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LONG-TERM TILLAGE, CROPPING SEQUENCE, AND NITROGEN FERTILIZATION EFFECTS ON SOIL CARBON AND NITROGEN DYNAMICS

A Dissertation

by

FUGEN DOU

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2005

Major Subject: Soil Science

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Approved as to style and content by:

Frank M. Hons (Chair of Committee)	David A. Zuberer (Member)
Thomas W. Boutton (Member)	Scott A. Senseman (Member)
Mark A. Hussey (Head of Department)	

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ABSTRACT

Long-term Tillage, Cropping Sequence, and Nitrogen Fertilization Effects on Soil Carbon and Nitrogen Dynamics. (May 2005)

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Chair of Advisory Committee: Dr. Frank M. Hons

Management practices that may increase soil organic matter (SOM) storage include conservation tillage, especially no till (NT), enhanced cropping intensity, and fertilization. My objectives were to evaluate management effects on labile [soil microbial biomass (SMB) and mineralizable, particulate organic matter (POM), and hydrolyzable SOM] and slow (mineral-associated and resistant organic) C and N pools and turnover in continuous sorghum [*Sorghum bicolor* (L.) Moench.], wheat (*Triticum aestivum* L.), and soybean [*Glycine max* (L.) Merr.], sorghum-wheat/soybean, and wheat/soybean sequences under conventional tillage (CT) and NT with and without N fertilization. A Weswood silty clay loam (fine, mixed, thermic Fluventic Ustochepts) in southern central Texas was sampled at three depth increments to a 30-cm depth after wheat, sorghum, and soybean harvesting. Soil organic C and total N showed similar responses to tillage, cropping sequence, and N fertilization following wheat, sorghum, and soybean. Most effects were observed in surface soils. NT significantly increased SOC. Nitrogen fertilization significantly increased SOC only under NT. Compared to NT or N addition, enhanced cropping intensity only slightly increased SOC. Estimates of C sequestration rates under NT indicated that SOC would reach a new equilibrium after 20 yr or less of imposition of this treatment. Labile pools were all significantly greater with NT than CT at 0 to 5 cm and decreased with depth. SMB, mineralizable C and N, POM, and hydrolyzable C were highly correlated with each other and SOC, but their slopes were significantly different, being lowest in mineralizable C and highest in hydrolyzable C. These results indicated that different methods determined various fractions of total SOC. Results from soil physical fractionation and ^{13}C concentrations further supported these observations. Carbon turnover rates increased in the sequence: ROC < silt- and clay-associated C < microaggregate-C < POM-C. Long-term incubation showed that 4 to 5% of SOC was in active pools with mean residence time (MRT) of about 50 days, 50% of SOC was in slow pools with an average MRT of 12 years, and the remainder was in resistant pools with an assumed MRT of over 500 years.

DEDICATION

To my parents, my wife, and my son.

ACKNOWLEDGEMENTS

I would like to thank all the people whose encouragement, support, and advice made the completion of this research possible. I am especially grateful to my major advisor, Dr. Frank M. Hons, for his guidance and continuous support through the five years at Texas A&M University. His curiosity about any new results, his stimulating discussion and comments on data interpretation, his strict requirements and constant inspiration made my study more fruitful and more enjoyable. He helped me collect most of my soil samples for this research.

Thanks are also due to Drs. Thomas Boutton, David A. Zuberer and Scott Senseman for serving on the advisory committee and offering suggestions and comments during various phases of this project. They provided constructive suggestions throughout the preparation of this dissertation. Dr. Boutton also performed the sample preparation for ^{13}C determination as well as other suggestions on running the isotope mass spectrometer. I also want to thank Dr. Zuberer for using his lab to determine soil microbial biomass.

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I also gratefully acknowledge the Tom Slick Fellowship that provided one year of financial support during my study.

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CHAPTER I

INTRODUCTION

Concerns about the effect of increasing concentrations of greenhouse gases in the atmosphere on global climate have increased interest in the soil carbon (C) cycle, with a focus on the potential for increasing C sequestration (Lal et al., 2004). The largest pool of terrestrial C is contained in soils. Compared to other ecosystems, agroecosystems are estimated to contain 178 * 10^9 mt C, and are second only to tropical forests which have approximately two times the area of agroecosystems (Schlesinger, 1997). On the other hand, agroecosystems are more manageable than other ecosystems. Furthermore, soil organic matter (SOM) plays essential roles in soil nutrient cycling as well as in soil physical, chemical, and biological properties. Therefore, a better understanding of SOM dynamics in agroecosystems as affected by various agricultural strategies is necessary.

LITERATURE REVIEW

Effect of Tillage on Soil Organic Matter

SOM storage is not only determined by intrinsic soil properties as well as environmental factors, but also by management strategies. Conventional tillage (CT) has caused reductions in C contents of agricultural soils by leading to increased decomposition rates and redistribution of C (Christensen, 1996). These reductions can be mitigated by utilizing sustainable management practices such as reduced tillage,

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decreased bare fallow, increased residue input and conversion to perennial vegetation (Paustian et al., 1997). Compared to other management practices, conservation tillage has received increasing focus due to its potential to increase soil C sequestration. A commonly accepted definition of conservation tillage is any tillage system that results in at least 30% of the soil surface covered with residues after a crop is planted (Unger, 1990). In the southern USA, Franzluebbers et al. (1994) reported that after 9 years, SOC under no tillage (NT) was 9% greater in continuous wheat [*Triticum aestivum* (L.)], 22% greater in rotated wheat-sorghum [*Sorghum bicolor* (L.) Moench.], and 30% greater in continuous wheat-soybean [*Glycine max* (L.) Merr.] than under CT and suggested that accumulation of SOC under NT compared to CT increased with increasing cropping intensity. Similar results have also been observed in other regions. Balota et al. (2004) found that no tillage practices increased total SOC concentrations over CT by 45, 34, and 14%, in 0- to 50-, 50- to 100, and 100- to 200-mm depths, respectively, in a tropical Oxisol.

Conservation tillage not only affects SOM storage, but also its distribution and turnover in different pools. Soil organic matter conceptually is divided into several pools with different turnover rates, although SOM represents a continuum. In general, SOM is often divided into active, slow, and passive pools. Active, or labile, pools are sometimes selected to reflect early changes in SOM due to management practices. Soil microbial biomass (SMB) is one of the labile pools which has been widely used as an early indicator of change in SOM. Many studies have reported that NT significantly increases SMB more than CT, especially in surface soil (Feng et al., 2003; Needelman et al., 1999; Saffigna et al., 1989). Similarly, Balota et al. (2004) observed that NT resulted in a significant increases in SMB in all crop rotations averaged across all depths compared to CT in a Brazilian Oxisol after 20 yrs. However, the greatest differences between NT and CT occurred in the surface soil layer (0 to 5 cm). Tillage not only affected the amount of SMB, but also the structure of the soil microbial community. Frey et al. (1999) reported that fungal abundance in surface soil (0 to 5 cm) in the southern USA was significantly higher in NT than CT at all sites, but significantly higher bacterial abundance was only observed in two of four sites. At 5 to 15 cm, no significant difference was observed for either bacterial or fungal abundance. In an early review of long-term effects of agricultural systems on soil biochemical and microbial parameters, Dick (1992) summarized that soil urease, acid phosphatase, protease, dehydrogenase, arylsulfatase, invertase, and amidase activities in surface soils were significantly higher in NT soils compared to plowed soils. Further, this author also reported the opposite result with increasing depth in several reviewed studies. Feng et al. (2003), using the phospholipid ester-linked fatty acid (PLFA) method, found that the soil microbial community shifted over time with soil depth to a greater number of soil bacteria. The above changes in SMB may be a consequence of positional differences in plant residue return and the soil physical environment resulting from different tillage management.

