Soil Carbon Sequestration in Sorghum Cropping Systems: Evidence From Stable Isotopes and Aggregate-Size Fractionation

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Abstract: Management practices can influence both the quantity of soil organic carbon (SOC) and its distribution into different fractions or pools. We investigated SOC sequestration potentials of cropping systems in near-surface (0–5 cm) samples through soil size and density fractionation coupled with acid hydrolysis and natural abundance of stable isotopes ($\delta^{13}C$) in a 20-year field study in the southern Great Plains in 2002. Treatments included two tillage regimens, conventional and no tillage (NT), in combination with two cropping systems: continuous grain sorghum [*Sorghum bicolor* (L.) Moench.] (CS) and a sorghum-wheat (*Triticum aestivum* L.)-soybean [*Glycine max* (L.) Merr.] (SWSoy) rotation, and with or without N fertilization. Tillage and cropping sequence significantly affected SOC distribution and the natural abundance of $\delta^{13}C$ in different fractions. Samples from CS exhibited $\delta^{13}C$ values ranging from −15‰ to −20‰, suggesting most SOC was derived from this C_4 crop species. The $\delta^{13}C$ values for soils from SWSoy varied from −20‰ to −22‰, reflecting a mixed input from C_3-derived and C_4-derived residue input. For whole soil and all aggregate-size fractions, SOC concentrations were significantly higher for NT than conventional tillage. However, the effects of cropping system and N fertilization on SOC interacted with tillage. Greater SOC for enhanced cropping (SWSoy) or N fertilization was observed only under NT. The fraction of <53 μm represented a greater proportion of soil than other aggregate-size fractions. Our long-term study indicated that SOC and its various fractions, including more resistant, can be increased by NT with enhanced cropping and N fertilization.

Key Words: Tillage, cropping, carbon, natural abundance, stable isotopes, aggregate-size fractionation

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Soil organic matter is broadly accepted as an important index of soil quality because of its influence on soil structure, nutrient supply, water-holding capacity, and soil microbial activity. To develop sustainable agricultural production, it is critical to maintain or increase soil organic carbon (SOC), a main component of soil organic matter. Although many environmental and biological factors affect the quantity of SOC (Borken and Matzner, 2009; Lu et al., 2009; Kaschuk et al., 2010; Soane et al., 2012), the most significant factors regulating the organic matter content in soils for specific sites are often vegetation and soil disturbance. The quantity and quality of crop residue inputs play an important role in the ability of soil to store carbon (C) (Dou et al., 2007; Blanco-Canqui and Lal, 2009; Lafond et al., 2009). In general, increased cropping intensity results in higher crop biomass deposition and thus more potential to sequester C compared with monoculture cropping (Wright et al., 2007; Gong, et al., 2009; Lal, 2009). In addition, West and Post (2002) reported that crop species differed in their ability to influence SOC sequestration. Although these results have been based largely on trials from cooler mid- to high-latitude soils, we suggest that the factor will also be important in warmer, humid environments such as the southern Great Plains region of the United States.

Tillage also impacts SOC storage and stability (Dou and Hons, 2006; Wright et al., 2008). Many studies have demonstrated that conservative tillage, including no till (NT), increases SOC sequestration more than conventional tillage (CT) (Mishra et al., 2010; Neto et al., 2010; Ogle et al., 2012). For example, Liu et al. (2014) reported that SOC under NT was 50% greater than that under CT in a 17-year trial. The underlying mechanism of increasing SOC with NT is mainly attributed to less disturbance and thus slower SOC turnover than that under CT.

Nitrogen fertilization has also been reported to enhance SOC sequestration (Fornara et al., 2013; Kirkby et al., 2014). Fornara et al. (2013) reported that SOC under N fertilization was 36% greater than that without N fertilization in a 19-year-old nutrient addition experiment, with increased root biomass and plant residue resulting in greater SOC. Thus theoretically, SOC sequestration should be further enhanced if one combines the management practices of enhanced cropping system, NT, and N fertilization. However, research determining the interactive effects of these practices on SOC storage is lacking.

