

## Carbon saturation and translocation in a no-till soil under organic amendments

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### ABSTRACT

Studies suggest that intensively tilled soils have lost 25–75% of their original soil organic C (SOC) content. No tillage (NT) can restore SOC in response to increased C inputs and reduced soil disturbance. Organic amendments provide faster recovery of SOC, ultimately promoting the saturation of soil layers. Here we present evidence of additional SOC accrual through translocation of SOC after saturation of the topsoil layer in a NT soil. Both processes were observed in a long-term (25 yr.) experiment comparing soil tillage systems (chisel tillage – CT, and NT) in central Kansas. Plots with continuous corn were amended with mineral (MF) and organic N (OF), in addition to a control treatment without N fertilization (CO). Accumulation of SOC was limited to the surface layer (0–5 cm) of the NT soil amended with MF. Organic fertilization increased SOC in the 0–5 cm soil layer from 9.5 Mg C ha<sup>-1</sup> to a level of 16.2 and 30.2 Mg C ha<sup>-1</sup> in CT and NT, respectively. Further analysis confirmed the saturation of physically protected SOC in the surface of NT soil under organic amendments. After saturation, significant SOC accrual (1.3 Mg C ha<sup>-1</sup> yr<sup>-1</sup>) was observed in the 5–15 cm soil layer. Isotopic assessment confirmed the occurrence of SOC translocation between saturated and C-depleted NT soil layers. No evidence of SOC translocation was observed between non-saturated soil layers. Further research is needed to elucidate the mechanisms regulating this process. Nonetheless, SOC translocation and subsequent SOC accrual suggests a greater C sink potential for NT soils than previously thought.

### 1. Introduction

Soil organic matter (1550 Pg C up to 1 m depth) contains about twice as much C as the earth's atmosphere (780 Pg C) and up to three times more C than vegetation (500–650 Pg C) (Smith et al., 2008). Agricultural systems can affect the C exchange between these pools and be an atmospheric C source or sink depending on soil management. Intensively tilled soils have lost up to 75% of SOC prior to cultivation (Lal, 2010; Sanderman et al., 2017). Conservation agriculture (CA), as defined as minimal soil disturbance, permanent soil cover and crop rotations, is a major global initiative to recover SOC and soil quality (Jat et al., 2014; Lal, 2016; Minasny et al., 2017). No-tillage (NT) provides minimum soil disturbance and is considered the key component of CA. Nonetheless, NT soils are considered a finite biological C sink with limited capacity for SOC storage for 20–50 years (Powlson et al., 2014; West and Post, 2002).

Soil organic C accumulation in agricultural soils is achieved by increasing C inputs and decreasing SOC losses with minimum soil disturbance, thus promoting the stabilization of newly added C (Fabrizzi et al., 2009; Stewart et al., 2008). The traditional understanding of SOC stabilization includes biochemical recalcitrance, organo-mineral interaction and bio-physical protection as the main mechanisms regulating SOC turnover (Christensen, 1996; Lehmann and Kleber, 2015; Lützw et al., 2006; Sollins et al., 1996). However, the recently proposed soil continuum model suggests that long-term SOC stabilization depends mostly on the multilayered adsorption of organic molecules on soil mineral surfaces, as organo-mineral interactions increases with the continued degradation of organic residues and closer contact with mineral surfaces (Kleber et al., 2007; Lehmann and Kleber, 2015; Lützw et al., 2006). Thus, although biochemical composition and N content play a crucial role on the initial decomposition of crop and organic residues (Grave et al., 2015; Schmatz et al., 2017), no evidence has

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been found regarding their influence on long-term SOC stabilization (Lehmann and Kleber, 2015). Moreover, the model highlights the composition of soil microbial communities, their energy requirements and availability of resources, such as oxygen and nutrients, as a key manageable factors regulating SOC turnover (Lehmann and Kleber, 2015). This proposition suggests soil aggregation as a management-sensitive factor controlling SOC stabilization in temperate soils, corroborating previous studies where SOC accumulation was associated with increased amounts of large soil macroaggregates ( $> 2000 \mu\text{m}$ ) as a result of minimum soil disturbance, increased C inputs and microbial biomass (Chung et al., 2008; Fabrizzi et al., 2009; Mikha and Rice, 2004; Six et al., 2004, 2002).

Studies have shown that increasing C inputs promotes a linear increase of SOC in C-depleted soils since soil aggregates and mineral surfaces that are less saturated with SOC are able to retain the newly added C (Carter et al., 2003; Hassink, 1997; Six et al., 2002). As SOC increases, however, soil aggregates and mineral surfaces lose efficiency in protecting SOC from microbial activity, thus limiting the soil's capacity to accumulate C (i.e., saturation) (Six et al., 2002, 2000, Stewart et al., 2008, 2007). When SOC is close to saturation, additional C inputs promote an asymptotic response to equilibrium (Six et al., 2002; Stewart et al., 2007) and only unprotected and therefore management sensitive SOC pools would accumulate (Gulde et al., 2008; Six et al., 2002). Thus, saturation would delineate the effective SOC stabilization capacity, thus limiting SOC accrual especially in those forms protected by soil aggregates and mineral surfaces (Six et al., 2002; Stewart et al., 2007).

West and Post (2002) reported that 85 and 15% of SOC accrual observed in NT soils occurred in the 0–7 and 7–15 cm layers, respectively, while no changes in SOC were noticed below 15 cm depth regardless of crop systems. This process is attributed to continuous and direct C input (stover and roots) to the soil surface layer and the lack of soil disturbance decreasing SOC mineralization (Bayer et al., 2006b; Corbeels et al., 2016; de et al., 2001; de Oliveira Ferreira et al., 2013; Santos et al., 2011). Major C sources for underlying soil layers in NT soils are the direct C input by crop roots (Santos et al., 2011), leaching of soluble C (Neff and Asner, 2001), and movement of SOC in the soil profile by bioturbation (Gabet et al., 2003; Jiménez and Lal, 2006; White and Rice, 2009). But, because these processes generally do not yield significant SOC accumulation (de Oliveira Ferreira et al., 2013) or may not suffice to maintain original SOC in subsoil layers of NT soils (Dimassi et al., 2014), it is possible that the saturation of the surface horizon could ultimately limit the potential of NT soil as a significant C sink (Stewart et al., 2008, 2007). However, if larger amounts of unprotected SOC pools were translocated and stabilized in underlying soil layers it would increase the capacity of NT soil to accumulate C. Translocation of SOC is a normal pedogenesis process, especially in Spodosols (Soil Survey Staff, 1999), but is also important in other soil orders (Andisols, Inceptisols, Entisols, Oxisols) under natural ecosystems (Fujii et al., 2011). Soluble C from organic horizons represents an important source of subsoil SOC (from 11 to 75% of total C input) to mineral horizons in boreal and temperate forests (Kleja et al., 2008; Zech et al., 1996). Nonetheless, bioturbation may also redistribute SOC over short vertical distances ( $< 5 \text{ cm}$ ) (Tonnejck and Jongmans, 2008).

Hypothetically, an analogous process could be expected when agricultural soils reach saturation and translocation of SOC from saturated surface soil layers would promote SOC accumulation in underlying layers. We assessed a long-term (25 yr.) experiment in a Mollisol from central Kansas (USA) including tillage systems and N sources to investigate this hypothesis. This experimental site has a unique dataset with high temporal and sampling depth resolution. Previous assessment revealed that SOC in the 0–5 cm layer of NT plots increased asymptotically in response C inputs from organic amendments, thus suggesting SOC saturation (Nicoloso et al., 2016). For the present study, we assessed temporal changes in SOC and total nitrogen (TN) in stratified 0–5, 5–15, and 15–30 cm soil layers, SOC within aggregates, and soil

**Table 1**

Soil texture and chemical characteristics of the 0–5 cm layer of a Hapludoll from central Kansas under chisel tillage (CT), no-tillage (NT), and tallgrass prairie as reported by Fabrizzi et al. (2009).

Tillage	pH	Bray-P mg kg <sup>-1</sup>	Ca <sup>2+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	CEC cmol <sub>c</sub> kg <sup>-1</sup>	Sand %	Silt	Clay
NT	5.8	55.0	2137	318	265	10.2	18.4	12	68	20
CT	6.2	54.9	2260	371	297	14.5	17.1	10	70	20
Prairie	5.7	65.0	2472	659	412	19.6	24.7	9	59	32

<sup>13</sup>C and <sup>15</sup>N isotopic signatures.

## 2. Material and methods

This study was based on a long-term experiment established at the North Farm of Kansas State University in Manhattan, KS (39° 12' 42"N, 96° 35' 39"W). The local annual mean precipitation and potential evapotranspiration were 800 and 1300 mm, respectively, with annual mean temperature of 11.4 °C. The soil was a moderately well-drained Kennebec silt loam (fine-silty, mixed, superactive mesic Cumulic Hapludoll). Basic soil properties of the areas used in this study are presented in Table 1 (Fabrizzi et al., 2009). Prior to establishment of the experiment the area was used for small grain production (wheat, oats and other C3 crops) under intensive tillage for at least 60 years. The experiment was initiated in 1990 when corn (*Zea mays* L.) was first introduced at this site. The experiment was arranged as split-plot randomized blocks with four replications. The tillage systems were the main plots and N sources for corn were the sub-plots. The sub-plots size was 7.5 m × 6 m. The tillage systems were fall chisel plow with pre-plant spring offset disk (chisel tillage: CT), and no-till (NT) by planting directly through the crop residues with minimal soil disturbance. The chisel plow and disking operations were performed to a depth of 15 and 10 cm, respectively. The fertilization treatments were different N sources applied just before planting: 168 kg ha<sup>-1</sup> of available N either as mineral fertilizer (ammonium nitrate or urea; MF) or organic N fertilizer (OF), besides a control without N (CO). Until 2001, fresh cattle manure was applied in the OF treatment and after that the organic N source was composted organic waste. The composted organic waste was collected at the North Farm's composting facility of the Kansas State University and consisted of a mixture of food waste, hay waste, and cattle manure. Each year, OF were analyzed for total N, organic N, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> contents and the application rate was calculated assuming that 30% of organic N and 100% of mineral N was available during the crop growing season (Mikha and Rice, 2004).

### 2.1. Soil sampling

Composite soil samples were collected in the 0–5, 5–15, and 15–30 cm soil layers using a 5 cm diameter soil probe after the end of cropping growing season in 1992, 1995, 1999, 2002, 2003, 2004, 2007 and 2014. About 2–3 subsamples were taken for one composite soil sample from each sub-plot. The undisturbed soil cores were separated into different depths in the field to prevent contamination between soil layers. Samples were air-dried, sieved ( $< 2 \text{ mm}$ ), visible roots were manually removed, and subsamples were finely ground for further analysis. Soil masses in the same depths were determined by collecting another two intact soil core samples from each sub-plot in 1992, 1999, 2003, 2004, 2007 and 2014 following the same sampling procedure. Samples were dried at 105 °C and weighed. The soil masses in 1995 and 2002 were assumed to be the same as those measured in 1992 and 2003, respectively. Samples of the 0–5 cm soil layer were also collected in 2007, using a shovel, for the analysis of aggregate-size distribution and SOC content within aggregate-size fractions. Samples were passed through an 8-mm sieve and the large macroaggregates that were

retained on top of a 4-mm sieve were collected. Roots and plant residues were removed. Samples were dried at 40 °C and stored at 4 °C until fractionation. A nearby tallgrass prairie with the same soil type was used for comparison by collecting four composite soil samples. The tallgrass prairie vegetation was dominated by big bluestem (*Andropogon gerardii* Vit.), little bluestem (*Schizachrium scoparium*), indiangrass (*Sorghastrum nutans* L.), and switchgrass (*Panicum virgatum* Michx.) along with other C4 grasses (Hartnett and Fay, 1998).

