kg^{-1}) within 30 days of incubation period in (A) Expt1, (B) Expt2, and (C) Expt3 and (D) Cumulative C mineralized (CO_2–C, mg kg^{-1}) in three experiments, as affected by strip-till (ST), no-till (NT), and conventional till (CT). Bars represent standard deviations (n = 6, Expt.1; n = 12, Expt.2; n = 12, Expt. 3). Different lowercase letters within a specific day and within an experiment indicate differences at 0.05 significance level.

et al., 2006; Cambardella and Elliott, 1992; Six et al., 2000). Studies have documented that SOC is physically protected from microbial decomposition within soil aggregates (Mikha and Rice, 2004; Gregorich and Beare, 2008; Mikha et al., 2013). Both, the SOC and the CPOM-C serve as energy source for soil microbial biomass and hence, their availabilities (SOC and CPOM-C) significantly influence soil microbial activities (Geisseler and Horwath, 2009). Furthermore, microbial biomass is considered as the agent for C mineralization (Haynes, 2005). Presence of high levels of microbial biomass and activity, due to increased substrate availability results in high quality soils and improves crop productivity (Lopes et al., 2012). The positive and significant correlations obtained in our present study suggested that SOC was a principal source of its fine estimators such as MBC, CPOM-C and Cmin, which could reflect early and sensitive changes in SOC. These results are consistent with previous studies (Dou et al., 2008; Chen et al., 2009; Liang et al., 2012; Carpenter-Boggs et al., 2003; Lucas and Weil, 2012; Mirsky et al., 2008; Jacinthe and Lal, 2005; Pandey et al., 2010; McLauchlan and Hobbie, 2004; Lutzow et al., 2002). Hence, it is not surprising to find positive correlations among different SOC fractions as they are inter-related with each other and are all appropriate to use as an index of labile SOC across a range of SOC levels.

Table 6
Pearson correlations among soil organic carbon (SOC), microbial biomass carbon (MBC), coarse particulate organic matter carbon (CPOM-C), permanganate oxidizable carbon ($KMnO_4$-C), and 30-d cumulative carbon mineralization (Cmin) in the three experiments.

C fractions	SOC	MBC	CPOM-C	$KMnO_4$-C	Cmin
	Experiment 1 (n = 18)				
SOC		0.47[a]	0.75[a]	NS	0.58[a]
MBC	0.47[a]		NS	NS	0.82[a]
CPOM-C	0.75[a]	NS		NS	0.44[a]
$KMnO_4$-C	NS	NS	NS		NS
Cmin	0.58[a]	0.82[a]	0.44[a]	NS	
	Experiment 2 (n = 36)				
SOC		NS	0.48[a]	NS	0.34[a]
MBC	NS		NS	NS	NS
CPOM-C	0.48[a]	NS		NS	0.37[a]
$KMnO_4$-C	NS	NS	NS		−0.36
Cmin	0.34[a]	NS	0.37[a]	−0.36	
	Experiment 3 (n = 36)				
SOC		NS	0.36[a]	NS	0.45
MBC	NS		NS	NS	NS
CPOM-C	0.36[a]	NS		0.32[a]	0.69[a]
$KMnO_4$-C	NS	NS	0.32[a]		NS
Cmin	0.45[a]	NS	0.69[a]	NS	

NS represents not significant.
[a] Significant at the 0.05 level.

In addition, significant correlation was also found between $KMnO_4$-C and CPOM-C in Expt3. According to Culman et al. (2012), $KMnO_4$-C reflects a more processed or decomposed fraction of SOC. The significant relationship between $KMnO_4$-C and CPOM-C has been also used to indicate a similar response of these fractions to management changes (Chen et al., 2009; Mirsky et al., 2008). However, in the present study, significant correlation was not found between $KMnO_4$-C and other measures of SOC pools. Previous investigations documented that $KMnO_4$-C was significantly correlated with SOC (Chen et al., 2009), MBC (Culman et al., 2012), and Cmin (Lutzow et al., 2002). In contrast, Melero et al. (2011), however, found $KMnO_4$-C to lack correlation with water soluble C (WSC) and other biological measures of SOC such as MBC, microbial biomass N, dehydrogenase activity, and glucosidase activity. Similarly, Tirol-Padre and Ladha (2004) did not find any significant correlation of $KMnO_4$-C with MBC or WSC but instead with lignified SOC. They suggested that the $KMnO_4$-C represent a more stable fraction of SOC than labile C. The lack of significant correlation of $KMnO_4$-C with SOC, MBC, and Cmin in our investigation may have also resulted from the short duration of time since the treatments were imposed.

The sensitivities of different SOC fractions to tillage effects greatly varied among each other, with MBC being irresponsive to tillage treatments in this study. However, across all the experiments, CPOM-C was 16.3–22.1%, MBC was 3.4–4.5%, cumulative Cmin was 0.7–1.4%, and $KMnO_4$-C was 1.6–1.7% of SOC. The highest correlation was observed between cumulative Cmin and MBC; however, CPOM-C and cumulative Cmin were the most highly correlated with SOC among all the SOC fractions measured (Table 3). The magnitude of changes in SOC fractions between conservation tillage practices (ST and NT) and CT ranged in the decreasing order of CPOM-C (17.2–41.8%) > cumulative Cmin (6.6–22.5%) > $KMnO_4$-C (2.6–4.8%). These suggest that tillage induced changes were sensitively reflected by the changes in physical (CPOM-C), chemical ($KMnO_4$-C), and biological (cumulative Cmin) SOC fractions and therefore, can be used to estimate early changes in SOC dynamics. CPOM-C was the most sensitive fractions of organic C to tillage effects than total SOC which showed 2.7–6.6% enrichment.

4. Conclusions

Across the management practices evaluated in the present study, tillage had the greatest effect on SOC and its various fractions (CPOM-C, $KMnO_4$-C, and Cmin) in two of the experiments (Expt2 and Expt3) in the surface (0–15 cm) soil after 6 years of tillage implementation, with positive results observed with conservation tillage practices (ST and NT) compared with conventional tillage. Expt1 was less responsive to tillage treatments. However, the trends observed with SOC fractions in this experiment (Expt1) indicated that conservation tillage managements were creating a more favorable plant growth environment relative to conventional tillage. Soil MBC was found to be sensitive to N management in Expt2. Our results supported the hypothesis that changes in surface SOC by tillage can be predicted by CPOM, $KMnO_4$-C and Cmin. Nonetheless, CPOM-C was the most sensitive to tillage managements among all the SOC fractions and represented the largest portion of the total SOC (19.2% on average). The adoption of conservation tillage practices offers soil C sequestration opportunity and soil health improvement under corn-sugarbeet-soybean rotation in the Red River Valley. However, SOC changes in clay enriched soils should be further investigated in relation to nitrogenous fertilizer management and crop rotation.

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