One of the most important tasks in studying SOC sequestration is to determine changes in both SOC pool sizes and turnover rates. In other words, one should be interested not only in whether a system is accruing SOC, but also whether the newly sequestered SOC is associated with active or resistant pools. Ideally, management practices would increase SOC pools with slower turnover rates. Research methods that integrate physical, chemical, and stable isotopic analyses may offer further insight into how management influences SOC dynamics. Soil physical fractionation has been a useful tool to relate soil fractions with different turnover rates. Liao et al. (2006) reported that the SOC fraction associated with silt and clay had a slower turnover rate than that of particulate organic C (POC). Similar results were also reported by Jastrow (1996). The mean residence time of nonhydrolyzable acid residue C was estimated to be 1,800 years by tandem accelerator mass spectrometer ^{14}C analysis (Leavitt et al., 1996), which was older than the remaining SOC pools. Stable isotopic analysis, such as for ^{13}C, may be used to determine relative changes in C source due to vegetation changes. Differences in $^{13}C/^{12}C$ ratios of plants

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utilizing the C_3 and C_4 photosynthetic pathways provide a natural tracer when a C_3 crop ($\delta^{13}C \approx -27‰$) is replaced by a C_4 crop ($\delta^{13}C \approx -14‰$) or vice versa, permitting the quantification of the accumulation of new C derived from the recent vegetation (Balesdent et al., 1988).

Soil organic C content is frequently low in the southern United States because of high temperature, which leads to enhanced SOC decomposition relative to northern climates (Hargrove and Luxmore, 1988). Integrating conservation tillage, enhanced cropping, and N fertilization may be an important strategy for increasing SOC sequestration in warmer regions. The objective of the present study was to determine the effects of NT, enhanced cropping, and N fertilization on total SOC, the pool sizes of different SOC fractions, and the accumulation of newly derived C from sorghum.

MATERIALS AND METHODS

Crop Management

A long-term field experiment was initiated in 1982 in the southern Great Plains in the Brazos River floodplain of south-central Texas (30°32′N, 94°26′W). Long-term mean annual temperature is 20°C, and annual rainfall is 978 mm. Sorghum was managed under CT and NT in continuous sorghum (CS) and rotated wheat (*Triticum aestivum* L.)/soybean [*Glycine max* (L.) Merr.]-grain sorghum [*Sorghum bicolor* (L.) Moench.] (SWSoy). Crop-growing seasons ranged from early November to mid-May for wheat, early June to late October for soybean, and late March to late July for sorghum. Continuous sorghum produced one crop each year, and SWSoy produced three crops every 2 years. Cropping intensity was defined as the fraction of the year when a crop was growing and was 0.33 for CS and 0.65 for SWSoy. Conventional tillage in soybean and sorghum consisted of disking to 10- to 15-cm depth after harvest, chiseling to 25 cm, a second disking, and ridging prior to winter. Soybean and sorghum under CT also received one to three in-season cultivations annually for weed control, while sorghum stalks were shredded for both tillage regimes. For wheat under CT, soil was disked three to four times after harvest. For NT, no soil disturbance occurred except during planting. Nitrogen fertilizer (NH_4NO_3) was broadcast on wheat at 0 or 68 kg N ha^{-1} during late winter or early spring. Soybean did not receive N fertilizer, whereas sorghum received 0 or 90 kg N ha^{-1} banded preplant. Soybean received 15 kg P ha^{-1} banded preplant. Wheat was planted in 0.18-m-wide rows, whereas sorghum and soybean were planted in 1-m-wide rows. Plots measured 4 m wide by 12.2 m long. No guard rows were used, but plot size was sufficient to avoid possible contamination between treatments. The depths of sowing were 2.5, 4, and 2.5 cm for sorghum, soybean, and wheat, respectively. A split, split plot within a randomized complete block design was established with crop sequence as the main treatment, tillage as the split treatment, and N fertilizer rate as the split, split treatment in 1982. Treatments were replicated four times.

Soil Sampling

The soil at the site is a Weswood silty clay loam [(fine-silty, mixed, superactive, thermic, Udifluventic Haplustepts) (Calcic Cambisol (FAO, 1988))] and contains 115, 452, and 433 g kg^{-1} of sand, silt, and clay, respectively. This soil has a pH of 8.2 (1:2, soil:water).

Soil samples for this study were collected in August 2002 shortly after sorghum harvest. Individual samples consisted of 25 composited cores (19-mm diameter) per plot at the 0- to 50-mm depth because most management practice effects occur in surface soil (Six et al. 2000; Dou and Hons, 2006). Wright and Hons (2005) showed that the primary changes in aggregation due to management occurred at 0 to 50 mm in a similar soil. Soil was sieved to pass a 4.7-mm screen (visible pieces of crop residues and roots removed) and oven dried for 24 h at 40°C. A portion of the sieved, moist soil was also dried at 60°C for 48 h for chemical and physical analysis.