## 2.2. Soil organic carbon stocks

The estimation of SOC stocks in different soil layers was performed on the basis of equivalent soil masses (ESM) (Wendt and Hauser, 2013). First, soil samples were analyzed for total organic carbon (TOC) content by dry combustion with a C/N elemental analyzer (Flash EA 1112 Series, ThermoScientific, Waltham, MA). Then, the SOC stocks were calculated considering the SOC contents and the measured soil masses in the 0–5, 5–15 and 15–30 cm soil layers in every sub-plot and sampling year. We used the average masses of 590, 1180, and 1800 Mg ha<sup>-1</sup> measured in the 0–5, 5–15, and 15–30 cm soil layers in the CT and NT plots in 1992 as references of ESM comparison. Thus, the cumulative reference soil masses for the 0–5, 0–15, and 0–30 cm soil layers were 0–590, 0–1770, and 0–3570 Mg ha<sup>-1</sup>, respectively. A cubic spline function which consisted of a piecewise series of cubic polynomial curves was used to calculate the cumulative SOC stocks present in the cumulative reference soil masses (Wendt, 2012). Soil organic C stocks in the intermediate reference soil masses (0–590, 590–1770, and 1770–3570 Mg ha<sup>-1</sup>) were calculated by subtraction. Although all SOC stock were calculated and compared as ESM, the results were expressed as 0–5, 5–15, and 15–30 cm soil layers for better clarity, always considering the 1992 soil sampling as the reference for all other years.

## 2.3. Aggregate-size distribution

Water-stable aggregates (WSA) within the 4–8 mm soil fraction obtained from soil samples collected in 2007 were separated using a modified Yoder wet-sieving apparatus (Mikha and Rice, 2004). An 80 g sub-sample was placed on the top of a 2000 µm sieve, slaked for 5 min and wet-sieved for 2 min with a stroke length of 4 cm, totaling 60 cycles. The WSA were collected and the remaining soil and water were passed through 250 and 53 µm sieves following the same procedure. Samples were oven dried at 60 °C and weighed. Subsamples of the isolated fractions, namely large macroaggregates (LM: > 2000 µm), small macroaggregates (SM: 250–2000 µm), microaggregates (m: 53–250 µm), and silt + clay (s + c: < 53 µm) were ground for determination of TOC content by dry combustion. Other subsamples were dispersed with 5 g L<sup>-1</sup> sodium hexametaphosphate solution for determination of sand content within WSA (Elliott et al., 1991). The sand content of the WSA fractions was subtracted for calculation of sand-free WSA and the C content within WSA fractions was corrected by dividing by the proportion of sand-free WSA (Gulde et al., 2008).

## 2.4. Carbon inputs

Corn plants present within 3.05 linear meters from two central rows in each sub-plot were sampled at harvest for determination of stover biomass production. Samples were dried at 65 °C and weighed. Carbon inputs were estimated assuming an average C content of 40% in stover biomass (Bayer et al., 2006a). The C contents of organic N sources were measured only sporadically. Thus, we estimated average C inputs from organic amendments considering measured application rates, total N content and, when data was available, the C/N ratio of organic N sources. The average C input by cattle manure was estimated as 2.6 Mg C ha<sup>-1</sup> yr<sup>-1</sup> from 1990 until 2001 and by composted organic waste as 5.2 Mg C ha<sup>-1</sup> year<sup>-1</sup> for the 2002–2014 period (Nicoloso et al., 2016). To account for the redistribution of corn residues and

organic fertilizers due to disking operations (0–10 cm) in CT treatments (Franzuebbers, 2002), we assumed that 50% of C inputs from corn stover and organic fertilizers were reallocated for the for 5–15 cm soil layer. The described procedures allowed the estimation of C inputs either for 0–5 and 0–15 cm layers.

## 2.5. Natural <sup>13</sup>C abundance

Soil samples collected in 1992 and 2014 were analyzed for natural <sup>13</sup>C and <sup>15</sup>N abundance ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) with a ThermoFinnigan ConFlo III interface and ThermoFinnigan Delta-plus Continuous Flow Stable Isotope Ratio Mass Spectrometer. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (parts per mille, ‰) were as follows:

$$\delta^{13}\text{C} = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 10^3 \quad (1)$$

$$\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3 \quad (2)$$

where, R is the ratio of <sup>13</sup>C:<sup>12</sup>C in the sample and in the standard Pee Dee Belemnite (PDB) from the Pee Dee River Formation (Hemingway, SC) and the ratio of <sup>15</sup>N:<sup>14</sup>N in the sample and in the atmospheric N<sub>2</sub> ( $R_{\text{standard}} = 0.003676$ ), respectively.

The relative contribution of C4 (corn and the original tallgrass prairie vegetation) and C3 (organic amendments in OF treatment) sources for SOC stocks were calculated by using a two end-member mixing model (Balesdent et al., 1987):

$$f_{\text{C4}} = (\delta^{13}\text{C}_{\text{SOC}} - \delta^{13}\text{C}_{\text{C3}})/(\delta^{13}\text{C}_{\text{C4}} - \delta^{13}\text{C}_{\text{C3}}) \quad (3)$$

$$f_{\text{C3}} = (\delta^{13}\text{C}_{\text{SOC}} - \delta^{13}\text{C}_{\text{C4}})/(\delta^{13}\text{C}_{\text{C3}} - \delta^{13}\text{C}_{\text{C4}}) \quad (4)$$

where,  $f_{\text{C4/C3}}$  were the fractions of SOC stocks derived from C4 and C3 sources, respectively,  $\delta^{13}\text{C}_{\text{SOC}}$  were the  $\delta^{13}\text{C}$  values of 1992 and 2014 soil samples, and  $\delta^{13}\text{C}_{\text{C4}}$  and  $\delta^{13}\text{C}_{\text{C3}}$  were the average  $\delta^{13}\text{C}$  values of C4 (-13‰) and C3 (-27‰) sources, respectively. The  $\delta^{13}\text{C}$  values used to discriminate C3 and C4 sources are typical for corn and wheat crops, respectively, and are within the range reported for the tallgrass prairie vegetation (-12 to -16‰) and organic amendments (-25.9 to -27.3‰) such as those used in this study (Dai et al., 2009; Das et al., 2010; Jessup et al., 2003; Lynch et al., 2006; Wang et al., 2015).

The C4 and C3-derived SOC stocks ( $\text{SOC}_{\text{C4/C3}}$ , Mg ha<sup>-1</sup>) were then calculated by multiplying SOC stocks (Mg ha<sup>-1</sup>) by  $f_{\text{C4/C3}}$  in the sample, as follows:

$$\text{SOC}_{\text{C4}} = \text{SOC} \times f_{\text{C4}} \quad (5)$$

$$\text{SOC}_{\text{C3}} = \text{SOC} \times f_{\text{C3}} \quad (6)$$

## 2.6. Statistical analysis

Repeated Measures Analysis of Variance (RM-ANOVA) was performed to assess differences in SOC stocks,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , C3- and C4-derived SOC stocks by using SAS PROC MIXED to consider the effects of blocks, soil tillage systems as the main plots, N sources as the subplots, time as sub-subplots and the interactions between the three last factors (SAS Institute Inc., Cary, NC, USA). We evaluated three covariance structure models for RM-ANOVA (type = UN@UN, UN@AR(1) and UN@CS) by using the Restrict Maximum Likelihood (REML) method and selecting the model with the lower Akaike information criterion (AIC) value (Littell et al., 2000). Analysis of variance (ANOVA) was also performed by using SAS PROC MIXED to assess differences in the mass and C content within WSA fractions considering the effects of blocks, soil tillage systems as the main plots and N sources as the subplots. All analyses were performed by soil depth. We used the LSMEANS statement with the protected *t*-test to assess the interactions between soil tillage systems and N sources within sampling years and the *F*-test to assess differences between sampling years for the interactions of soil tillage systems and N sources. All results were considered statistically

significant at  $p < 0.05$ . Regression analysis was performed to assess SOC dynamics on the interaction of soil tillage systems and N sources. For treatments receiving OF, we differentiated the regressions analysis in two periods (1992–2002 and 2002–2014) according to the organic N source used in each period (cattle manure and composted organic waste, respectively). We also used regression analysis to assess the correlations between C inputs and SOC stocks and between SOC stocks and C within WSA fractions by using SigmaPlot 12.5 (Systat Software, San Jose, CA). We employed linear ( $y = a + bx$ ), quadratic ( $y = a + bx + cx^2$ ), logarithmic with two or three parameters [ $y = a \ln(x - x_0)$  or  $y = y_0 + a \ln(x - x_0)$ ] and exponential [ $y = y_0 + a(1 - e^{-bx})$ ] regressions to assess the relationships between variables, where:  $y$  and  $x$  are the dependent and explanatory variables;  $y_0$  and  $x_0$  are the initial values for  $y$  and  $x$  variables;  $a$ ,  $b$ , and  $c$  are the regression coefficients;  $\ln$  is the natural logarithm of a given number and;  $e$  is the base of the natural logarithm. The F-test was used to assess the significance of the adjusted functions ( $p < 0.05$ ). The adjusted functions with greater adjusted  $R^2$  values were selected (Gulde et al., 2008).

### 3. Results

#### 3.1. SOC and TN dynamics

Soil organic C dynamics were significantly affected by both tillage and N sources in the 0–5, 5–15, and 15–30 cm soil layers (Fig. 1, Tables S1 and S2). The SOC remained stable during the evaluation period (25 yr) in the cultivated CT CO and CT MF treatments at 8.9 and 10.1 Mg C ha<sup>-1</sup>, respectively, in the 0–5 cm soil layer, and at 16.8 and 17.7 Mg C ha<sup>-1</sup> in the 5–15 cm soil layer (Fig. 1a/c). However, in the 15–30 cm soil layer it decreased by 0.25 and 0.24 Mg C ha<sup>-1</sup> yr<sup>-1</sup> (Fig. 1e). In the NT CO and NT MF treatments, SOC in the 0–5 cm layer increased by 42 and 44.9% (1992–2014) (Fig. 1a), but no changes in SOC were apparent in the 5–15 or 15–30 cm soil layers of either treatment (Fig. 1c/e).

The C inputs from organic N sources increased significantly with the change from cattle manure (2.6 Mg C ha<sup>-1</sup> yr<sup>-1</sup> from 1990 to 2001) to compost organic waste (5.2 Mg C ha<sup>-1</sup> yr<sup>-1</sup> from 2002 to 2014). We thus evaluated SOC dynamics in CT OF and NT OF treatments considering these two distinct periods (Fig. 1a/c/e). We observed significant SOC accumulation rates of 0.53, 0.61, and 0.16 Mg C ha<sup>-1</sup> yr<sup>-1</sup> in the 0–5, 5–15, and 15–30 cm soil layers, respectively, of the CT OF treatment during the first period (1992–2002). However, in the later period (2002–2014), SOC levels stabilized in the topsoil layer (0–5 cm) although a significant accumulation of SOC of 0.61 Mg C ha<sup>-1</sup> yr<sup>-1</sup> was observed in the underlying soil layer (5–15 cm). Soil organic C increased in the 0–5 cm soil layer of the NT OF treatment by 0.96 Mg C ha<sup>-1</sup> yr<sup>-1</sup> between 1992 and 2002, but no changes were noticed in the underlying 5–15 cm soil layer during the same period. In contrast, SOC in the 15–30 cm soil layer of NT OF decreased by 0.48 Mg C ha<sup>-1</sup> yr<sup>-1</sup> between 1992 and 2002. With the application of composted organic waste (2002–2014), SOC increased asymptotically in both 0–5 (19.4 to 31.3 Mg C ha<sup>-1</sup>) and 5–15 cm soil layers (19.3 to 34.6 Mg C ha<sup>-1</sup>) and linearly in the 15–30 cm soil layer (0.46 Mg C ha<sup>-1</sup> yr<sup>-1</sup>) in the NT OF treatment.

Soil TN dynamics generally followed the same pattern observed for SOC in the tillage and fertilization treatments (Fig. 1 b/d/f, Table S2). However, no changes in soil TN were apparent in the 0–5 cm soil layer of the NT CO and NT MF treatments that averaged 0.96 and 1.08 Mg N ha<sup>-1</sup> (1992–2014), respectively. The application of cattle manure increased soil TN in 0–5 and 5–15 cm soil layers of the CT OF treatment between 1992 and 2002 by 19 and 15 kg N ha<sup>-1</sup> yr<sup>-1</sup>, respectively (Fig. 1b/d). In the following period (2002–2014), soil TN stabilized at 1.50 Mg N ha<sup>-1</sup> in the 0–5 cm soil layer but increased by 123 kg N ha<sup>-1</sup> yr<sup>-1</sup> in the underlying 5–15 cm soil layer of CT OF soil. Soil TN increased by 72 kg N ha<sup>-1</sup> yr<sup>-1</sup> in the 0–5 cm soil layer of the NT OF treatment between 1992 and 2002, but no significant changes

were observed in the underlying soil layers during the same period. In the later period (2002–2014), soil TN increased asymptotically in the 0–5 cm soil layer (1.6 to 4.0 Mg N ha<sup>-1</sup>) and linearly in the underlying 5–15 cm soil layer (171 kg N ha<sup>-1</sup> yr<sup>-1</sup>) following the application of composted organic waste in the NT OF treatment.