Compared to CT, plant residues are usually left on the soil surface with NT. Increased surface accumulation of residues may reduce gas and energy exchange between the soil surface and the atmosphere (Grant et al., 1997). A number of studies have documented effects of tillage practices on several soil water, temperature, and aeration regimes (Black, 1973; Licht and Al-Kaisi, 2005). Franzluebbers et al. (1995b) found that soil water content under NT was greater than with CT in surface soil during the fallow period of sorghum-wheat/soybean, and greater or equal water content as CT was observed at deeper depths. Soil temperature was lower under NT than CT, and bulk density was greater under NT than CT. Similar results were estimated by the mathematical model, *ecosys*, with elevated CO_2 (Grant et al., 1997).

Particulate organic matter (POM) is significantly affected by tillage practices. After examining tillage treatments, Cambardella and Elliott (1992) reported that POM under NT was 36% greater than with CT to a depth of 20 cm in a Duroc loam in Sidney, NE after 20 years of treatment imposition. Furthermore, these researchers suggested that decomposition even in a stubble-mulch treatment was more rapid than in NT, considering that the inputs from crop production were not different between tillage treatments. This hypothesis was partially supported by significantly higher C/N (20:1) under NT than with CT (14:1) for POM. In general, fresh plant residues have higher C/N ratios depending on specific species compared to more decomposed materials. The C/N ratio decreases with decomposition, and usually approximates 10:1 for highly humified materials. Therefore, higher C/N ratio may indicate slower decomposition or residues in an early stage of decomposition. However, significantly higher C/N for POM was only observed in surface soil (0 to 5 cm) by Franzluebbers and Arshad (1997) in four sites in northern Alberta and British Columbia. These authors found no significant differences when C/N of POM was averaged to 200-mm depth. One possible reason for the observed difference may be due to colder weather because soil temperature, moisture, or substrate availability between tillage treatments could affect POM decomposition (Cambardella and Elliott, 1992).

Other labile pools, such as mineralizable and hydrolyzable SOM pools, are changed by tillage treatments as well. Using aerobic incubation at 25 ^{0}C for 24 days, Franzluebbers et al. (1994) observed that mineralizable C was significantly greater under NT than CT for several cropping sequences, especially in surface soil. Because slow and passive C pools contribute less to short-term incubation, results of mineralizable C usually mirror changes in POM or SMB. Compared to mineralizable C, estimates of minerlizable N are frequently more complicated and more variable due to N reimmobilization and relatively low concentration. Studies on effects of tillage treatments on soil hydrolyzable C are few compared to other labile C pools. One possible reason may be that this C pool includes all labile C pools as well as part of slow C pools after 18 hr of digestion at high temperature using strong acid. Another reason may be the relatively complicated procedures and specific equipment used.

Compared to labile C pools, slow and passive C pools are thought to be more stable and less affected by cultivation. Slow and passive C pools are conceptual terms. Our discussion of these pools will focus mostly on mineral-associated C and resistant organic C (ROC). In fact, although mineral-associated C is considered a protected pool, it is still affected by tillage practices. Cambardella and Elliott (1992) reported that mineral-associated C was 2492, 2813, 2803, and 2566 g m^{-2} for bare fallow, stubble mulch, no till, and native sod, respectively. In this case, CT significantly reduced the pool size of this slow C pool. Conversely, NT stored more C in this fraction than native sod which was thought to be near equilibrium for C dynamics. These researchers suggested that the enrichment in mineral-associated C may have resulted from decomposition of the POM originally contained in the grassland soils when first plowed and the subsequent movement of this organic matter into the slow C pool.

Resistant organic C is defined as unhydrolyzable C after acid hydrolysis for 18 hrs (Rovira and Vallejo, 2002). The turnover rate for this C pool is much slower than other C pools. According to the results of ^{14}C dating by Paul et al. (1997), the turnover time of this resistant C pool averages more than one thousand years and increases with depth. However, the quantity of C is still affected by cultivation. After examining nine soils from long-term experimental sites in the Corn Belt region of the East-Central United States, Collins et al. (2000) found that ROC was significantly greater under NT than CT at 0 to 5 cm. These authors also reported that the proportion of SOC as ROC decreased with soil depth. Similar results were also reported by Follet et al. (1997).

Cropping System and Fertilization

Compared to tillage practices, cropping system affects SOM and its different pools mainly through quantity and quality of plant residue (Janzen et al., 1997). SOM levels depend on the balance between soil respiration and annual C inputs to soil. Therefore, soil with more C inputs will have more SOM if all other conditions are similar. Larson et al. (1972) reported that SOM in agroecosystems is positively correlated with the amount of C inputs. However, plant residue quality such as lignin content or the ratio of lignin to N also affects decomposition, and therefore, amount of SOM. Parton et al. (1994) hypothesized that lignin concentration is a species-specific characteristic and controls decomposition rates. Using the conceptual model, CENTURY, Schimel et al. (1994) found that soil C storage was linearly related to lignin content over the range of lignin values simulated. Furthermore, cropping system changed soil C pools, especially labile C pools. After reviewing the effect of crop rotation on soil biochemical and microbial parameters, Dick (1992) summarized that intensified crop rotation results in significantly higher levels of microbial biomass and soil enzyme activities than cropping sequences that are either continuously monocultured or have more limited crop rotations. Similar results were also reported by Franzluebbers et al. (1994), and these authors suggested that the difference in SMB was partially caused by higher SOC from enhanced cropping intensity compared with continuous monoculture.

Nitrogen fertilization usually has a positive effect on SOM. A number of studies have shown that alleviation of nutrient deficiencies by the addition of nutritive amendments can enhance crop residue inputs, and consequently SOC content (Campbell et al., 1991b; Christensen, 1986; Franzluebbers et al., 1994). However, Halvorson et al. (2002) reported that although N addition increased the mass of crop residue returned to the soil, it generally did not increase SOC sequestration in the examined cropping systems. Therefore, the influence of N addition on soil C storage may vary with other factors, such as soil texture or climate.