Size and Density Fractionation

Size and density fractionations were conducted on soil samples to isolate the SOC fractions described in the conceptual model of Six et al. (2000, 2002a,b). Soil (20 g) was gently immersed in deionized water on a 250-μm mesh screen and shaken with 50 glass beads (diameter = 4 mm). A continuous and steady water flow through the screen ensured that microaggregates were flushed onto a 53-μm sieve and not exposed to any further disruption by the beads. After all macroaggregates were broken, material on the 53-μm screen was sieved to ensure that isolated microaggregates were water-stable. A subsample of microaggregates from each sample was taken at this stage and saved for C analyses. Intermicroaggregate particulate organic matter (POM) that was retained together with microaggregates on the sieve was isolated by density flotation in a 1.85 g cm^{-3} sodium polytungstate solution and removed (Six et al., 2000). This procedure resulted in the following fractions: coarse (>250 μm) unprotected POM, fine (53–250 μm) unprotected POM, protected POM (53–250 μm) (POM), SOM in the fraction <53 μm inside microaggregates (mineral-associated organic matter [MAOM]), and SOM in the fraction <53 μm outside microaggregates (OMAOM). Because the coarse unprotected POM had very low yield in the CS treatment, C and $\delta^{13}C$ were not analyzed for this fraction. The overall conceptual scheme used for integrated size fractionation and acid hydrolysis (described in the following section) is presented in Fig. 1.

Chemical Analyses

Nonhydrolyzable Organic Matter

Nonhydrolyzable organic matter (NHOM) in the <53-μm fractions either inside or outside microaggregates was determined using the method suggested by Rovira and Vallejo (2002) with the following modifications. One gram of oven-dried soil of the fraction <53 μm was hydrolyzed with 25 mL of 6 *N* HCl at 110°C for 18 h with occasional shaking. After cooling, the nonhydrolyzed residue was recovered by centrifugation at 1680*g* after decanting the liquid. The process of centrifugation and decantation was repeated several times with deionized water until neutral pH was reached. The NHOM was then dried at 60°C to constant weight before C analysis. Because NHOM either inside or outside microaggregates had approximately the same values, only the results for the NHOM outside microaggregates are presented.

Elemental and Isotopic Analyses

Following the size fractionation procedures described in Fig. 1, we collected coarse and fine POM, protected POM, MAOM, and NHOM fractions. Because we were more interested in the changes associated with more passive or important SOC pools, only the results for POM, MAOM, NHOM, and whole-soil samples are presented. Organic C and $\delta^{13}C$ were determined for POM, MAOM, NHOM, and whole-soil samples following the method of Jessup et al. (2003). Oven-dried samples were ground and homogenized to pass a 0.25-mm sieve. Four- or 40-mg soil samples, depending on C concentration, were weighed into silver capsules, and inorganic carbonate was removed by exposure to saturated HCl in a desiccator (Harris et al., 2001), and $\delta^{13}C$ and

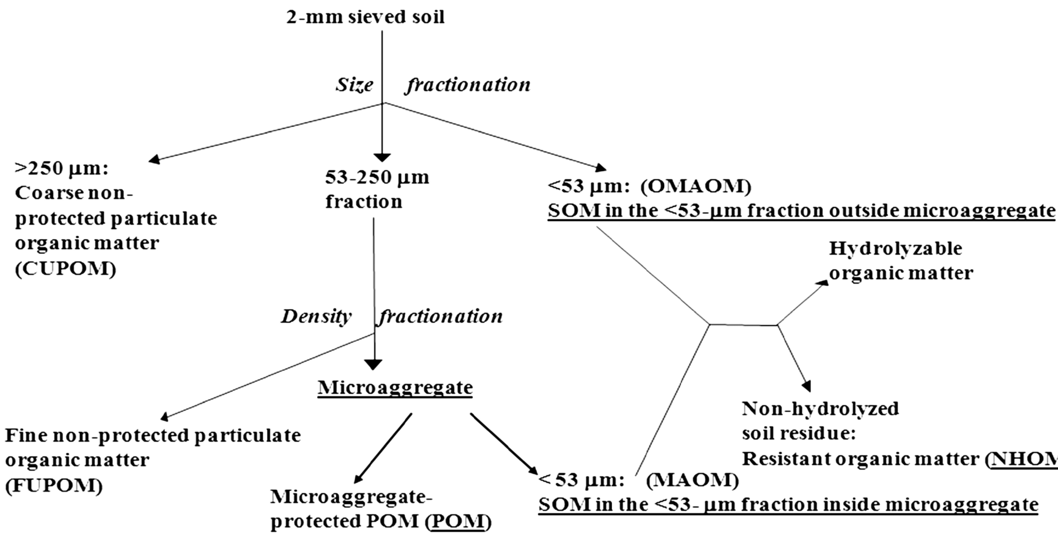

FIG. 1. A conceptual scheme of integrated size fractionation and chemical analysis used for soil and various fraction analysis [Six et al. (2000, 2002a,b)].