#### 3.2. C inputs and SOC

We investigated the relationship between C inputs and SOC in both 0–5 and 0–15 cm by using regression analysis (Fig. 2, Table S3). The CT CO and CT MF treatments had no increase in SOC stocks in response to cumulative C inputs regardless sampling layer. Cumulative C inputs for the 0–5 cm soil layer in CT CO and CT MF treatments were 18 and 23 Mg C ha<sup>-1</sup> (1992–2014), averaging 0.8 and 1.3 Mg C ha<sup>-1</sup> yr<sup>-1</sup>, respectively. For the 0–15 cm soil layer, cumulative C inputs in the respective treatments were 36 and 57 Mg C ha<sup>-1</sup> during the same period, averaging 1.6 and 2.6 Mg C ha<sup>-1</sup> yr<sup>-1</sup>. In contrast, NT CO and NT MF treatments showed respective linear and quadratic responses of SOC stocks to cumulative C inputs for the 0–5 cm soil layer. Nonetheless, SOC in both treatments responded linearly to C inputs for the 0–15 cm soil layer. Cumulative C inputs for these treatments were 35 and 59 Mg C ha<sup>-1</sup> (averaging 1.6 and 2.7 Mg C ha<sup>-1</sup> yr<sup>-1</sup>, respectively). The relationship between cumulative C inputs and SOC in CT OF and NT OF treatments followed a distinct pattern according to sampling depth. Cumulative C inputs of 148 and 149 Mg C ha<sup>-1</sup> (stover + organic amendments) had linear relationships with SOC in the 0–15 cm soil layer of respective NT OF and CT treatments. However, cumulative C inputs (148 and 74 Mg C ha<sup>-1</sup> in NT OF and CT OF treatments, respectively) had quadratic relationships with SOC in the 0–5 cm soil layer of the same treatments.

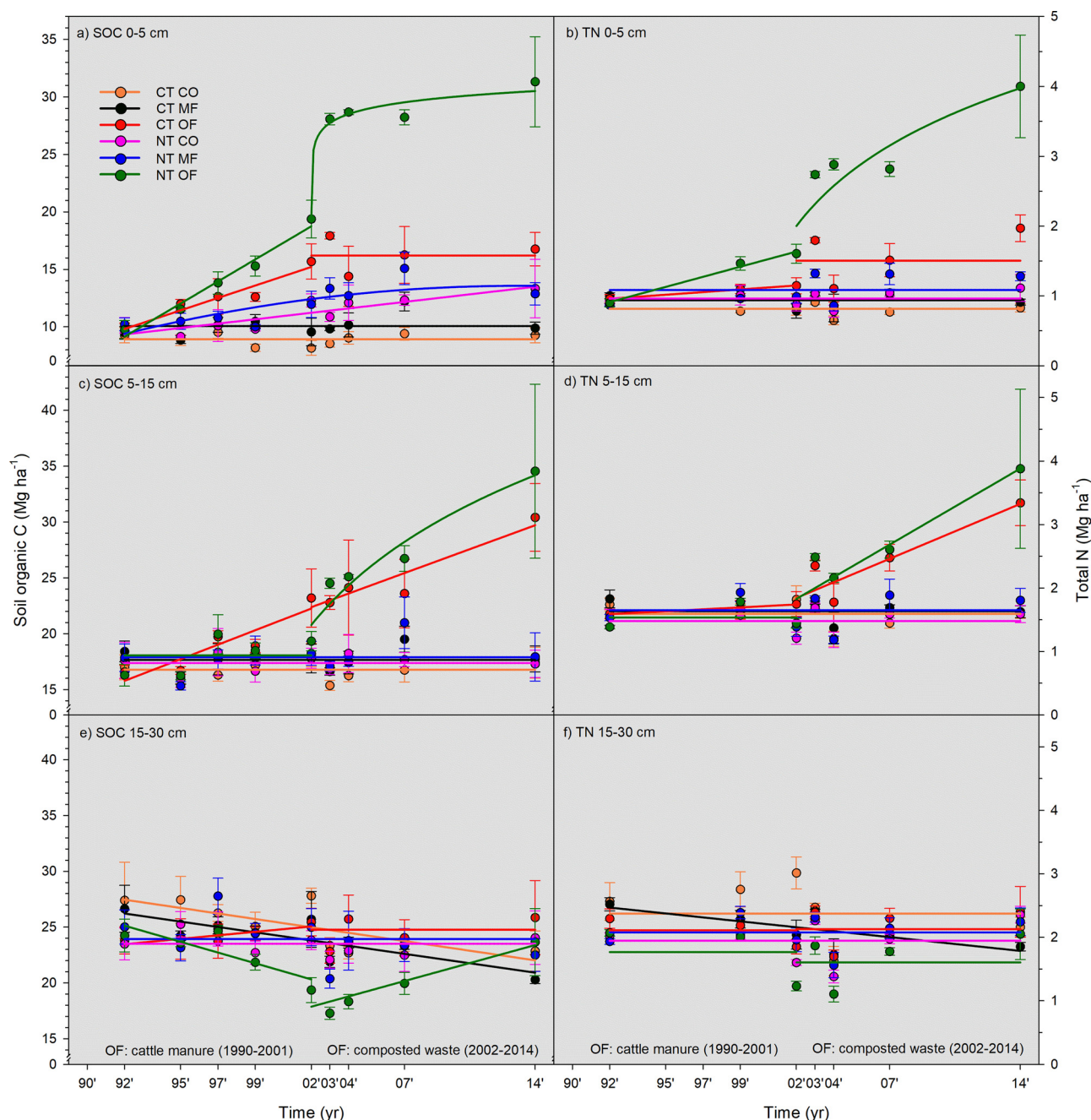
#### 3.3. SOC within soil aggregates

We assessed the soil aggregate size distribution and the C content within water-stable aggregates (WSA) in the 0–5 cm soil layer of the tillage and fertilization treatments in comparison with a nearby tallgrass prairie soil (Fig. 3, Table S4). The NT soils had higher proportion of LM than CT soils, averaging 30.0 compared with 10.1%. However, the proportion of LM in both tillage systems was had considerably lower than in the prairie soil (54.7%), which also had the highest SOC (43 Mg C ha<sup>-1</sup>). Organic amendments (OF) doubled the proportion of LM compared with the MF and CO treatments (30.7, 15.2, and 14.5%, respectively). The C content within the isolated WSA increased from the smaller to larger aggregate size fractions following the same hierarchy observed for aggregate size distribution (NT > CT and OF > MF ≈ CO; Fig. 3). However, despite the lower SOC in the NT OF treatment (28.2 Mg C ha<sup>-1</sup> in 2007) in comparison to the tallgrass prairie (43 Mg C ha<sup>-1</sup>), the C content within the WSA fractions isolated from both soils were similar (e.g. 5.5 and 5.4 g C 100 g<sup>-1</sup> sand-free WSA within LM fraction, respectively). Nonetheless, the C content within WSA fractions in the CT OF treatment was lower than in the other two soils (e.g., 3.6 g C 100 g<sup>-1</sup> sand-free WSA within LM fraction). The best fit for the relationship between SOC and the C content in the SM and LM microaggregates was an exponential expression (Fig. 3). For the silt + clay fraction, the C content increased linearly to 2.5 g C 100 g<sup>-1</sup> s + c fraction in the tallgrass prairie soil.

#### 3.4. Soil $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures

The SOC  $\delta^{13}C$  isotopic signatures of CT and NT plots in 1992 were  $-18.9$  and  $-19.1\text{‰}$  in the 0–5 cm soil layer, gradually enriching to  $-15.8$  to  $-16.5\text{‰}$  in the 15–30 cm soil layer, respectively (Fig. 4a/c). By 2014, the use of continuous corn (C4 plant) had enriched the  $\delta^{13}C$  signature in the CO and MF treatments, especially in the 0–5 and 5–15 cm soil layers where the  $\delta^{13}C$  ranged from  $-17.0$  to  $-17.7\text{‰}$  for both CT and NT soils. An enriched SOC  $\delta^{13}C$  signature, in comparison





**Fig. 1.** Soil organic C (a, c, e) and total N (b, d, f) dynamics in the 0–5 (a, b), 5–15 (c, d) and 15–30 cm (e, f) soil layers of a Hapludoll from central Kansas as affected by no-tillage (NT), chisel tillage (CT), control without nitrogen (CO), mineral fertilizer (MF), and organic fertilizer (OF) management practices. The vertical bars are the mean's standard errors (n = 4). The colored lines represent the best fit regressions as reported in Table S2. Arrows indicate the OF. Source: cattle manure (1990–2001) and composted organic waste (2002–2014).

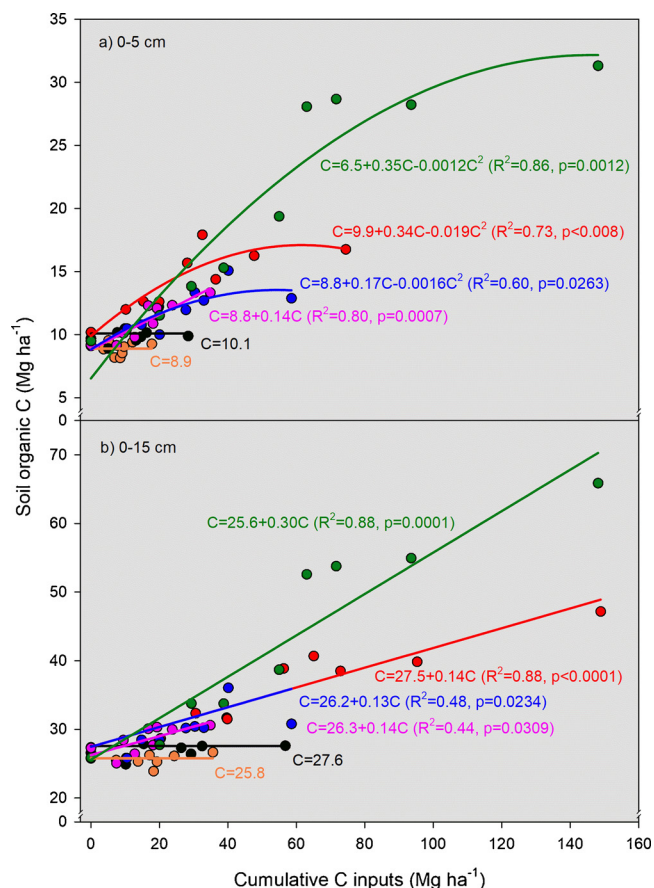
with NT in 1992, was also apparent in the 15–30 cm soil layer of the NT CO and NT MF treatments (–15.3 to –16.0‰). In contrast, the soil  $\delta^{13}\text{C}$  isotopic signatures of CT OF and NT OF treatments in 2014 were more depleted in relation to CT and NT soils in 1992. The  $\delta^{13}\text{C}$  ranged from –19.4 and –21.2‰ in the topsoil layer gradually enriching to –16.9 and –17.4‰ in the 15–30 cm soil layer for CT OF and NT OF treatments, respectively.

The  $\delta^{15}\text{N}$  of CT and NT plots as measured in 1992 ranged from 5.9 to 6.7‰ in the 0–30 cm soil layer (Fig. 4b/d). In 2014, the  $\delta^{15}\text{N}$  in all soil layers of both CT and NT soils with and without mineral N fertilizer was more depleted than in 1992. This effect was more pronounced in the 0–5 cm soil layer of both CT and NT soils without N fertilization where  $\delta^{15}\text{N}$  was depleted to 4.3 and 4.4‰, respectively. The

application of organic N sources significantly enriched the  $\delta^{15}\text{N}$  natural abundance of the 0–5 and 5–15 cm soil layers in both CT OF and NT OF plots following the soil TN accumulation in these treatments (Fig. 4b/d).