Research Methods

Chemical procedures have long been used to explore SOM dynamics. Soil organic matter may generally be divided into three major categories: the fulvic acid fraction that is soluble in both acid and alkali; the humic acid fraction that is soluble in alkali and insoluble in acid; and the humin fraction that is insoluble in both acid and alkali (Oades, 1989). The principle behind the use of chemical analysis is that chemical characteristics affect SOM transformation or decomposition. For example, ROC extracted by chemical procedures has a turnover time of over thousand years, as dated with ^{14}C techniques (Paul et al., 1997). Although humic molecules are undoubtedly more recalcitrant natural biopolymers, their intrinsic chemical recalcitrance does not account for the observed stabilization of organic matter in soils (Golchin et al., 1997). Supporting work reported by Duxbury et al. (1989) found that old humic acid fractions of SOM, with ages in the range of thousands of years, have half lives on the orders of weeks when extracted and added to unextracted soils. Therefore, Ladd and Foster (1996) suggested that studies of C dynamics in soils involving extractive chemical procedures have not provided much useful information.

Alternatively, physical fractionation methods have been extensively used in the last two decades. These methods are based on the assumption that SOM dynamics are mainly controlled by turnover of soil aggregates. Aggregate hierarchy has been used to explain SOM storage and distribution. In a recent review, Jastrow and Miller (1997) summarized this hypothesis of aggregate hierarchy. In soils where OM is the major binding agent, primary particles are bound together with bacterial and fungal debris into extremely stable silt-sized microaggregates (2- to 20-μm diameter), which may be bound together with additional debris and fragments into larger microaggregates (20- to 50-μm diameter). These microaggregates, in turn, are bound into macroaggregates (>250 μm diameter) by transient and temporary binding agents such as fine roots and fungal hyphae. In this conceptual model, organic matter is divided into particulate organic matter and adsorbed organic matter (Golchin et al., 1997). Particulate organic matter can be divided into three categories: free POM, macroaggregate-POM, and microaggregate-POM according to their function in aggregate stabilization, as well as their physical position. Free POM consists of relatively fresh plant root and other residues with slight decomposition, and thus can be floated out of the soil with gentle shaking using a solution of density 1.6 g ml^{-1}. Macroaggregate POM is more decomposed and tightly associated with soil minerals of microaggregates and functions more as the core of macroaggregates than does free POM. Compared to the extraction of free POM, more energy should be used to separate macroaggregates to obtain occluded POM. A number of researchers indicate that this fraction is responsible for C loss during conversion of pasture or forest to cultivated soils. Microaggregate POM is usually separated by ultrasonic energy and consists of materials with various densities ranging from 1.6 to 2.0 g ml^{-1} depending on the degree of decomposition. However, soil POM is a continuum from relatively fresh plant residues to deeply degraded residues, and therefore, the above definition of POM is not absolute. Recently, Six et al. (2000a) proposed additional mechanisms of C storage under NT. These authors suggested that new microaggregates can be produced within macroaggregates. Compared to CT, more new microaggregates can be formed under NT, and therefore more C may be sequestered under NT because the C associated with microaggregates is more stable.

Isotopes, especially stable isotopes such as ^{13}C have been coupled with physical fractionation techniques to study soil C turnover. Boutton (1996) and Balesdent and Mariotti (1996) reviewed the theory, techniques, case studies, and issues on ^{13}C fractionation during decomposition as well as environmental factors. The basic principle behind the ^{13}C natural abundance technique is related to a change from C3 to C4 vegetation or vice versa. C3 and C4 plants have different ^{13}C discrimination, or fractionation, because of different photosynthetic pathways. The extent of discrimination against ^{13}C is greater for C3 than C4 plants. Plants with the C3 photosynthetic pathway have ^{13}C values ranging from approximately -32 to -22 ‰, with a mean of -27‰, and the corresponding values for C4 plants is -17 to -9‰, with a mean of -13‰. These authors have also indicated that some variations of ^{13}C abundance in plant residues are also caused by other environmental and biological factors (Fu et al., 1993). In addition, soil microbes and macrofauna contribute to ^{13}C fractionation during decomposition. Thus, final data analysis should interpret these effects to help explain C turnover. Boutton (1996) used ^{13}C and physical fractionation methods to study SOC dynamics of savanna ecosystems in the subtropical Rio Grande Plains of southern Texas. The results showed that the sand fraction contained new C derived from the current C3 woodland vegetation and conversely, older C derived from previous C4 grassland was mainly found in finer fractions. Similar results were reported by Jastrow et al. (1996b).

OBJECTIVES

Access to a long-term experiment in the southern USA with treatments of cropping sequence (wheat, sorghum, and soybean systems), tillage (CT and NT), and N fertilization (with and without) provides an opportunity to explore the effects of agricultural practices on soil C storage and turnover. Specifically, we wanted to evaluate the effects of cropping sequences, tillage, and N fertilization on: i) SOC and total N storage, as well as C sequestration rates in the last decade, ii) labile SOC pools such as SMB, mineralizable C and N, POM C, and hydrolyzable C, iii) slow and ROC pools (mineral-associated organic C and unhydrolyzable C), iv) C distribution in physical size fractions, and v) C turnover rates and pool sizes using a long-term incubation technique.

CHAPTER II

SOIL ORGANIC CARBON AND NITROGEN STORAGE AS AFFECTED BY TILLAGE, CROPPING SEQUENCE, AND NITROGEN FERTILIZATION

INTRODUCTION

Accumulation of soil organic matter (SOM) affects soil quality, greenhouse gas mitigation, and agricultural ecosystem dynamics (Lal et al., 2004). SOM storage is determined not only by intrinsic soil properties as well as environmental factors, but also by management practices. Management practices that may increase SOM storage include conservation tillage, especially no-till (NT), enhanced cropping intensity, and fertilization. Increasing SOM accumulation by NT compared to conventional tillage (CT) has been extensively reported (Lal et al., 2004). However, the potential of NT to sequester C is not unlimited since previous studies have reported that soil organic C (SOC) concentration reaches a maximum peak, or a new equilibrium, one or more decades after initiation of NT. West and Post (2002), summarizing global data, reported that C sequestration rates, with a change from CT to NT, can be expected to peak in 5 to 10 yr, with SOC reaching a new equilibrium in 15 to 20 yr. Furthermore, these authors also indicated that following initiation of an enhancement in rotation complexity, SOC may reach a new equilibrium in approximately 40 to 60 yr. These conclusions, however, are based on global data, and specific SOM accumulation and dynamics are affected by various factors such as soil, climate, and management practices, etc. Therefore, to better manage SOM, more specific information is needed.

In this study, our objectives were to explore: (i) the response of SOC and soil N to tillage, cropping sequence, and N fertilization, and (ii) to estimate average SOC accumulation rates.