organic C were subsequently measured using an elemental analyzer (Carlo Erba EA-1108, Lakewood, NJ) interfaced with a Delta Plus isotope ratio mass spectrometer (ThermoFinnigan, Bremen, Germany) operating in continuous flow mode. Precision for the $\delta^{13}C$ measurements was less than 0.1‰. $\delta^{13}C$ values were expressed relative to the V-PDB standard (Coplen, 1995). Because a C_4 crop (sorghum) was grown on soils that originally supported C_3 vegetation, the $\delta^{13}C_{PDB}$ signature of the soils at the time of sampling reflected the combined inputs of organic matter from both C_4 and C_3 vegetation. Thus, the $\delta^{13}C_{V\text{-}PDB}$ of each isolated fraction from the soil was partitioned as in Eq. (1):

$$\delta_S \approx f^* \delta_{C3} + (1{-}f)^* \delta_{C4} \qquad (1)$$

where δ_S is the $\delta^{13}C_{V\text{-}PDB}$ of whole soil or a given fraction isolated from soil, δ_{C3} is the $\delta^{13}C_{V\text{-}PDB}$ of that fraction before the switch from C_3 to C_4 vegetation, δ_{C4} is the $\delta^{13}C_{V\text{-}PDB}$ of organic inputs from C_4 sorghum plant residue, f is the proportion or fraction of SOC derived from C_3 vegetation, and $(1 - f)$ is the proportion derived from C_4 sorghum. The value of δ_{C4} was estimated by the average $\delta^{13}C_{PDB}$ value measured for sorghum plant residue (−14‰). Values for δ_{C3} were estimated by the $\delta^{13}C_{PDB}$ values of isolated fractions from a continuous wheat treatment with the same implementation time as CS and SWSoy treatments.

Statistical Analysis

Data were analyzed with SAS (2009). A PROC GLM model was used for individual treatment comparisons at $P < 0.05$. Briefly, two test statements were included because we used nonstandard error structures. A general model was used.

$$\text{dependent variables} = \begin{matrix} \text{rep} & \text{rotation} & & \text{rep*rotation} \\ \text{tillage} & \text{rotation*tillage} & & \text{rotation*tillage*rep} \\ \text{nitrogen} & \text{nitrogen*rotation} & \text{nitrogen*tillage} & \text{nitrogen rotation tillage} \end{matrix}$$

where rep refers to replication. If equal variance was not met (Levene's test), then data were transformed according to the minimal lambda value.

RESULTS

Distribution of Soil Fractions and C

Soil was initially separated into three size fractions: >250 μm, 53 to 250 μm, and <53 μm (Table 1). The average total recovery after wet sieving was greater than 97%. The 53- to 250-μm and <53-μm fractions comprised the majority of soil (47% and 51%, respectively). The >250-μm fraction (unprotected particulate organic matter) comprised less than 1% of the total soil. Although only a small fraction, the >250-μm fraction was more sensitive to tillage and cropping sequence than all other fractions, being 218% higher under NT than CT. The >250-μm fraction was also affected by crop sequence, with SWSoy having slightly more of this fraction than CS. Total recovery of SOC in the various fractions averaged 93% compared with whole soil.

Total organic C in the whole soil was not affected by the three-way interaction but was significantly affected by two-way interactions of rotation by tillage and tillage by N (Table 2). Although SWSoy numerically increased SOC more than CS, it was significant only under NT (Table 3). Soil organic C under SWSoy and NT was 19% greater than that under CS and NT. A similar effect of N fertilization on SOC was also observed.

TABLE 1. Percentage of Soil Mass (0- to 5-cm Depth) in Different Physical Fractions as Affected by Tillage, Nitrogen Addition, and Cropping Sequence (n = 4)

			Cropping Sequence	
Fraction	Tillage	Nitrogen	CS	SWSoy
			%	
>250 μm	CT	Without	0.2 ± 0.0	0.4 ± 0.1
		With	0.3 ± 0.1	0.4 ± 0.1
	NT	Without	0.9 ± 0.2	0.9 ± 0.2
		With	0.9 ± 0.2	1.2 ± 0.2
53–250 μm	CT	Without	47.0 ± 3.6	48.5 ± 2.9
		With	42.4 ± 3.3	47.8 ± 3.5
	NT	Without	39.8 ± 2.9	49.8 ± 2.7
		With	42.0 ± 3.9	47.9 ± 2.4
<53 μm	CT	Without	48.2 ± 4.4	50.3 ± 3.2
		With	54.4 ± 2.8	50.8 ± 3.5
	NT	Without	56.7 ± 2.9	47.7 ± 2.7
		With	54.0 ± 4.0	48.3 ± 2.2

S.E. follows the means.