### 3.5. SOC derived from C3 and C4 sources

The fractions of SOC derived from C3 and C4 sources in 1992 and 2014 were calculated by using a two end-member mixing model (Fig. 5). The C3-derived SOC represented 40–46, 34–46, and 17–27% of total SOC (range for all treatments) in the 0–5, 5–15, and 15–30 cm soil layers in 1992, respectively. For CT CO and CT MF treatments, we observed a loss of C3-derived SOC (–2.2 and –1.9 Mg C ha<sup>-1</sup>,



**Fig. 2.** Relationship between soil organic C and cumulative C inputs in the (a) 0–5 and (b) 0–15 cm soil layers of a Hapludoll from central Kansas as affected by no-tillage (NT), chisel tillage (CT), control without nitrogen (CO), mineral fertilizer (MF), and organic fertilizer (OF) management practices. Each data point represents SOC and cumulative C inputs as measured in each sampling year within the same treatment. The colored lines represent the best fit regressions ( $p < 0.05$ ). Errors bars were omitted for clarity.

respectively) in the 0–5 and 5–15 cm soil layers from 1992 to 2014, which were compensated by a similar increase of C4-derived SOC (+2.7 and +1.6 Mg C ha<sup>-1</sup>, respectively). The 15–30 cm soil layer of CT CO and CT MF treatments had no significant changes of C3-derived SOC (+0.9 and +0.3 Mg C ha<sup>-1</sup>, respectively) but showed a significant loss of C4-derived SOC (-5.4 and -6.7 Mg C ha<sup>-1</sup>, respectively). We observed a similar pattern for C3- and C4-derived SOC dynamics for NT CO and NT MF treatments. Loss of C3-derived SOC (-3.4 and -3.2 Mg C ha<sup>-1</sup>) were offset by an increase of C4-derived SOC (+6.3 and +5.9 Mg C ha<sup>-1</sup>) in the 0–5 and 5–15 cm soil layers, respectively. In addition, the loss of C3-derived SOC in the 15–30 cm soil layer was compensated by an increase of C4-derived SOC, resulting in no changes in total SOC in the NT CO treatment. In contrast, SOC derived from C3 sources in CT OF treatment increased by 3.7 and 8.2 Mg C ha<sup>-1</sup> in the 0–5 and 5–15 cm soil layers, respectively (Fig. 5). The SOC derived from C4-sources also increased in the CT OF soil by 3.4 and 5.6 Mg C ha<sup>-1</sup> in the same soil layers. Similar of observed in CT soil, the NT OF treatment also showed increased C3- (14.6 and 11.7 Mg C ha<sup>-1</sup>) and C4-derived SOC (7.0 and 6.5 Mg C ha<sup>-1</sup>) in both 0–5 and 5–15 cm soil layers, respectively.

## 4. Discussion

### 4.1. SOC and TN dynamics

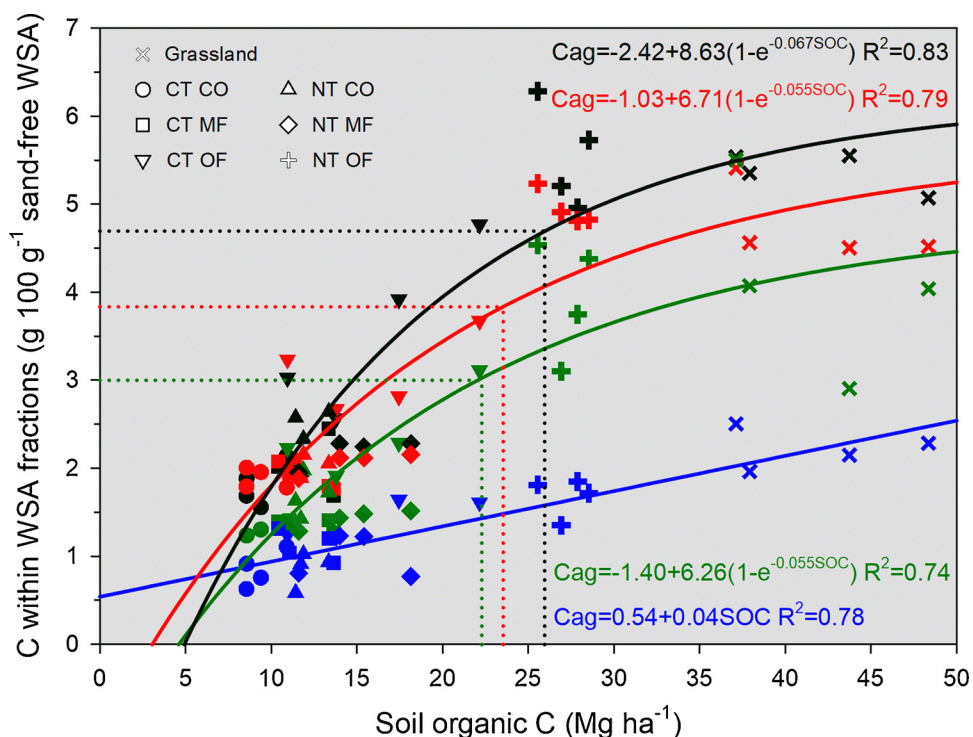
The long-term (60 yr.) cultivation of tallgrass prairie with wheat

and other small grains under CT decreased SOC in the 0–30 cm layer by 64% during the pre-experimental period (data not shown), in agreement with previous research (Sanderman et al., 2017). The introduction of continuous corn since 1990 did not promote any recovery of SOC in CT CO and CT MF treatments. In fact, SOC decreased in the 15–30 cm soil layer of the respective treatments. In contrast, SOC increased by 7.7% and 9.3%, respectively, from 1992 to 2014 in the 0–30 cm layer of NT CO and NT MF treatments. Nonetheless, SOC accrual in these treatments was limited to the surface soil layer (0–5 cm). Moreover, SOC accumulation in the 0–5 cm soil layer of the NT MF treatment had the best fit with a quadratic function (Table S2), indicating that SOC accrual rates in the surface soil layer were decreasing with time approaching new steady-state SOC levels. Further increase of SOC could be achieved either by improving crop fertilization, intensifying cropping systems or by using organic amendments as an external C source (de Oliveira Ferreira et al., 2013; Smith et al., 1997). The relative annual SOC accrual rate observed in the 0–30 cm soil layer of the NT CO and NT MF treatments (3.5 and 4.2‰, respectively) closely matches the goal of the “4 per Thousand” Initiative launched by France during COP 21 in order to halt the annual increase in CO<sub>2</sub> concentrations in the atmosphere and mitigate climate change (Lal, 2016).

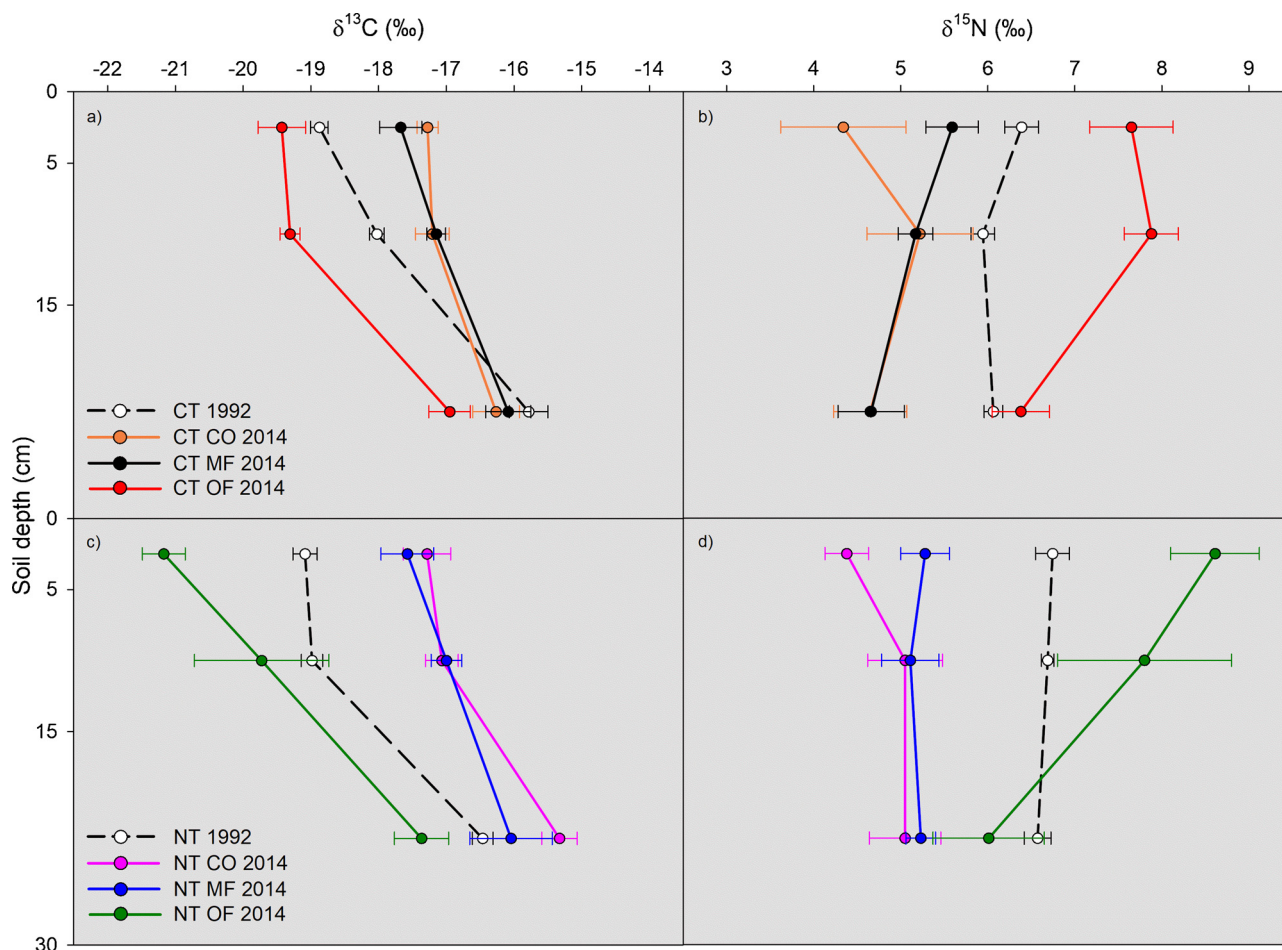
The application of external C (cattle manure) increased SOC accrual rates in both CT and NT soils (1992–2002). We observed significant SOC accrual in all 0–5, 5–15, and 15–30 cm soil layers of the CT OF treatment due to mixing of the crop residues cattle manure with chiseling and disking operations (Franzluebbers, 2002). In contrast, SOC accrual was limited to the 0–5 cm soil layer in the NT OF treatment. Moreover, SOC in the 15–30 cm soil layer of NT OF treatment decreased by 0.48 Mg C ha<sup>-1</sup> yr<sup>-1</sup> in the same period. The depletion of SOC in the 15–30 cm layer of the NT OF treatment in the first period (1992–2001) was twice that in the CT CO and CT MF treatments. Previous studies have reported increased SOC in the soil surface and decreased SOC in deeper layers of NT soil for review, see (Angers and Eriksen-Hamel, 2008; Luo et al., 2010). Depletion of SOC in the 15–30 cm soil layer of the CT CO, CT MF, and NT OF treatments (1992–2001) was probably related to the lack of direct C inputs by corn roots necessary to maintain the original SOC in this soil layer (Franzluebbers, 2002). Although we did not assess maize root biomass in our study, MCGowan (2015) reported that 82, 7 and 3% of corn root biomass measured to a depth of 120 cm were found in the 0–5, 5–15, and 15–30 cm layers of NT plots carried out nearby our experimental site. The concentration of the crop root system in the soil surface has already been reported to limit C input to deeper soil layers thus impacting SOC (Baker et al., 2007).