MATERIALS AND METHODS

Crop Management and Site Characteristics

A long-term field experiment was initiated in 1982 in the Brazos River floodplain in south-central Texas ($30^0$32'N, $94^0$26'W). Sorghum [*Sorghum bicolor* (L.) Moench.] was managed under CT and NT in continuous sorghum (CS), and rotated wheat (*Triticum aestivum* L.)/soybean [*Glycine max* (L.) Merr.]-sorghum (SWS) cropping sequences. Soybean was managed under CT and NT in continuous soybean (CS), continuous wheat/soybean (WS), and rotated SWS cropping sequences. Wheat was managed under CT and NT in continuous wheat (CW), WS, and rotated SWS cropping sequences. Crop growing seasons were from early November to late May for wheat, early June to late October for soybean, and late March to late July for sorghum. Continuous monocultures produced one crop each year, WS produced two crops each year, and SWS produced three crops every two years. Cropping intensity was defined as the fraction of the year when a crop was growing, and was 0.42 for continuous sorghum, 0.38 for continuous soybean, 0.5 for CW, 0.65 for SWS, and 0.88 for WS. Nitrogen fertilizer (NH_4NO_3) was broadcast on wheat at 0 or 6.8 g N m^{-2} during late winter or early spring. Soybean did not receive N fertilizer, while sorghum received 0 or 9 g N m^{-2} banded preplant.

The experimental design was a split-split plot within a randomized complete block design, with cropping sequence as the main plot, tillage treatment as the sub plot, and N fertilizer rate as the sub-sub plot. Plots measured 4 x 12.2 m, and treatments were replicated four times.

The soil used is classified as Weswood silty clay loam (fine-silty, mixed, superactive, thermic, Udifluventic Haplustepts) and contains an average of 115, 452, and 433 g kg^{-1} of sand, silt, and clay, respectively. Under cultivation, this soil has a pH of 8.2 (1:2, soil:water) and an organic C content of approximately 8 g C kg^{-1} soil. Annual temperature is 20 ^{0}C and annual rainfall is 978 mm.

Soil Sampling and Stover Harvest

Soil samples were collected shortly after wheat, sorghum, and soybean harvesting in May, August, and October 2002, respectively. Individual samples consisted of 25 composited cores (19-mm diameter) per plot that were divided into depth increments of 0 to 5, 5 to 15, and 15 to 30 cm. 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 ^{0}C. A portion of the sieved, moist soil was also dried at 60 ^{0}C for 48 h for chemical analysis.

Wheat and sorghum stover yields in 2002 were estimated by hand harvesting 2 m^2 or 3 m^2 of each plot, respectively, at ground level, removing panicles, weighing, drying at 60 ^{0}C for 48 h, and reweighing. Soybean stover was not harvested for yield determination.

Soil Organic C and N

Soil that had been dried at 60 ^{0}C was further ground to pass a 0.5-mm screen and was analyzed for SOC by the modified Mebius method (Nelson and Sommers, 1982) and total

N (TN) following the procedure of Gallaher et al. (1976). Ammonium-N was determined from the diluted digest using an automated salicylic acid modification of the indophenol blue method (Technicon Industrial Systems, 1977a).

Carbon Sequestration Rates

Annual sequestration rates for SOC since 1992 were calculated based on the equation proposed by West and Post (2002):

$$\Delta SOC_R\%\ yr^{-1} = \{[(NT_2 - CT_2) - (NT_1 - CT_1)]/(NT_1 - CT_1)/(t_2 - t_1)\} * 100 \qquad (2\text{-}1)$$

where NT_1 and NT_2 and CT_1 and CT_2 are SOC under NT and CT measured in 1992 and 2002, respectively; SOC_R is the estimated annual rate of soil SOC sequestration; and t_1 and t_2 are the respective years of soil sample collection (1992, 2002). Because soil sample depths in 1992 and 2002 were from 0 to 5, 5 to 12.5, and 12.5 to 20 cm, and 0 to 5, 5 to 15, and 15 to 30 cm, respectively, only data from 0 to 15 cm were used. Data collected in 1992 from 5 to 12.5 cm was normalized to 5 to 15 cm, assuming soil bulk density and SOC concentration were the same for these two depths. Soil bulk density values used in 2002 were those of Franzluebbers et al. (1994). Soil samples used to estimate C sequestration rates were collected after wheat or sorghum harvest. Samples collected in 1992 represented 10 years of treatment imposition.

Statistical Analysis

Analysis of variance, correlation, and regression were conducted as appropriate (SPSS, 2001). All differences discussed are significant at the $P<0.05$ probability level, unless otherwise stated. Fisher's protected least significant difference was calculated only when the analysis of variance F-test was significant at P<0.05.

RESULTS AND DISCUSSION

Sorghum and Wheat Stover Yield

Sorghum stover yield was significantly affected by tillage and cropping sequence. Yield of sorghum stover with CT was significantly greater than for NT, especially for CS (Fig. 2.1). Compared to NT, stover yield under CT was 28 and 5% greater for CS and SWS, respectively. Enhanced cropping intensity with SWS also increased sorghum stover yield. Yield was 18% greater for SWS than for CS. Nitrogen addition did not affect stover yield.

Nitrogen fertilization, however, significantly increased wheat stover yield (Fig. 2.1). Averaged across cropping sequence and tillage, N addition increased wheat stover yield by 70%. Averaged across N application and cropping sequence, yield of wheat stover under NT was significantly higher by 19% than with CT. Our results contrasted with those observed by Fenster and Peterson (1979), who reported that residue inputs from crop production were not different between tillage treatments. In addition, Destain et al. (1989) observed that wheat stover yield under conventional tillage was greater than with minimum tillage in a silt loam soil. One possible reason for these discrepancies may be due to a difference in experimental period. Our study was conducted for 20 years instead of one year for that by Destain et al. (1989). Compared with tillage, N fertilization significantly contributed to observed differences in wheat stover production. Nitrogen

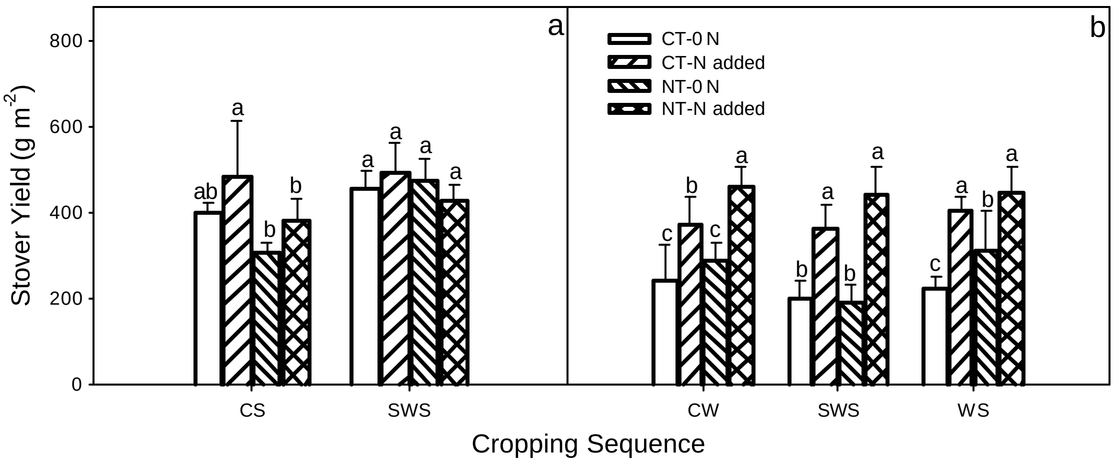

Figure 2.1. Sorghum (a) and wheat (b) residue production in 2002 as affected by tillage, cropping sequence, and N fertilization. CS, CW, SWS, and WS indicate continuous sorghum, continuous wheat, sorghum-wheat-soybean, and wheat-soybean. CT and NT refer to conventional and no tillage. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