TABLE 2. Analysis of Variance *P* Values for POC, MAOC, and NHOC Based on Soil Fraction or Whole Soil (0- to 5-cm Depth) and Total SOC as Affected by Cropping Sequence (Rotation), Tillage, and Nitrogen Fertilization in a 20-Year Study on a Silty Clay Loam Soil

		Organic C Based on Soil Fraction			Organic C Based on Whole Soil			
Source	**DF**	**POC**	**MAOC**	**NHOC**	**SOC**	**POC**	**MAOC**	**NHOC**
				P				
Replication (rep)	3	0.48	0.97	0.88	0.17	0.22	0.89	0.85
Rotation	1	<0.0001	<0.0001	0.41	<0.0001	<0.0001	<0.0001	0.61
Rotation*rep	3	0.86	0.22	0.67	0.99	0.48	0.53	0.60
Tillage	1	<0.0001	<0.0001	0.001	<0.0001	<0.0001	<0.0001	0.001
Tillage*rotation	1	0.06	0.96	0.81	<0.0001	<0.0001	0.11	0.33
Tillage*rotation*rep	6	0.46	0.40	0.67	0.72	0.17	0.37	0.59
Nitrogen	1	0.001	0.03	0.13	<0.0001	0.001	0.04	0.07
Nitrogen*rotation	1	0.97	0.18	0.38	0.75	0.24	0.44	0.38
Nitrogen*tillage	1	0.01	0.02	0.11	<0.0001	0.001	0.15	0.45
Nitrogen*tillage*rotation	1	0.86	0.44	0.38	0.88	0.85	0.79	0.69

Particulate organic C and mineral-associated organic C (MAOC) on a whole-soil basis were both increased by SWSoy compared with CS whether under CT or NT, but values for both cropping sequences were greatest with NT. Nonhydrolyzed organic C (NHOC) on a whole-soil basis was relatively unaffected by tillage or sequence.

Particulate organic C and MAOC on a soil fraction basis were affected by the interaction of tillage and nitrogen (Tables 2 and 4). Nitrogen fertilization significantly increased POC (46%) and MAOC (15%), but only under NT. Both POC and MAOC were also affected by cropping intensity. Sorghum-wheat-soybean significantly increased POC and MAOC by 98% and 46%, respectively, compared with CS. Nonhydrolyzed organic C was affected only by tillage and was 28% greater under NT than CT. Overall, POC had the highest C concentrations, whereas NHOC had the lowest. No tillage increased C concentrations in all fractions, but especially for POC and MAOC. When considered on a whole-soil basis, MAOC was not affected by the interaction of tillage by nitrogen and was affected only by the main factors of rotation, tillage, and nitrogen (Tables 2 and 3). Mineral-associated organic C was increased 33%, 35%, and 10%, respectively, by rotation (increased intensity), NT, and N addition. Particulate organic C and SOC were significantly affected by rotation, tillage, rotation by tillage, nitrogen, and nitrogen by tillage. Particulate organic C and SOC were increased by N addition only with NT. Nonhydrolyzed organic C was affected only by tillage. The greatest C concentrations on a whole-soil basis were observed with MAOC and the lowest with POC (Table 3).

Unlike C concentrations based either on respective fractions or whole soil, the C:N ratio for each fraction or whole soil was less affected by treatment variables (Table 5). The C:N ratio of whole soil was affected by cropping intensity and tillage. Sorghum-wheat-soybean had greater C:N ratio of whole soil than CS. Compared with CT, NT significantly decreased C:N ratio of whole soil. The C:N ratio of MAOM was significantly greater with SWSoy

TABLE 3. Particulate Organic C, MAOC, and NHOC Based on Whole Soil (0- to 5-cm Depth) and Total SOC as Affected by Cropping Sequence (Rotation), Tillage, and Nitrogen Fertilization in a 20-Year Study on a Silty Clay Loam Soil

			Cropping Sequence	
Component	**Tillage**	**Nitrogen**	**CS**	**SWSoy**
			g C m^{-2} soil	
SOC	CT	Without	562 ± 87	651 ± 32
		With	589 ± 97	695 ± 17
	NT	Without	809 ± 64	981 ± 27
		With	965 ± 121	1,122 ± 90
			g C kg^{-1} soil	
POC	CT	Without	0.3 ± 0.1	0.7 ± 0.1
		With	0.3 ± 0.0	0.9 ± 0.1
	NT	Without	0.8 ± 0.2	2.5 ± 0.4
		With	1.2 ± 0.2	3.1 ± 0.1
MAOC	CT	Without	2.5 ± 0.2	4.1 ± 0.2
		With	2.8 ± 0.1	4.0 ± 0.3
	NT	Without	3.7 ± 0.2	4.7 ± 0.4
		With	4.5 ± 0.7	5.2 ± 0.5
NHOC	CT	Without	1.3 ± 0.1	1.5 ± 0.2
		With	1.5 ± 0.1	1.5 ± 0.1
	NT	Without	1.7 ± 0.1	1.8 ± 0.2
		With	2.1 ± 0.5	1.9 ± 0.1