The substitution of cattle manure for composted organic waste in the later period (2002–2014) had no effect on SOC in the topsoil layer (0–5 cm) of the CT OF treatment. Nonetheless, SOC increased by 0.61 Mg C ha<sup>-1</sup> yr<sup>-1</sup> in the underlying soil layer (5–15 cm) of the CT OF treatment. For the NT OF treatment, SOC increased asymptotically in both 0–5 and 5–15 cm soil layers and linearly in the 15–30 cm soil layer during the same period. The higher SOC accumulation with the application of composted organic waste was attributed to the increased external C inputs in comparison with cattle manure. Additionally, composted organic waste has a higher proportion of humic and fulvic acids that are known to be thermodynamically resistant to decomposition (Huang et al., 2006), promoting slower decomposition rates (Grave et al., 2015) and prolonged soil storage than noncomposted organic sources (Lynch et al., 2006). The application of composted organic sources was shown to yield a greater C recovery as SOC (up to 95% depending on composted source) than noncomposted manure [for review, see (Biala, 2011)]. Although biochemical recalcitrance was recently found to have a weak relationship with long-term SOC stabilization (Kleber et al., 2011; Lehmann and Kleber, 2015) it was reported to play a crucial role in early residue decomposition in the soil (Schmatz et al., 2017). Larger accumulation of compost-added C as SOC is indeed expected in the first years of composted fertilizer amendment (Lynch et al., 2006), thus helping to explain the sharp increase of SOC observed





**Fig. 3.** Relationship between soil organic C and the C content within sand-free WSA fractions in the 0–5 cm soil layer of a Hapludoll from central Kansas in 2007 as affected by no-tillage (NT), chisel tillage (CT), control without nitrogen (CO), mineral fertilizer (MF), and organic fertilizer (OF) management practices. A nearby native prairie was included for comparison (Table S1). The colored lines are the best fitted regressions for silt + clay (blue), microaggregates (green), small macroaggregates (red), and large macroaggregates (black). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 4.** Soil  $\delta^{13}\text{C}$  (a, c)  $\delta^{15}\text{N}$  (b, d) isotopic signatures and in the 0–30 cm soil layer of a Hapludoll from central Kansas in 1992 and 2014 as affected by (a, b) chisel tillage (CT), (c, d) no-tillage (NT), control without nitrogen (CO), mineral fertilizer (MF), and organic fertilizer (OF) management practices. The horizontal bars represent the mean's standard errors ( $n = 4$ ).

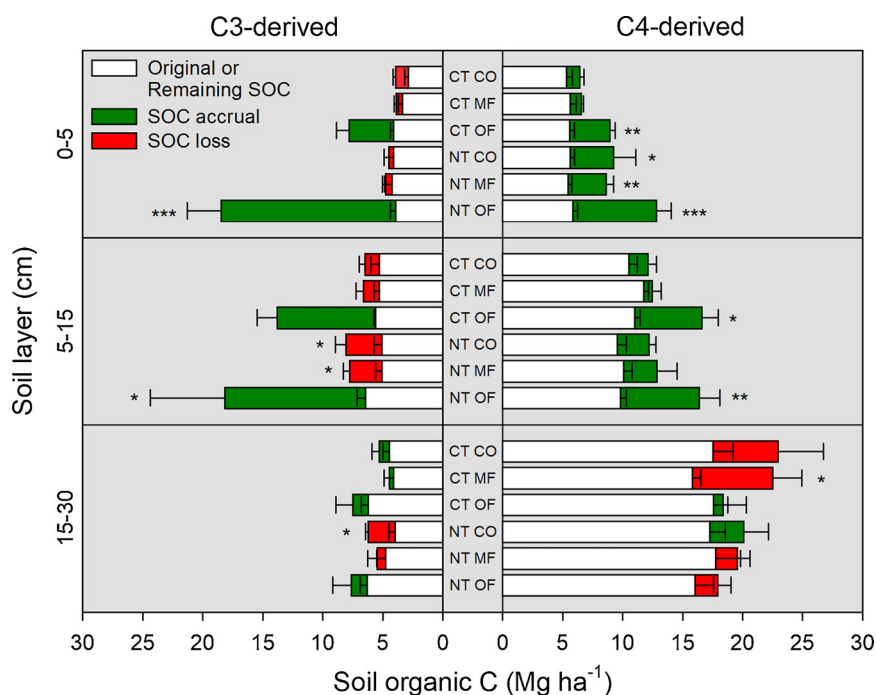


Fig. 5. Soil organic carbon derived from C3 and C4 sources in the 0–30 cm soil layer of a Hapludoll from central Kansas in 1992 and 2014 as affected by chisel tillage (CT), no-tillage (NT), control without nitrogen (CO), mineral fertilizer (MF), and organic fertilizer (OF) management practices. The error bars are the mean's standard errors ( $n = 4$ ). The symbols \*, \*\*, and \*\*\* indicate the statistical significance levels (0.05, 0.01, and 0.001, respectively) for the comparison of the lost (red) and recovered (green) C3- and C4-derived SOC between 1992 and 2014. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in the 0–5 cm soil layer within the first years of compost applications in the NT OF treatment.

Overall, SOC in the 0–5 and 5–15 cm soil layers of the NT OF treatment increased by 228 and 91%, respectively, between 1992 and 2014. Compost amendments (2002–2014) reversed previous SOC losses in the 15–30 cm layer of the NT OF treatment, recovering to 93% of the soil SOC measured in 1992. Nonetheless, the relative annual SOC accretion rate observed in the 0–30 cm soil layer of CT OF and NT OF treatments (17.8 and 30.7%, respectively) were much greater than the French's "4 per Thousand" Initiative (Lal, 2016), but were similar to observations from the long-term experiments at Rothamsted in plots under organic amendments (Poulton et al., 2018). Although there is still discussion about whether SOC accretion promoted by external C sources should be considered C sequestration (Olson et al., 2014), the benefits of increasing SOC through organic amendments regarding soil health and productivity are unquestionable.

The stabilization of SOC at 16.2 and 30.5 Mg C ha<sup>-1</sup> in the respective 0–5 cm soil layers of the CT OF and NT OF treatments suggests that new steady-state SOC levels were achieved in these soil layers. Similarly, soil TN also achieved a new steady-state level at 1.5 Mg N ha<sup>-1</sup> in the 0–5 cm soil layer of CT OF treatment (Fig. 1b, Table S2). However, a new steady-state N level for the NT OF treatment was not clearly identifiable in the same soil layer. Nonetheless, maximum soil TN storage was found to be constrained by SOC and a minimum soil C:N ratio (Schipper et al., 2004). An assessment of SOC and TN distribution in the 0–30 cm layer of soils of the world found a minimum C:N ratio of 8.9, while the majority of soils had C:N ratios higher than 10 (Batjes, 1996). Organic compounds with lower C:N ratios were found to be unstable in the soil and rapidly mineralized by soil microbes (Amato and Ladd, 1988; Kassem and Diethelm, 1987). Thus, considering the steady-state SOC levels measured in the CT OF and NT OF treatments and a minimum C:N ratio of 9 as indicative of maximum N storage (Schipper et al., 2004), soil N would achieve steady-state levels at 1.8 and 3.4 Mg N ha<sup>-1</sup> for the same treatments, respectively. These values are within the standard error of the soil N as measured in the 0–5 cm layer of CT OF and NT OF treatments in 2014, respectively.

#### 4.2. SOC saturation

Organic amendments are a well-known strategy to recover SOC (Biala, 2011; Jarecki and Lal, 2003; Powlson et al., 2012; Smith et al., 1997; Xia et al., 2017). Continuous application of cattle manure was reported to increase C content in both labile or more stable SOC pools, promoting the saturation of SOC stabilization mechanisms (Gulde et al., 2008). The increase and the subsequent stabilization of SOC in the 0–5 cm soil layer of CT OF and NT OF treatments suggests the same phenomenon (Fig. 1a). Soil organic C saturation is typically identified by an asymptotic response of SOC to C inputs at steady-state (Six et al., 2002; Stewart et al., 2007). When soils are close to SOC saturation levels, soil aggregates and mineral surfaces lose efficiency in protecting the newly added C from microbial activity, thus limiting the soil's capacity to accumulate SOC in physically-protected forms (Six et al., 2002, 2000; Stewart et al., 2008, 2007).

We investigated the relationship between cumulative C inputs and measured SOC stocks in both 0–5 and 0–15 cm soil layers in order to identify this process. The linear relationships between cumulative C inputs and SOC in the 0–15 cm soil layer do not provide evidence of SOC saturation for any treatments tested in our study. For the 0–5 cm soil layer, there was no increase of SOC in the CT CO and CT MF treatments in response to cumulative C inputs. In contrast, the SOC increased linearly in response to cumulative C inputs in the same soil layer of the NT CO treatment. For NT MF, CT OF and NT OF treatments, we observed a quadratic relationship between SOC and cumulative C inputs for the 0–5 cm soil layer, thus confirming that all treatments were approaching new steady-state SOC levels in the soil surface layer. However, soils can achieve steady-state levels either when SOC accumulation is limited by C inputs or when soils achieve their effective SOC stabilization capacity thus indicating SOC saturation (Stewart et al., 2007). When SOC reaches saturation levels, any increase in C inputs can only promote SOC accumulation within unprotected SOC pools (Gulde et al., 2008; Six et al., 2002). Nonetheless, further SOC accumulation in the 5–15 cm layer of CT OF and NT OF treatments after both treatments had achieved steady-state levels in the 0–5 cm soil layer, suggest that C inputs were not limiting SOC accumulation in the soil surface layer. Although these results suggest that the effective stabilization capacity of CT and NT soils was achieved in the surface soil layer of the CT OF



and NT OF treatments, further evidence demonstrating the saturation of SOC stabilization mechanisms is necessary to support this hypothesis. Thus, we assessed the soil C content within water-stable aggregates (WSA) in the 0–5 cm soil layer to look for evidences of saturation in physically-protected SOC pools.

Previous studies proposed that macroaggregate turnover (allowing the formation of stable microaggregates within macroaggregates) plays a crucial role in SOC stabilization in agroecosystems (Deneff et al., 2001; Six et al., 2004, 2000). Studies also reported that SOC saturation follows a hierarchy from smaller to larger aggregate size fractions (Gulde et al., 2008; Stewart et al., 2008). Thus, we assumed that the saturation of soil LM would be indicative of the biophysical protection capacity as a SOC stabilization mechanism. Therefore, our assessment focused on the isolated 4–8 mm soil fraction that would have a higher proportion of LM than the whole soil. For this assessment, samples were collected from the 0–5 cm soil layer in 2007 when the first evidence for SOC saturation was noticed in this experiment. We also included a nearby tallgrass prairie from the same soil type as a reference for comparison (Fabrizzi et al., 2009). As expected, SOC in the tallgrass prairie soil was higher (43 Mg C ha<sup>-1</sup> in the 0–5 cm soil layer) than in the investigated long-term experiment. We assumed that the reference soil would have the maximum amount of LM (> 2000 μm), since the soil was not disturbed and C inputs were significant and constant by the perennial tallgrass vegetation.

Decreased disturbance in the NT soils compared with CT soils and increased C inputs by OF promoted higher aggregate stability and increased C storage in larger aggregate size fractions. These results agree with previous findings from this experimental site and elsewhere (Fabrizzi et al., 2009; Gulde et al., 2008; Mikha and Rice, 2004; Stewart et al., 2008). The LM, SM and also the microaggregates (53–250 μm) fractions showed saturation behaviors as indicated by the fitted exponential regressions between SOC and C content within each WSA fraction (Fig. 3). By calculating the first derivative of the exponential functions and assuming  $dy/dx < 0.1$  as indicative of the lower limit of the SOC saturation levels, we estimated that the LM, SM and microaggregates fractions would reach saturation at 4.7, 3.8, and 3.0 g C 100 g<sup>-1</sup> of the respective sand-free WSA fractions isolated from the 4–8 mm sieved soil. Those levels were achieved with SOC at 25.9, 23.5, and 22.3 Mg C ha<sup>-1</sup> for the same WSA fractions, respectively, following the same saturation hierarchy from smaller to larger aggregate size fractions previously reported (Gulde et al., 2008; Stewart et al., 2008). These results indicate that only WSA from the tallgrass prairie and NT OF soils could be considered C saturated, since the C content within the LM, SM and microaggregates fractions were higher than the estimated WSA saturation levels. Nonetheless, the C content within the silt + clay fraction followed a linear pattern increasing to 2.5 g C 100 g<sup>-1</sup> in the tallgrass prairie soil. Although the saturation levels and maximum SOC storage capacity of the silt + clay fraction could not be determined in this assessment, SOC storage within silt + clay fraction in the tallgrass prairie soil was lower than the maximum SOC storage capacity predicted for 90% silt + clay soils in other studies [i.e. 2.8 to 3.7 g 100 g<sup>-1</sup> silt + clay (Carter et al., 2003; Hassink, 1997; Six et al., 2002)]. However, results could not be compared directly due to differences in the methodology used in this study.