addition increased stover yield in all cropping sequences, whether produced with CT or NT, compared to no N controls. Since grain was removed, the difference in wheat stover yield reflected the primary difference in plant residue input, assuming a consistent difference in root production. Root dry matter response to increased soil N level has been reported as positive (Bulisani and Warner, 1980). However, N has also been shown to increase the shoot:root ratio in crops (Reed et al., 1988; Bulisani and Warner, 1980) and this increase is due mainly to the increase in new leaf formation and leaf area expansions (Reed et al., 1988). Moroke (2002) reported that tillage did not significantly affect root length density of cowpea [*Vigna unguiculate* (L.) Walp], sunflower (*Helianthus annuus* L.), and sorghum in Texas during a two-year observation.

Soil Organic C and N

In wheat systems, SOC was affected by two significant interactions in surface soil samples: tillage by N fertilization and tillage by cropping sequence (Fig. 2.2a). With NT, SOC was significantly higher than without N fertilization. The difference was insignificant under CT when averaged across all cropping sequences. Although N fertilization increased the amount of crop residue input with CT (Fig. 2.1), it did not increase concentrations of SOC. SWS and WS under NT, although not different from each other, exhibited significantly higher SOC concentration than CW.

Compared to surface soil (0 to 5 cm), concentrations of SOC in wheat systems decreased with depth (Fig. 2.2b, c). Few differences in SOC concentration between treatments were noted at a depth of 5 to 15 cm (Fig. 2.2b). However, at 15 to 30 cm, NT significantly increased SOC for SWS and WS compared to CT.

Soil total N under wheat showed similar patterns as SOC (Fig. 2.3). The C:N ratio of SOM was > 10:1 at all soil depths (Fig. 2.4), which was slightly greater than 9:1 reported by Franzluebbers et al. (1994) 10 years after treatment establishment.

In sorghum systems, soil organic C in surface soil (0 to 5 cm) varied with tillage, N, and cropping intensity (Fig. 2.2). A significant interaction between tillage and N fertilization was observed (Fig. 2.2a). Under NT, SOC was 20% greater with than without N fertilization in both CS and SWS. This result contrasted with that observed by Franzluebbers et al. (1994), who reported that N fertilization had little effect on SOC under both CT and NT. Nitrogen addition had no effect on SOC with sorghum under CT in the current study.

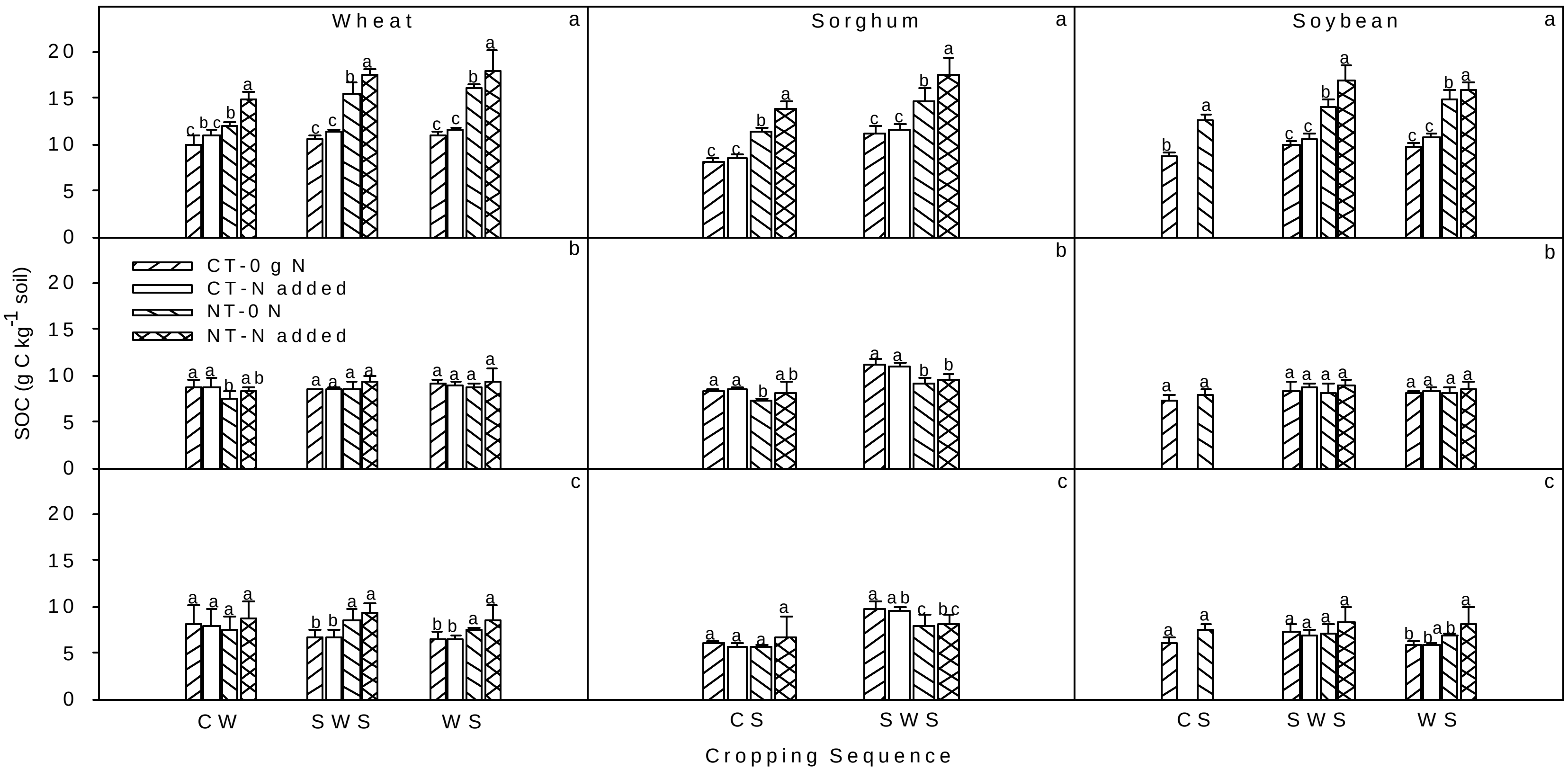

Figure 2.2. Soil organic C (SOC) with depth as affected by cropping sequence, tillage, and N fertilization at a) 0- to 5-, b) 5- to 15-, and c) 15- to 30-cm depths. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage, respectively. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean legends refer to soil samples collected after wheat, sorghum, and soybean harvesting. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