TABLE 4. Particulate Organic C, MAOC, and NHOC on a Soil Fraction Basis (0- to 5-cm Depth) as Affected by Cropping Sequence (Rotation), Tillage, and Nitrogen Fertilization in a 20-Year Study on a Silty Clay Loam Soil

			Cropping Sequence	
Component	**Tillage**	**Nitrogen**	**CS**	**SWSoy**
			g C kg^{-1} Fraction	
POC	CT	Without	3.1 ± 0.8	8.7 ± 2.8
		With	3.5 ± 1.0	9.2 ± 1.7
	NT	Without	9.2 ± 1.6	19.1 ± 3.1
		With	15.8 ± 3.9	25.5 ± 2.3
MAOC	CT	Without	5.1 ± 0.1	8.1 ± 0.2
		With	5.2 ± 0.2	7.9 ± 0.1
	NT	Without	6.6 ± 0.2	9.9 ± 0.8
		With	8.3 ± 1.3	10.7 ± 0.6
NHOC	CT	Without	3.6 ± 0.3	3.8 ± 0.2
		With	3.6 ± 0.3	3.7 ± 0.2
	NT	Without	3.9 ± 0.2	4.6 ± 0.6
		With	5.2 ± 1.3	5.0 ± 0.4

TABLE 5. The C:N Ratios for POM, MAOM, and NHOM Based on Whole Soil (0- to 5-cm Depth) and SOM as Affected by Cropping Sequence (Rotation), Tillage, and Nitrogen Fertilization in a 20-Year Study on a Silty Clay Loam Soil

			Cropping Sequence	
Component	Tillage	Nitrogen	CS	SWSoy
			C:N	
SOM	CT	Without	10.2 ± 0.3	11.8 ± 0.8
		With	10.4 ± 0.1	11.6 ± 0.3
	NT	Without	9.6 ± 0.2	11.1 ± 0.4
		With	9.5 ± 0.2	10.4 ± 0.4
POM	CT	Without	13.6 ± 3.1	12.9 ± 0.6
		With	12.7 ± 2.3	12.6 ± 0.8
	NT	Without	11.8 ± 0.2	11.7 ± 0.4
		With	12.3 ± 1.0	11.9 ± 0.3
MAOM	CT	Without	8.5 ± 0.2	10.8 ± 1.1
		With	8.3 ± 0.3	10.6 ± 0.8
	NT	Without	8.5 ± 0.3	11.2 ± 1.5
		With	8.7 ± 0.5	10.1 ± 0.8
NHOM	CT	Without	18.1 ± 1.3	18.9 ± 1.0
		With	22.3 ± 7.9	18.3 ± 0.8
	NT	Without	19.6 ± 1.1	23.1 ± 2.9
		With	23.0 ± 3.5	22.7 ± 2.8

than CS. The C:N ratio of either POM or NHOM was not affected by treatment. The greatest C:N ratio was observed for NHOM, whereas the lowest occurred with MAOM.

Stable Isotope Ratios of $\delta^{13}C$ in Bulk Soil and Size/Density Fractions

Because all field treatments were located in very close proximity and were the same soil series, we assumed that soil $\delta^{13}C$ values were once similar across all plots, and differences in soil $\delta^{13}C$, therefore, resulted primarily from differences in tillage and crop species.

$\delta^{13}C$ values of whole-soil SOC, MAOC, and NHOC were affected by an interaction of tillage by rotation, whereas POC was influenced only by rotation (Table 6). Compared with SWSoy, CS significantly increased the $\delta^{13}C$ of whole-soil SOC (Table 7). However, both responded differently to tillage. For CS, NT slightly but significantly increased $\delta^{13}C$ compared with CT, whereas the opposite effect of tillage on $\delta^{13}C$ was observed for SWSoy. Similar patterns were observed for MAOC and NHOC. $\delta^{13}C$ of POC was affected only by cropping sequence (Table 6), with greater values occurring for CS. The highest $\delta^{13}C$ values were noted for CS in the POC fraction, whereas the lowest were found for SWSoy in the NHOC fraction. Samples from CS exhibited $\delta^{13}C$ ranging from −15‰ to −20‰, typical of C derived from C_4 species. The SWSoy cropping sequence had $\delta^{13}C$ values smaller than CS but greater than those for continuous wheat (C_3 crop; data not shown), indicating a mixed input of C derived from both C_3 and C_4 crops.