Nonetheless, this study gathered evidence (i.e., increasing SOC in the 5–15 cm after the stabilization of the surface soil layer, non-linear response of SOC stocks to C inputs and C content within WSA fractions) suggesting that the NT OF treatment achieved the effective SOC stabilization capacity of the surface layer of NT soil (Gulde et al., 2008; Stewart et al., 2008). Additional increase in SOC stocks in the 0–5 cm soil layer of NT OF treatment would have resulted either from the accumulation of unprotected SOC fractions (Gulde et al., 2008) or by increasing the amount of LM in NT soil, since further increase of C content is unlikely to occur within saturated WSA fractions. In contrast, SOC accrual in the CT OF and NT MF treatment was not limited by saturation, since the C content within WSA were below SOC saturation

levels determined for this soil. Thus, SOC accumulation in NT MF treatment was only limited by the lower C inputs from crop residues in comparison with NT OF treatment receiving external C sources. These results also support the notion that CT limited the capacity of the soil to accumulate SOC by disrupting LM and decreasing SOC stabilization in more stable aggregate size fractions, resulting in faster LM turnover, lower C recovery and higher SOC mineralization (Nicoloso et al., 2016; Six et al., 2004, 2002).

#### 4.3. SOC and TN translocation

Our results also suggest that saturation of the surface soil layer of NT soils did not impair SOC or TN accrual as previously believed. No changes in SOC or TN were noticed in the 5–15 cm soil layer of NT OF treatment before 2002 when SOC in the topsoil layer was 60% of the SOC saturation level that was observed in the later period. However, after SOC approached saturation levels in the 0–5 cm soil layer, a significant increase of both SOC and TN were noticed in the underlying layer. We investigated the sources for this significant SOC and TN accumulation by comparing the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  natural abundance signature in the soil samples collected in 1992 and 2014 (Fig. 4).

The introduction of continuous corn (C4 plant) enriched the soil  $\delta^{13}\text{C}$  signature of the CO and MF treatments in both CT and NT soils. In contrast, the soil  $\delta^{13}\text{C}$  isotopic signatures of CT OF and NT OF treatments in 2014 were more depleted in relation to 1992. This would indicate that OF sources used in this study were derived from C3 vegetation being more depleted in  $\delta^{13}\text{C}$  than SOC and corn residues. Cattle manure (from animals fed with C3 forage: e.g. alfalfa hay) and composted organic waste from different sources have been reported to have a  $\delta^{13}\text{C}$  ranging from -25.9 to -27.3 (Dai et al., 2009; Lynch et al., 2006). The composting process is also known to deplete the  $\delta^{13}\text{C}$  signature of organic residues (Lynch et al., 2006) since C4 sources decompose faster than C3 sources (Wynn and Bird, 2007).

Soil organic C derived from C3 sources decreased in CT and NT soils where corn residues were the only C source returned to the soil in CO and MF treatments. The same treatments showed an increase in C4-derived SOC which overcame (NT) or partially compensated (CT) losses in C3-derived SOC. In contrast, both C4- and C3-derived SOC increased in the 0–5 and 5–15 cm soil layer of CT OF treatment. The accumulation of both C4- and C3-derived SOC in the 0–5 and 5–15 cm soil layers of CT OF treatment was already expected due to incorporation of both corn residue (C4 source) and organic fertilizers (C3 source) into the soil with chisel and disking operations (Franzluebbers, 2002). Similarly, the accumulation of C4-derived SOC in NT OF treatment could be attributed to direct C inputs from corn stover (0–5) and roots (0–5 and 5–15 cm) as observed in the other NT treatments. However, the NT OF treatment also had an increase of C3-derived SOC in both 0–5 and 5–15 cm soil layers. The accumulation of C3-derived SOC in the 5–15 cm soil layer of the NT OF treatment provides evidence that SOC derived from organic fertilizers (C3 source), which were applied at soil surface, was translocated from the saturated topsoil layer to the underlying soil layer. Thus, considering that long-term SOC and TN dynamics were coupled in both CT and NT soils (Fig. 1) we further investigated soil  $\delta^{15}\text{N}$  isotopic signature to gather more evidences of the proposed SOC translocation process.

The CO and MF treatments were more depleted in  $\delta^{15}\text{N}$  in 2014 than 1992, regardless of sampling layer and soil tillage. Depletion of soil  $\delta^{15}\text{N}$  isotopic signature would be expected following the application of  $^{15}\text{N}$  depleted mineral fertilizers such as urea and ammonium nitrate [ $\delta^{15}\text{N} = -0.2 \pm 2.1$  to  $-1.5 \pm 0.5\text{‰}$  (Bateman and Kelly, 2007; Lim et al., 2010)] or deposition of atmospheric N [ $\delta^{15}\text{N} = -3$  to  $+3\text{‰}$  (Boutton and Liao, 2010)]. In contrast, the application of organic N sources significantly enriched the  $\delta^{15}\text{N}$  natural abundance of the 0–5 and 5–15 cm soil layers in both CT and NT soils. Organic N sources are known to have significantly higher  $\delta^{15}\text{N}$  values (3.5 to 20.9‰) than mineral fertilizers due to  $^{15}\text{N}$ -depleted N losses during manure storage

or composting process (Angnes et al., 2013; Bateman and Kelly, 2007; Lim et al., 2010). Thus, the application of organic N sources was expected to increase soil  $\delta^{15}\text{N}$  natural abundance as reported elsewhere (Chen et al., 2011). Nonetheless, the enriched  $\delta^{15}\text{N}$  natural abundance of the 5–15 cm soil layer in the NT OF treatment, where organic fertilizers were applied on the soil surface, also suggests translocation of TN from the 0–5 to the underlying soil layer. Thus, both SOC and TN dynamics and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic signatures were independent evidences of the occurrence of both SOC and TN translocation from SOC/TN-saturated to SOC/TN-depleted soil layers.

Translocation of SOC is a process observed in many soil orders, characteristic of Spodosols (Soil Survey Staff, 1999), but also reported in Andisols, Inceptisols, Entisols, and Oxisols under forest ecosystems (Fujii et al., 2011). Soluble C translocation rates between organic (O) and mineral (A) horizons in soils under temperate forests range from 0.11 to 0.36 kg C ha<sup>-1</sup> yr<sup>-1</sup> in Spodosols, 0.28 kg C ha<sup>-1</sup> yr<sup>-1</sup> in Oxisols, 0.05 to 0.48 kg C ha<sup>-1</sup> yr<sup>-1</sup> in Andisols, 0.15 to 0.40 kg C ha<sup>-1</sup> yr<sup>-1</sup> in Inceptisols (Fujii et al., 2011). In tropical forests, as much as 0.96 kg C ha<sup>-1</sup> yr<sup>-1</sup> could be translocated in Entisols (Fujii et al., 2011). The observation of such significant SOC translocation in natural forest ecosystems supports the notion that SOC saturation would limit but not impair SOC accumulation in those soils.

Translocation of soluble C between mineral soil layers is more pronounced in soils with low Fe- and Al-oxides content where soluble C is less adsorbed by soil colloids (Fujii et al., 2011), such as the Mollisol in our study. Higher soil porosity (Da Silveira Nicoloso et al., 2008) and the better architecture, continuity and stability of soil pores (Williams and Weil, 2004) may contribute to soluble C translocation between soil layers. Soil organic C mineralization rates was also suggested to increase as SOC approach saturation levels (Nicoloso et al., 2016). The products of the partial degradation of SOC are more easily desorbed from mineral surfaces, being dissolved in water and translocated through the soil profile until stabilized in C-depleted subsoil layers (Kaiser and Kalbitz, 2012). Newly-added C was also reported to be strongly adsorbed to soil minerals displacing already stabilized and older SOC, which then could migrate to underlying soil layers (Kaiser and Kalbitz, 2012). Although translocation of soluble C would also be expected in C-depleted agricultural soils, significant SOC accumulation as promoted by this process was until now only recognized in soils presenting a pedogenic organic horizon (Kaiser and Kalbitz, 2012) or mineral soils under natural forest ecosystem (Fujii et al., 2011).

Another possible mechanism for the SOC translocation observed in the NT OF treatment was bioturbation (Gabet et al., 2003; Jiménez and Lal, 2006). Previous studies reported that bioturbation enhanced the movement of both soluble and particulate SOC in forests and also in agricultural soils under minimum disturbance and organic amendments (Butenschoen et al., 2007; Gabet et al., 2003; Jiménez and Lal, 2006; Tonnejck and Jongmans, 2008). However, movement of SOC due to bioturbation would be expected to occur since the beginning of the our experiment, regardless of SOC saturation (Butenschoen et al., 2007). However, no SOC or TN accrual was observed in the 5–15 cm soil layer of NT soils prior to the saturation of the surface soil layer in the NT OF treatment. Thus, although bioturbation may contribute to SOC translocation, it was likely not the dominant mechanism. Further research assessing the accumulation of coarse C and N fractions in the 5–15 cm soil layer, such as particulate organic carbon (POC) and nitrogen (PON), as well as other markers indicative of soil fauna activity would provide evidence regarding the importance of bioturbation for the translocation of SOC from the saturated topsoil layer.

Although the mechanisms regulating SOC translocation still needs to be verified by further research, this process clearly increases the potential of agricultural soils to serve as a larger C sink in both quantity and time than previously thought (Lal, 2004; West and Post, 2002). Moreover, SOC accrual in deeper soil layers maybe more stable and resilient to changes in agricultural practices and climate conditions that may favor SOC losses in topsoil layers (Fontaine et al., 2007).

Nonetheless, to our knowledge, the results presented in this study are the first evidence of significant SOC accrual that can be attributed to the translocation of SOC between saturated and depleted soil layers in agricultural mineral soils. The pioneering of these results relies on the fact that SOC saturation is a recent research topic, and few studies have been able to assess this process in agricultural soils (Chung et al., 2008; Gulde et al., 2008; Hassink, 1997; Six et al., 2002; Stewart et al., 2008, 2007). Moreover, no-till experiments are relatively recent (< 50 yr.) and generally evaluate systems without high C inputs (i.e., organic N sources) to drive soil C towards saturation. We were able to identify both SOC saturation and translocation processes by assessing SOC and TN dynamics in a long-term experiment with contrasting soil tillage and fertilization practices using high temporal (9 samplings in 25 years) and depth sampling resolution. Although one-time sampling of paired CT and NT plots may provide relevant information regarding SOC and N storage in agricultural soils, this approach lacks of crucial piece of information necessary for the assessment of SOC and TN accrual, saturation and translocation processes. Thus, we should intensify our studies efforts in long-term experiments from different regions of the world in order to improve our knowledge on the potential of agricultural soils for SOC sequestration and climate change mitigation.

## 5. Conclusions

The use of organic amendments over 25 years increased SOC in the 0–5 cm layer of a prairie soil (Hapludoll) from central Kansas by up to 65 and 228% under CT and NT, respectively. The quadratic relationship between SOC and cumulative C inputs suggests that saturation limits SOC accumulation in both CT OF and NT OF treatments. However, C content within aggregate size fractions reached saturation levels only in the NT OF treatment, suggesting that SOC accumulation in CT soil was limited by faster turnover due to chiseling and disking operations. Thus, no evidence of saturation in the surface soil layer was observed in CT soils. Significant SOC accumulation was observed in the underlying soil layer (5–15 cm) after the saturation of the topsoil layer in the NT OF treatment. Partitioning of SOC through the assessment of  $\delta^{13}\text{C}$  natural abundance demonstrated that SOC accrual in the 5–15 cm soil layer was at least partially derived from the translocation of C derived from organic fertilizers applied to the soil surface in NT soil. Soil TN dynamics and the  $\delta^{15}\text{N}$  isotopic signature followed the same pattern, providing further evidence for SOC and TN translocation. Further research is needed to elucidate the mechanisms regulating SOC translocation between saturated and depleted layers in agricultural soils. Nonetheless, this process clearly increases the potential of agricultural soils managed under NT to serve as a C sink than previously thought.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.agee.2018.05.016>.