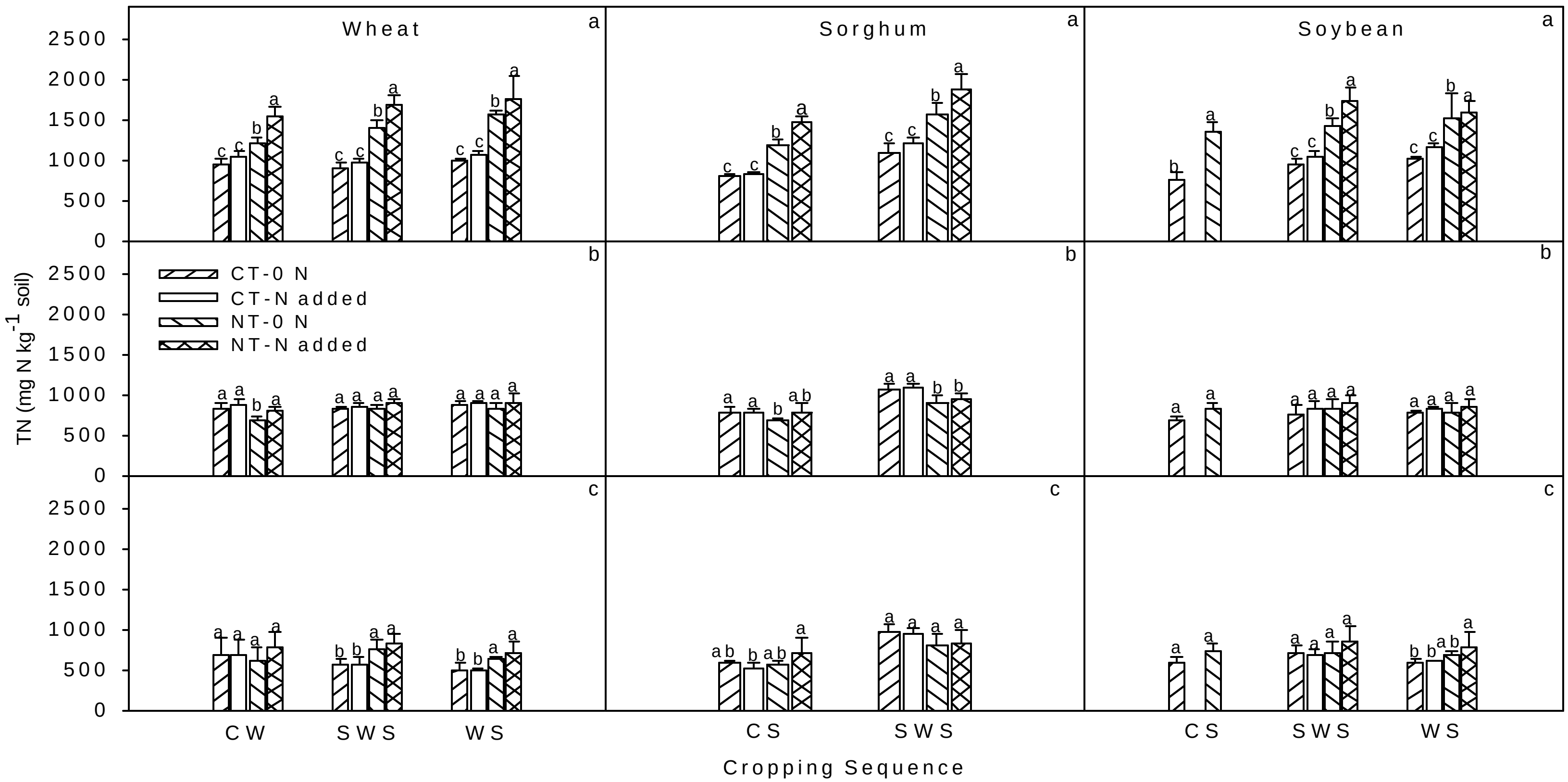

Figure 2.3. Soil total nitrogen (TN) with depth as affected by cropping sequence, tillage, and N fertilization at a) 0- to 5-, b) 5- to 15-, and c) 15- to 30-cm depths. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage, respectively. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean legends refer to soil samples collected after wheat, sorghum, and soybean harvesting. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