TABLE 6. Analysis of Variance *P* Values for δ13C Values of SOC, POC, MAOC, and NHOC as Affected by Cropping Sequence (Rotation), Tillage, and Nitrogen Fertilization in a 20-Year Study on a Silty Clay Loam Soil

Source	DF	SOC	POC	MAOC	NHOC
			P		
Replication (rep)	3	0.44	0.64	0.75	0.80
Rotation	1	<0.0001	<0.0001	<0.0001	<0.0001
Rotation*rep	3	0.70	0.52	0.85	0.15
Tillage	1	0.01	0.14	0.00	0.65
Tillage*rotation	1	<0.0001	0.47	0.001	0.001
Tillage*rotation*rep	6	0.07	0.46	0.14	0.38
Nitrogen	1	0.22	0.28	0.36	0.96
Nitrogen*rotation	1	0.10	0.09	0.13	0.81
Nitrogen*tillage	1	0.97	0.90	0.32	0.61
Nitrogen*tillage*rotation	1	0.36	0.89	0.74	0.45

TABLE 7. δ13C Values of POC, MAOC, and NHOC Based on Whole Soil (0- to 5-cm Depth) and Total SOC as Affected by Cropping Sequence (Rotation), Tillage, and Nitrogen Fertilization in a 20-Year Study on a Silty Clay Loam Soil

			Cropping Sequence	
Component	Tillage	Nitrogen	CS	SWSoy
			$\delta^{13}C$, ‰	
SOC	CT	Without	−17.6 ± 0.3	−19.5 ± 0.1
		With	−17.1 ± 0.5	−19.7 ± 0.3
	NT	Without	−16.4 ± 0.3	−20.0 ± 0.6
		With	−16.1 ± 0.6	−19.9 ± 0.2
POC	CT	Without	−15.9 ± 0.4	−20.4 ± 0.3
		With	−15.5 ± 0.7	−20.5 ± 0.3
	NT	Without	−15.6 ± 0.2	−20.2 ± 0.5
		With	−15.1 ± 0.3	−20.3 ± 0.2
MAOC	CT	Without	−17.9 ± 0.1	−19.8 ± 0.1
		With	−17.7 ± 0.4	−19.9 ± 0.1
	NT	Without	−17.1 ± 0.4	−19.9 ± 0.5
		With	−16.7 ± 0.4	−19.9 ± 0.0
NHOC	CT	Without	−19.8 ± 0.1	−20.9 ± 0.2
		With	−19.9 ± 0.3	−21.0 ± 0.2
	NT	Without	−19.6 ± 0.1	−21.3 ± 0.6
		With	−19.4 ± 0.2	−21.4 ± 0.1

Net Inputs of C_4 Organic Carbon Estimated by Stable Isotope Ratios

After 20 years, tillage had significant effects on the sequestration of recent C_4-derived C within the CS cropping sequence. No tillage significantly increased the sequestration of recent C_4-derived sorghum C for all SOC pools, except POC (Fig. 2A). More than 66% of SOC under CS and CT was from recent C_4-derived sorghum C, while this percentage was even larger for NT (78%). Compared with SOC and other soil C pools, NHOC sequestered the least recent C_4-derived sorghum C, amounting to only 34% and 40% for CT and NT, respectively. Recent sorghum C in CS comprised the largest fraction (approximately 90%) of POC.

In contrast to CS, NT and CT in the SWSoy cropping sequence exhibited similar sequestration of recent C_4-derived sorghum C for all studied C pools (Fig. 2B). The greatest sequestration of sorghum C was again observed in POC, and the lowest was in NHOC, with values ranging from 56% to 20%, respectively.

DISCUSSION

Although our results indicated that NT increased SOC and its various fractions, cropping sequence and N fertilization had interactive effect with tillage on SOC (Tables 2 and 3). The more intensive SWSoy cropping sequence significantly increased SOC only