## References

Amato, M., Ladd, J.N., 1988. Assay for microbial biomass based on ninhydrin-reactive



- nitrogen in extracts of fumigated soils. *Soil Biol. Biochem.* 20, 107–114.
- Angers, D.A., Eriksen-Hamel, N.S., 2008. Full-inversion tillage and organic carbon distribution in soil profiles: a meta-analysis. *Soil Sci. Soc. Am. J.* 72, 1370. <http://dx.doi.org/10.2136/sssaj2007.0342>.
- Anges, G., Nicoloso, R.S., da Silva, M.L.B., de Oliveira, P.A., V Higarashi, M.M., Mezzari, M.P., Miller, P.R.M., 2013. Correlating denitrifying catabolic genes with N<sub>2</sub>O and N<sub>2</sub> emissions from swine slurry composting. *Bioresour. Technol.* 140, 368–375. <http://dx.doi.org/10.1016/j.biortech.2013.04.112>.
- Baker, J.M., Ochsner, T.E., Venterea, R.T., Griffis, T.J., 2007. Tillage and soil carbon sequestration—What do we really know? *Agric. Ecosyst. Environ.* 118, 1–5. <http://dx.doi.org/10.1016/j.agee.2006.05.014>.
- Balesdent, J., Mariotti, A., Guillet, B., 1987. Natural <sup>13</sup>C abundance as a tracer for studies of soil organic matter dynamics. *Soil Biol. Biochem.* 19, 25–30. [http://dx.doi.org/10.1016/0038-0717\(87\)90120-9](http://dx.doi.org/10.1016/0038-0717(87)90120-9).
- Bateman, A.S., Kelly, S.D., 2007. Fertilizer nitrogen isotope signatures. *Isotopes Environ. Health Stud.* 43, 237–247. <http://dx.doi.org/10.1080/10256010701550732>.
- Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. *Eur. J. Soil Sci.* 47, 151–163. <http://dx.doi.org/10.1111/ejss.12120>.
- Bayer, C., Lovato, T., Dieckow, J., Zanatta, J.A., Mielniczuk, J., 2006a. A method for estimating coefficients of soil organic matter dynamics based on long-term experiments. *Soil Tillage Res.* 91, 217–226. <http://dx.doi.org/10.1016/j.still.2005.12.006>.
- Bayer, C., Martin-Neto, L., Mielniczuk, J., Pavinato, A., Dieckow, J., 2006b. Carbon sequestration in two Brazilian Cerrado soils under no-till. *Soil Tillage Res.* 86, 237–245. <http://dx.doi.org/10.1016/j.still.2005.02.023>.
- Biala, J., 2011. Short Report: The Benefits of Using Compost for Mitigating Climate Change. *Org. Force* 42.
- Boutton, T.W., Liao, J.D., 2010. Changes in soil nitrogen storage and  $\delta^{15}\text{N}$  with woody plant encroachment in a subtropical savanna parkland landscape. *J. Geophys. Res. Biogeosci.* 115, 1–10. <http://dx.doi.org/10.1029/2009JG001184>.
- Butenschon, O., Poll, C., Langel, R., Kandeler, E., Marhan, S., Scheu, S., 2007. Endogeic earthworms alter carbon translocation by fungi at the soil–litter interface. *Soil Biol. Biochem.* 39, 2854–2864. <http://dx.doi.org/10.1016/j.soilbio.2007.05.028>.
- Carter, M.R., Angers, D.A., Gregorich, E.G., Bolinder, M.A., 2003. Characterizing Organic Matter Retention for Surface Soils in Eastern Canada Using Density and Particle Size Fractions 6.
- Chen, R., Hu, J., Dittert, K., Wang, J., Zhang, J., Lin, X., 2011. Soil total nitrogen and natural <sup>15</sup>Nitrogen in response to long-term fertilizer management of a maize-wheat cropping system in northern China. *Commun. Soil Sci. Plant Anal.* 42, 322–331. <http://dx.doi.org/10.1080/00106324.2011.538883>.
- Christensen, B.T., 1996. Carbon in primary and secondary organomineral complexes. In: Carter, M.R., Stewart, B.A. (Eds.), *Advances in Soil Science – Structure and Organic Matter Storage in Agricultural Soils*. CRC Lewis Publishers, Boca Raton, pp. 97–165.
- Chung, H., Grove, J.H., Six, J., 2008. Indications for soil carbon saturation in a temperate agroecosystem. *Soil Sci. Soc. Am. J.* 72, 1132. <http://dx.doi.org/10.2136/sssaj2007.0265>.
- Corbeels, M., Marchão, R.L., Neto, M.S., Ferreira, E.G., Madari, B.E., Scopel, E., Brito, O.R., 2016. Evidence of limited carbon sequestration in soils under no-tillage systems in the Cerrado of Brazil. *Sci. Rep.* 6, 21450. <http://dx.doi.org/10.1038/srep21450>.
- Da Silveira Nicoloso, R., Amado, T.J.C., Schneider, S., LanzaNova, M.E., Girardello, V.C., Braganolo, J., 2008. Eficiência da escarificação mecânica e biológica na melhoria dos atributos físicos de um latossolo muito argiloso e no incremento do rendimento de soja. *Rev. Bras. Cienc. do Solo* 32, 1723–1734. <http://dx.doi.org/10.1590/S0100-06832008000400037>.
- Dai, X., Vietor, D.M., Hons, F.M., Provin, T.L., White, R.H., Boutton, T.W., Munster, C.L., 2009. Effect of composted biosolids on soil organic carbon storage during establishment of transplanted sod. *HortScience* 44, 503–507.
- Das, O., Wang, Y., Hsieh, Y.P., 2010. Chemical and carbon isotopic characteristics of ash and smoke derived from burning of C3 and C4 grasses. *Org. Geochem.* 41, 263–269. <http://dx.doi.org/10.1016/j.orggeochem.2009.11.001>.
- Denef, K., Six, J., Paustian, K., Merckx, R., 2001. Importance of macroaggregate dynamics in controlling soil carbon stabilization: short-term effects of physical disturbance induced by dry-wet cycles. *Soil Biol. Biochem.* 33, 2145–2153. [http://dx.doi.org/10.1016/S0038-0717\(01\)00153-5](http://dx.doi.org/10.1016/S0038-0717(01)00153-5).
- Dimassi, B., Mary, B., Wylleman, R., Labreuche, J., Couture, D., Piroux, F., Cohan, J.P., 2014. Long-term effect of contrasted tillage and crop management on soil carbon dynamics during 41 years. *Agric. Ecosyst. Environ.* 188, 134–146. <http://dx.doi.org/10.1016/j.agee.2014.02.014>.
- Elliott, E.T., Palm, C.A., Reuss, D.E., Monz, C.A., 1991. Organic matter contained in soil aggregates from a tropical chronosequence: correction for sand and light fraction. *Agric. Ecosyst. Environ.* 34, 443–451. [http://dx.doi.org/10.1016/0167-8809\(91\)90127-J](http://dx.doi.org/10.1016/0167-8809(91)90127-J).
- Fabrizzi, K.P., Rice, C.W., Amado, T.J.C., Fiorin, J., Barbagelata, P., Melchiori, R., 2009. Protection of soil organic C and N in temperate and tropical soils: effect of native and agroecosystems. *Biogeochemistry* 92, 129–143. <http://dx.doi.org/10.1007/s10533-008-9261-0>.
- Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* 450, 277–280. <http://dx.doi.org/10.1038/nature06275>.
- Franzluebbers, A., 2002. Soil organic matter stratification ratio as an indicator of soil quality. *Soil Tillage Res.* 66, 95–106. [http://dx.doi.org/10.1016/S0167-1987\(02\)00018-1](http://dx.doi.org/10.1016/S0167-1987(02)00018-1).
- Fujii, K., Funakawa, S., Shinjo, H., Hayakawa, C., Mori, K., Kosaki, T., 2011. Fluxes of dissolved organic carbon and nitrogen throughout Andisol, Spodosol and Inceptisol profiles under forest in Japan. *Soil Sci. Plant Nutr.* 57, 855–866. <http://dx.doi.org/10.1080/00380768.2011.637304>.
- Gabet, E.J., Reichman, O.J., Seabloom, E.W., 2003. The effects of bioturbation on soil processes and sediment transport. *Annu. Rev. Earth Planet. Sci.* 31, 249–273. <http://dx.doi.org/10.1146/annurev.earth.31.109091.141314>.
- Grave, R.A., Nicoloso, R.D.S., Cassol, P.C., Aita, C., Corrêa, J.C., Costa, M.D., Fritz, D.D., 2015. Short-term carbon dioxide emission under contrasting soil disturbance levels and organic amendments. *Soil Tillage Res.* 146, 184–192. <http://dx.doi.org/10.1016/j.still.2014.10.010>.
- Guidle, S., Chung, H., Amelung, W., Chang, C., Six, J., 2008. Soil carbon saturation controls labile and stable carbon pool dynamics. *Soil Sci. Soc. Am. J.* 72, 605. <http://dx.doi.org/10.2136/sssaj2007.0251>.
- Hartnett, D.C., Fay, P.A., 1998. Plant populations: patterns and processes. In: Knapp, A.K., Briggs, J.M., Hartnett, D.C., Collins, S.L. (Eds.), *Grassland Dynamics: Long-Term Ecological Research in Tallgrass Prairie*. Oxford University Press, New York, pp. 81–100.
- Hassink, J., 1997. The capacity of soils to preserve organic C and N by their association with clay and silt particles. *Plant Soil* 191, 77–87. <http://dx.doi.org/10.1023/A:1004213929699>.
- Huang, G.F., Wu, Q.T., Wong, J.W.C., Nagar, B.B., 2006. Transformation of organic matter during co-composting of pig manure with sawdust. *Bioresour. Technol.* 97, 1834–1842. <http://dx.doi.org/10.1016/j.biortech.2005.08.024>.
- Jarecki, M.K., Lal, R., 2003. Crop management for soil carbon sequestration. *CRC. Crit. Rev. Plant Sci.* 22, 471–502. <http://dx.doi.org/10.1080/713608318>.
- Jat, R.A., Sahrawat, K.L., Kassam, A.H., Friedrich, T., 2014. Conservation agriculture for sustainable and resilient agriculture: global status, prospects and challenges. *Conserv. Agric. Glob. Prospect. Challenges* 1–25.
- Jessup, K.E., Barnes, P.W., Boutton, T.W., 2003. Vegetation dynamics in a Quercus – Juniperus savanna: an isotopic assessment. *J. Veg. Sci.* 14, 841–852. <http://dx.doi.org/10.1111/j.1654-1103.2003.tb02217.x>.
- Jiménez, J.J., Lal, R., 2006. Mechanisms of C sequestration in soils of latin america. *CRC. Crit. Rev. Plant Sci.* 25, 337–365. <http://dx.doi.org/10.1080/0735268060094240>.
- Kaiser, K., Kalbitz, K., 2012. Cycling downwards – dissolved organic matter in soils. *Soil Biol. Biochem.* 52, 29–32. <http://dx.doi.org/10.1016/j.soilbio.2012.04.002>.
- Kassem, A., Diethelm, K., 1987. Estimation of anaerobic microbial activities in soils by arginine ammonification and glucose-dependent CO<sub>2</sub>-production. *Soil Biol. Biochem.* 19, 683–686.
- Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. *Biogeochemistry* 85, 9–24. <http://dx.doi.org/10.1007/s10533-007-9103-5>.
- Kleber, M., Nico, P.S., Plante, A., Filley, T., Kramer, M., Swanston, C., Sollins, P., 2011. Old and stable soil organic matter is not necessarily chemically recalcitrant: implications for modeling concepts and temperature sensitivity. *Glob. Chang. Biol.* 17, 1097–1107. <http://dx.doi.org/10.1111/j.1365-2486.2010.02278.x>.
- Kleja, D.B., Svensson, M., Majdi, H., Jansson, P.-E., Langvall, O., Bergkvist, B., Johansson, M.-B., Weslien, P., Truus, L., Lindroth, A., Ågren, G.I., 2008. Pools and fluxes of carbon in three Norway spruce ecosystems along a climatic gradient in Sweden. *Biogeochemistry* 89, 27. <http://dx.doi.org/10.1007/s10533-008-9179-6>.
- Lützw, M.V., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. *Eur. J. Soil Sci.* 57, 426–445. <http://dx.doi.org/10.1111/j.1365-2389.2006.00809.x>.
- Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. *Science* (80-) 304, 1623–1627. <http://dx.doi.org/10.1126/science.1097396>.
- Lal, R., 2010. Depletion and restoration of carbon in the pedosphere. *Jpn. Soc. Pedol.* 53, 19–32.
- Lal, R., 2016. Beyond COP 21: potential and challenges of the 4 per thousand initiative. *J. Soil Water Conserv.* 71, 20A–25A. <http://dx.doi.org/10.2489/jswc.71.1.20A>.
- Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. *Nature* 0–8. <http://dx.doi.org/10.1038/nature16069>.
- Lim, S., Lee, S., Lee, S., Choi, W., 2010. Nitrogen isotope compositions of synthetic fertilizer, raw livestock manure slurry, and composted livestock manure. *Korean J. Soil Sci. Fertil.* 43, 453–457.
- Littell, R.C., Pendergast, J., Natarajan, R., 2000. Modelling covariance structure in the analysis of repeated measures data. *Stat. Med.* 19, 1793–1819. [http://dx.doi.org/10.1002/1097-0258\(20000715\)19:13<1793::AID-SIM482>3.0.CO;2-Q](http://dx.doi.org/10.1002/1097-0258(20000715)19:13<1793::AID-SIM482>3.0.CO;2-Q).
- Luo, Z., Wang, E., Sun, O.J., 2010. Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. *Agric. Ecosyst. Environ.* 139, 224–231. <http://dx.doi.org/10.1016/j.agee.2010.08.006>.
- Lynch, D.H., Voroney, R.P., Warman, P.R., 2006. Use of <sup>13</sup>C and <sup>15</sup>N natural abundance techniques to characterize carbon and nitrogen dynamics in composting and in compost-amended soils. *Soil Biol. Biochem.* 38, 103–114. <http://dx.doi.org/10.1016/j.soilbio.2005.04.022>.
- Mcgowan, A.R., 2015. *Biofuel Cropping System Impacts on Soil C, Microbial Communities and N<sub>2</sub>O Emissions*. Kansas State University.
- Mikha, M.M., Rice, C.W., 2004. Tillage and manure effects on soil and aggregate-Associated carbon and nitrogen. *Soil Sci. Soc. Am. J.* 68, 809. <http://dx.doi.org/10.2136/sssaj2004.0809>.
- Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovov, V., Stockmann, U., Sulaeman, Y., Tsui, C.C., Vågen, T.G., van Wesemael, B., Winowiecki, L., 2017. Soil carbon 4 per mille. *Geoderma* 292, 59–86. <http://dx.doi.org/10.1016/j.geoderma.2017.01.002>.
- Neff, J.C., Asner, G.P., 2001. Dissolved organic carbon in terrestrial ecosystems: synthesis and a model. *Ecosystems* 4, 29–48. <http://dx.doi.org/10.1007/s100210000058>.
- Nicoloso, R.S., Rice, C.W., Amado, T.J.C., 2016. Kinetic to saturation model for