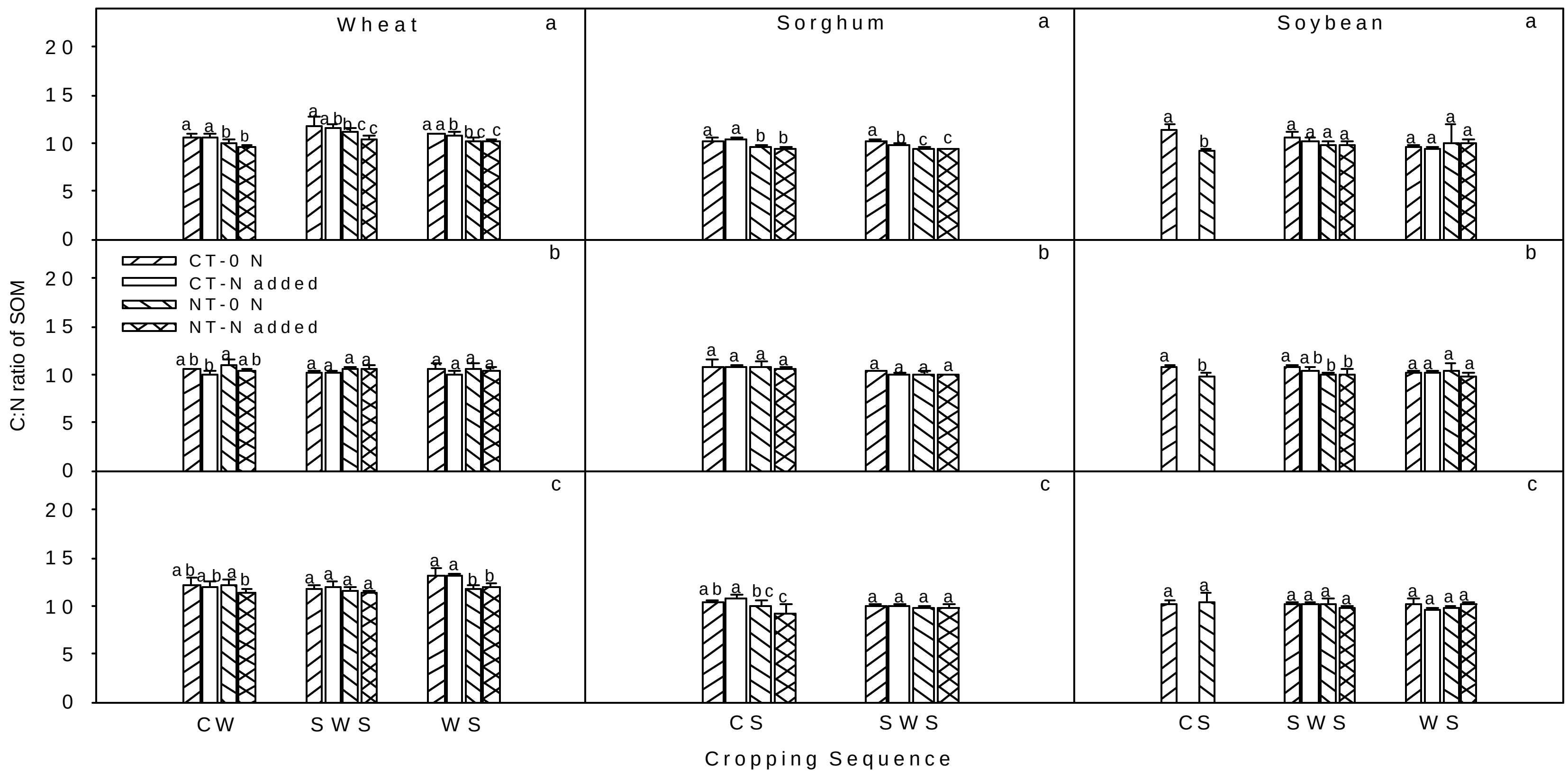

Figure 2. 4. The C:N ratio of soil organic matter (SOM) with depth as affected by cropping sequence, tillage, and N fertilization at a) 0- to 5-, b) 5- to 15-, and c) 15- to 30-cm depths. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage, respectively. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean legends refer to soil samples collected after wheat, sorghum, and soybean harvesting. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at P<0.05.

Compared to CT, SOC under NT with sorghum was 50 and 42% greater for CS and SWS, respectively. Increased cropping intensity significantly enhanced SOC storage under both CT and NT. Soil organic C in SWS was 35% and 28% greater for CT and NT compared with CS, respectively. Using global data analysis, West and Post (2002) reported that both NT and increased crop intensity increased SOC. Furthermore, NT increased SOC over two-fold compared with enhanced crop intensity.

However, SOC with sorghum was lower under NT than CT in deeper soil (5 to 30 cm) (Fig. 2.2b, c). Soil organic C under NT was 9 and 18% lower for CS and SWS, respectively, than with CT at a depth of 5 to 15 cm. The difference was smaller at 15 to 30 cm. The stratification effect of NT on SOC was also observed by other studies (Doran, 1987; Six et al., 2000b).

Soil total N under sorghum was highly related with SOC (data not shown). Therefore, TN showed patterns similar to SOC (Fig. 2.3). The C:N ratio of SOM with sorghum under CT was greater than with NT through all soil depths (0 to 30 cm) (Fig. 2.4). Differences, however, were only significant ($P<0.05$) in surface soil. These results were consistent with our study for wheat, where we also observed greater C:N ratios under CT.

In soybean cropping systems, SOC in surface soil after 20 years was significantly affected by tillage, cropping sequence, and previous N fertilization of crops (Fig. 2.2a). Soil organic C under NT was 45, 49, and 50% higher in CS, WS, and SWS, respectively, than under CT. Averaged across tillage and previous crop-N fertilization, SOC was 20% higher in WS and 21% higher in SWS compared to CS. Overall, previous crop-N fertilization resulted in 3% more SOC than with no N. Soil organic C increased with cropping intensity, but there was no difference between SWS and WS. No major significant differences in SOC were observed among treatments at deeper depths (Fig. 2.2b, 2c). Similar results for TN and C:N ratio of SOM following soybean were observed as in sorghum and wheat systems (Figs. 2.3 and 2.4).

Significant increases in SOC due to NT have been observed by many studies. Sá et al. (2002) reported SOC was significantly higher in soils under long-term NT than under native vegetation and CT treatments in the 0-to 5-cm layer in Brazil. These authors also reported that SOC in the soil surface was significantly higher at 22 years than at 10 years after conversion to NT. Halvorson et al. (2002) also observed similar results in the central Great Plains of the US. Greater crop residue return may partially explain differences in SOC between NT and CT. Under NT, the higher concentration of SOC with N fertilization was consistent with that reported by Christensen (1988), who suggested that mineral fertilizers influence SOM levels by increasing crop productivity, thereby, causing a greater return of plant residues to soil.

As was found in our study, increased cropping intensity has also been reported to increase soil C sequestration (Balota et al., 2004; Campbell et al., 1991a; Paustian et al., 2000; West and Post, 2002). Lal (1997) suggested that cropping sequences and combinations influence SOC contents through their effects on: (i) total amount and rate of biomass production, (ii) shoot:root ratio, and (iii) ratio of C to other nutrients in the biomass. Furthermore, West and Post (2002) suggested that enhancing rotation complexity did not result in as much SOC sequestration as did a change to NT. These authors also observed that enhancing rotation complexity, while already using NT, did not result in a significant increase in SOC. However, our study indicated 34% more SOC in SWS than in continuous sorghum with NT (Fig. 2.2).

Various mechanisms of C accumulation under NT have been proposed. Franzluebbers et al. (1994) reported that the lack of soil disturbance under NT contributed to the accumulation of SOC compared to CT. After investigating a chronosequence of restored tallgrass prairie, Jastrow (1996a) observed that most of the accumulated C occurred in the mineral-associated fraction of macroaggregates. Six et al. (2000a) proposed a conceptual model to explain the loss or accumulation of SOC under CT or NT, respectively. The main mechanism for C sequestration under NT was hypothesized to be due to slowed soil macroaggregate turnover and increased microaggregate formation (Six et al., 2000a). According to this conceptual model, the rate of macroaggregate degradation is reduced under NT compared to CT, resulting in microaggregates in which C is stabilized and sequestered over the long term.

Different crop species also differentially affected SOC storage. SOC with CW was greater than with continuous sorghum or soybean, regardless of tillage treatment (Fig. 2.5). In surface soil, SOC under CT was 25 and 21% greater with CW than with continuous sorghum and soybean, respectively. Under NT, however, the difference in SOC was significantly smaller (Fig. 2.5). Differences decreased with depth and were smallest at 5- to 15-cm soil depth. The return of aboveground crop stover was greater with continuous sorghum than CW. If we that assume these results occur annually, then