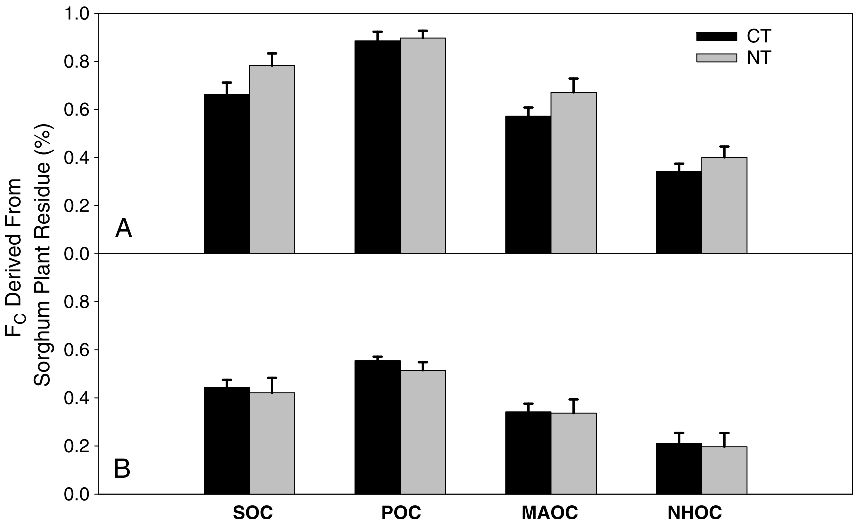

FIG. 2. A, The fraction of C derived from CS for whole soil and size/density fractions (0- to 5-cm depth) with respect to CT versus NT. FC was calculated by mass balance using the δ13C values of whole soil and soil fractions from CS and continuous wheat, with wheat assumed to have δ13C similar to the original C3 species on this soil. B, Recently sequestered C from sorghum in the SWSoy rotation as affected by tillage and estimated by natural stable isotope C values and size fractions. Error bars indicate 1 S.E.

under NT, indicating that input plant residue was more protected under NT than CT. In contrast, West and Post (2002) reported that enhancing rotation complexity under NT did not result in significant SOC increase. These authors suggested that SOC under NT is closer to a maximum steady level than when under CT and therefore stands to gain less SOC under rotation enhancement. This difference might be attributed to at least two reasons. First, the SOC levels in our study might not be close to the maximum level. Second, our study was conducted in a warmer region than the colder temperate areas reported by West and Post (2002). The difference in climatic conditions may also have contributed to the different observed effects of cropping system on SOC. Similar explanations might also apply to the interaction between N fertilization and tillage.

Another interesting result from our study was the effect of management practices on slow or resistant SOC pools. Mineral-associated organic C and NHOC have been broadly accepted as either not or less affected by management practices like tillage (Christensen, 2001; Plante et al., 2005). Our results showed that MAOC was affected by tillage, cropping sequence, and N fertilization (Tables 2–4). Similar effects of long-term tillage on SOC pools were reported by de Moraes Sa et al. (2014), but not by Blanco-Moures et al. (2013). Both de Moraes Sa et al. (2014) and our long-term studies were conducted in subtropical regions with annual precipitation greater than 900 mm. The study reported by Blanco-Moures (2013) was conducted in a Mediterranean climate with less than 600-mm annual precipitation. Thus, climatic variation may at least partly contribute to observed differences. For more resistant and slower turnover pools such as NHOC, tillage also had a significant effect, suggesting that NT may protect even more resistant SOC pools from decomposition by soil microbes (Tables 2–4). Huang et al. (2008) also reported that soil mulching significantly affected NHOC. We did not observe a significant effect of cropping sequence on NHOC.

The results from size fractionation and stable isotopic analyses also provided meaningful information on the accumulation of plant residue (Fig. 2). For more labile SOC pools such as POC, the majority of C in this fraction under CS was derived from recent sorghum inputs, regardless of tillage. Even the most resistant SOC pool, NHOC, had more than 30% of C derived from recent sorghum residue. Similar results were reported by Yamashita et al. (2006). In addition, the difference in the proportion of recent sorghum C in various SOC pools suggested that the turnover of SOC pools decreased in the order of POC > MAOC > NHOC, which was consistent with our general hypothesis that POC was more labile, whereas NHOC was the most resistant pool.

CONCLUSIONS

No tillage and enhanced cropping significantly increased SOC compared with CT and monoculture sorghum in a 20-year field study in the southern Great Plains. Increased SOC resulting from NT was observed in whole soil and all physical and chemical fractions. However, the effects of cropping system and N fertilization on SOC interacted with tillage. Significantly increased SOC due to enhanced cropping or N fertilization was observed only under NT. Continuous grain sorghum had $\delta^{13}C$ values ranging from −15‰ to −20‰, typical of C derived largely from C_4 species. The SWSoy cropping sequence had $\delta^{13}C$ values intermediate between continuous C_3 species and CS, indicating a mixed input of C derived from both C_3 and C_4 crops. No tillage increased sequestration of recent organic C inputs and decreased C decomposition compared with CT. $\delta^{13}C$ results suggested that more resistant SOC pools, including MAOC and NHOC, were also affected by management practices, especially tillage. We concluded that SOC and its fractions can be increased by using NT with enhanced cropping and N fertilization, even for more resistant fractions.

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