- simulation of soil organic carbon increase to steady state. *Soil Sci. Soc. Am. J.* 80, 147. <http://dx.doi.org/10.2136/sssaj2015.04.0163>.
- Olson, K.R., Al-Kaisi, M.M., Lal, R., Lowery, B., 2014. Experimental consideration, treatments, and methods in determining soil organic carbon sequestration rates. *Soil Sci. Soc. Am. J.* 78, 348. <http://dx.doi.org/10.2136/sssaj2013.09.0412>.
- Poulton, P., Johnston, J., MacDonald, A., White, R., Powlson, D., 2018. Major limitations to achieving 4 per 1000<sup>+</sup> increases in soil organic carbon stock in temperate regions: evidence from long-term experiments at Rothamsted Research, UK. *Glob. Chang. Biol.* 1–22. <http://dx.doi.org/10.1111/gcb.14066>.
- Powlson, D.S., Bhogal, A., Chambers, B.J., Coleman, K., Macdonald, A.J., Goulding, K.W.T., Whitmore, A.P., 2012. The potential to increase soil carbon stocks through reduced tillage or organic material additions in England and Wales: a case study. *Agric. Ecosyst. Environ.* 146, 23–33. <http://dx.doi.org/10.1016/j.agee.2011.10.004>.
- Powlson, D.S., Stirling, C.M., Jat, M.L., Gerard, B.G., Palm, C.A., Sanchez, P.A., Cassman, K.G., 2014. Limited potential of no-till agriculture for climate change mitigation. *Nat. Clim. Chang.* 4, 678–683. <http://dx.doi.org/10.1038/nclimate2292>.
- Sanderman, J., Hengl, T., Fiske, G.J., 2017. Soil carbon debt of 12,000 years of human land use. *Proc. Natl. Acad. Sci.* 0610, 3. <http://dx.doi.org/10.1073/PNAS.1706103114>.
- Santos, N.Z., dos Dieckow, J., Bayer, C., Molin, R., Favaretto, N., Pauletti, V., Piva, J.T., 2011. Forages, cover crops and related shoot and root additions in no-till rotations to C sequestration in a subtropical Ferralsol. *Soil Tillage Res.* 111, 208–218. <http://dx.doi.org/10.1016/j.still.2010.10.006>.
- Schipper, L.A., Schipper, L.A., Percival, H.J., Sparling, G.P., 2004. An approach for estimating when soils will reach maximum nitrogen storage. *Soil Use Manag.* 20, 281–286. <http://dx.doi.org/10.1079/SUM2004255>.
- Schmatz, R., Recous, S., Aita, C., Tahir, M.M., Schu, A.L., Chaves, B., Giacomini, S.J., 2017. Crop residue quality and soil type influence the priming effect but not the fate of crop residue. *C. Plant Soil* 414, 229–245. <http://dx.doi.org/10.1007/s11104-016-3120-x>.
- Six, J., Elliott, E.T., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biol. Biochem.* 32, 2099–2103. [http://dx.doi.org/10.1016/S0038-0717\(00\)00179-6](http://dx.doi.org/10.1016/S0038-0717(00)00179-6).
- Six, J., Feller, C., Denef, K., Ogle, S.M., de Moraes, J.C., Albrecht, A., 2002. Soil organic matter, biota and aggregation in temperate and tropical soils – effects of no-tillage. *Agronomie* 22, 755–775. <http://dx.doi.org/10.1051/agro:2002043>.
- Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil Tillage Res.* 79, 7–31. <http://dx.doi.org/10.1016/j.still.2004.03.008>.
- Smith, P., Powlson, D.S., Glendinning, M.J., Smith, J.U., 1997. Potential for carbon sequestration in European soils: preliminary estimates for five scenarios using results from long-term experiments. *Glob. Chang. Biol.* 3, 67–79. <http://dx.doi.org/10.1046/j.1365-2486.1997.00055.x>.
- Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G., Romanenkov, V., Schneider, U., Towprayoon, S., Wattenbach, M., Smith, J., 2008. Greenhouse gas mitigation in agriculture. *Philos. Trans. R. Soc. B Biol. Sci.* 363, 789–813. <http://dx.doi.org/10.1098/rstb.2007.2184>.
- Soil Survey Staff, 1999. *Soil Taxonomy: a basic system of soil classification for making and interpreting soil surveys*. Natural Resources Conservation Service, 2nd ed. USDA, Washington, DC.
- Sollins, P., Homann, P., Caldwell, B. a., 1996. Stabilization and destabilization of soil organic matter. *pdf. Geoderma* 74, 65–105. [http://dx.doi.org/10.1016/S0016-7061\(96\)00036-5](http://dx.doi.org/10.1016/S0016-7061(96)00036-5).
- Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J., 2007. Soil carbon saturation: concept, evidence and evaluation. *Biogeochemistry* 86, 19–31. <http://dx.doi.org/10.1007/s10533-007-9140-0>.
- Stewart, C.E., Plante, A.F., Paustian, K., Conant, R.T., Six, J., 2008. Soil carbon saturation: linking concept and measurable carbon pools. *Soil Sci. Soc. Am. J.* 72, 379. <http://dx.doi.org/10.2136/sssaj2007.0104>.
- Tonneijck, F.H., Jongmans, A.G., 2008. The influence of bioturbation on the vertical distribution of soil organic matter in volcanic ash soils: a case study in northern Ecuador. *Eur. J. Soil Sci.* 59, 1063–1075. <http://dx.doi.org/10.1111/j.1365-2389.2008.01061.x>.
- Wang, J., Wang, X., Xu, M., Feng, G., Zhang, W., Yang, X., Huang, S., 2015. Contributions of wheat and maize residues to soil organic carbon under long-term rotation in north China. *Sci. Rep.* 5, 11409. <http://dx.doi.org/10.1038/srep11409>.
- Wendt, J.W., Hauser, S., 2013. An equivalent soil mass procedure for monitoring soil organic carbon in multiple soil layers. *Eur. J. Soil Sci.* 64, 58–65. <http://dx.doi.org/10.1111/ejss.12002>.
- Wendt, J.W., 2012. ESM Sample Spreadsheets. Xlsm [WWW Document]. URL <https://docs.google.com/open?id=OBzxNffzLbFjSG9RWlpwQ0FXc0k>. (Accessed 9 August 16).
- West, T.O., Post, W.M., 2002. Soil organic carbon sequestration rates by tillage and crop rotation: a global data analysis. *Soil Sci. Soc. Am. J.* 66, 1930. <http://dx.doi.org/10.2136/sssaj2002.1930>.
- White, P.M., Rice, C.W., 2009. Tillage effects on microbial and carbon dynamics during plant residue decomposition. *Soil Sci. Soc. Am. J.* 73, 138. <http://dx.doi.org/10.2136/sssaj2007.0384>.
- Williams, S.M., Weil, R.R., 2004. Crop cover root channels may alleviate soil compaction effects on soybean crop. *Soil Sci. Soc. Am. J.* 68, 1403. <http://dx.doi.org/10.2136/sssaj2004.1403>.
- Wynn, J.G., Bird, M.I., 2007. C4-derived soil organic carbon decomposes faster than its C3 counterpart in mixed C3/C4 soils. *Glob. Chang. Biol.* 13, 2206–2217. <http://dx.doi.org/10.1111/j.1365-2486.2007.01435.x>.
- Xia, L., Lam, S.K., Yan, X., Chen, D., 2017. How does recycling of livestock manure in agroecosystems affect crop productivity, reactive nitrogen losses, and soil carbon balance? *Environ. Sci. Technol.* 51, 7450–7457. <http://dx.doi.org/10.1021/acs.est.6b06470>.
- Zech, W., Guggenberger, G., Haumaier, L., Pöhhacker, R., Schäfer, D., Amelung, W., Miltner, A., Kaiser, K., Ziegler, F., 1996. Organic matter dynamics in forest soils of temperate and tropical ecosystems. In: Piccolo, A. (Ed.), *Humic Substances in Terrestrial Ecosystems*. Elsevier B.V., Amsterdam, pp. 101–170.
- de Oliveira Ferreira, A., Jorge Carneiro Amado, T., da Silveira Nicoloso, R., de Moraes Sa, J.C., Ermani Fiorin, J., Santos Hansel, D.S., Menefee, D., 2013. Soil carbon stratification affected by long-term tillage and cropping systems in southern Brazil. *Soil Tillage Res.* 133, 65–74. <http://dx.doi.org/10.1016/j.still.2013.05.011>.
- de, M., Sá, J.C., Cerri, C.C., Dick, W.A., Lal, R., Filho, S.P.V., Piccolo, M.C., Feigl, B.E., 2001. Organic matter dynamics and carbon sequestration rates for a tillage chronosequence in a Brazilian oxisol. *Soil Sci. Soc. Am. J.* 65, 1486. <http://dx.doi.org/10.2136/sssaj2001.6551486x>.