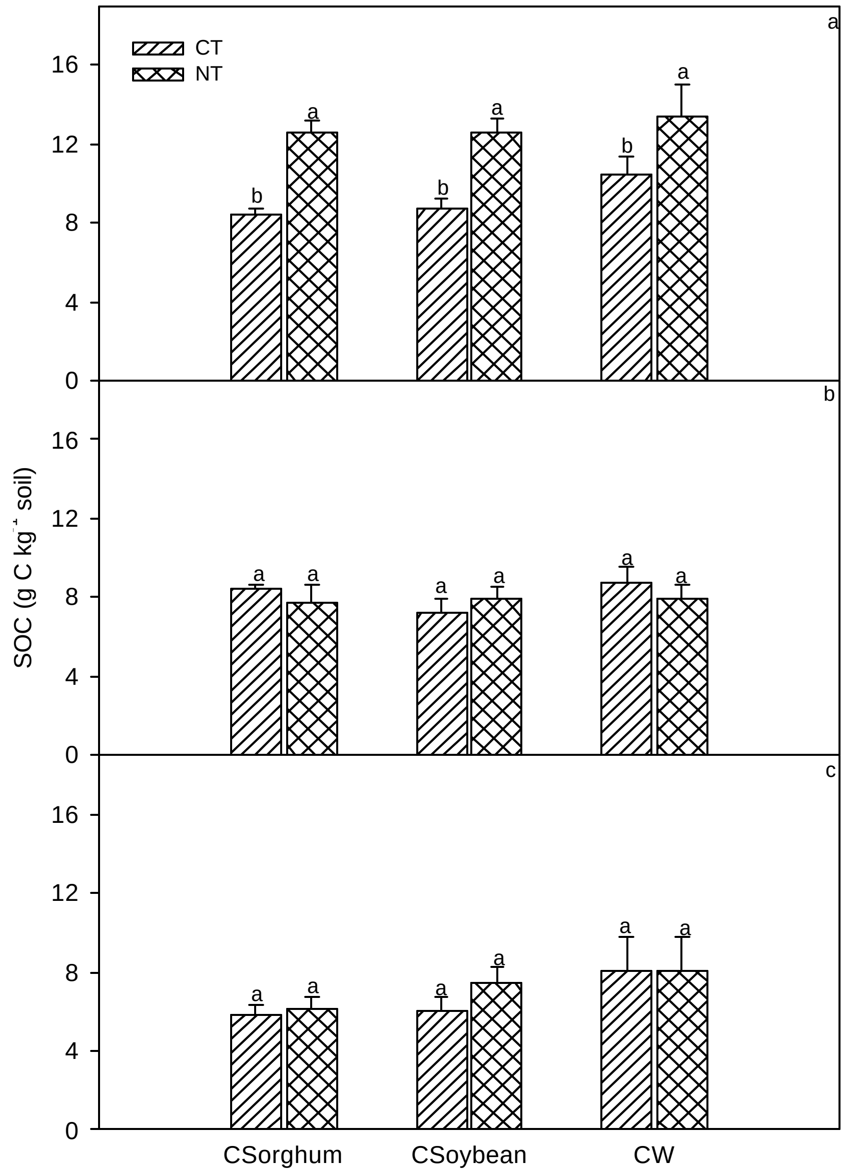

Figure 2.5. Soil organic C (SOC) with depth as affected by cropping sequence, and tillage at a) 0- to 5-, b) 5- to 15-, and c) 15- to 30-cm depths. CSorghum, CSoybean, and CW indicate continuous sorghum, soybean, and wheat, respectively. CT and NT refer to conventional and no tillage. Error bars represent standard deviation. Comparisons are across all crops. Means followed by the same letter are not different at $P<0.05$.

one possible reason for this difference may be due to a difference in crop residue quality, or possibly due to differences in root distribution.

Rate of C Storage

Using the equation suggested by West and Post (2002), C sequestration rates in this study were negative compared with data collected in 1992 (Franzluebbers, unpublished data), except in sorghum systems with N addition (Table 2.1). Nitrogen addition tended to reduce the negative trend. One reason for negative sequestration rates was a decrease over time in the difference of SOC between CT and NT. Concentrations of SOC under CT in 2002 were greater than those in 1992; however, SOC under NT in 2002 was very similar to that in 1992. Thus, according to the suggested equation, it is possible to get low or negative values for C sequestration rate. Soil organic C may have reached saturation or a new equilibrium under NT. Hassink et al. (1997) hypothesized that soil has a finite capability to protect or sequester C. In our case, SOC under NT may have approached or reached carbon saturation, while SOC under CT still has capacity to store C. Our results with NT were consistent with the hypothesis that SOC reaches a new equilibrium in 15 or 20 yrs after converting CT to NT. West and Post (2002) also reported minimal or negative rates of SOC accrual for soil converted from CT to NT after 7 or 10 years. Compared with monoculture, enhanced cropping intensity did not show consistent trends for wheat and sorghum systems with tillage treatments (Table 2.2).

Table 2.1. Annual C sequestration rates for no-till vs. conventional till for sorghum and wheat systems. Values compared at 9 and 20 years after treatment imposition.

Crop Systems	Cropping sequence†	N Addition (g N m^{-2})	C sequestration rate (% yr^{-1})
Wheat	CW	0	-7.5
		6.8	-4.3
	SWS	0	-2.6
		6.8	-0.94
	WS	0	-4.4
		6.8	-2.3
Sorghum	CS	0	-1.04
		9.0	3.9
	SWS	0	-7.1
		9.0	0.9

†CW, SWS, WS, and CS refer to continuous wheat, sorghum-wheat-soybean, wheat-soybean, and continuous sorghum, respectively.

Table 2.2. Annual C sequestration rates for enhanced cropping intensity vs. monoculture across N addition for sorghum and wheat systems. Values compared at 9 and 20 years after treatment imposition.

Crop Systems	Cropping sequence†	Tillage treatment‡	C sequestration rate (% yr^{-1})
Wheat	SWS	CT	232.82
		NT	1.70
	WS	CT	-9.45
		NT	1.54
Sorghum	SWS	CT	1.84
		NT	-0.76

†SWS and WS refer to sorghum-wheat-soybean and continuous wheat-soybean, respectively.

‡ CT and NT refer to conventional and no till.

CONCLUSIONS

Crop stover production was differentially affected by tillage, cropping sequence, and N fertilization for wheat and sorghum. Nitrogen addition significantly increased wheat stover yield regardless of tillage. NT also increased wheat stover yield compared to CT. No significant difference from N fertilization was observed for yield of sorghum stover. In contrast to wheat, NT also slightly decreased sorghum stover yield compared to CT.

Soil organic C and TN showed similar responses to tillage, cropping sequence, and N fertilization in soils collected following wheat, sorghum, or soybean. Most effects were observed in surface soils. NT significantly increased SOC. Nitrogen addition significantly increased SOC under only NT. Compared to NT or N fertilization, enhanced cropping sequences only slightly increased SOC. At a soil depth of 5 to 15 cm, SOC often was slightly greater under CT than NT. Crop species also affected SOC storage. In general, soil cropped to wheat sequestered more C than with sorghum or soybean, especially with CT. Soil TN was highly correlated with SOC regardless of soil depth or crop species. The C:N ratio of SOM in current samples was slightly wider than 10 years earlier.

Based on the assumption that soil bulk density did not change significantly during the past 10 years, the SOC sequestration rate for NT compared to CT generally was negative, indicating that SOC reached a maximum or a new equilibrium between 9 and 20 years after imposition of NT.

CHAPTER III

LABILE SOIL ORGANIC CARBON AND NITROGEN POOLS: RESPONSE TO TILLAGE, CROPPING SEQUENCE, AND NITROGEN FERTILIZATION

INTRODUCTION

The accumulation of soil organic matter (SOM) is critical because SOM plays important beneficial roles in soil physical, chemical, and biological characteristics. Recently, SOM has received additional attention because of the magnitude of terrestrial SOM in global C sequestration and its potential effect on global climate change (Schlesinger, 1997). The quantity of SOM is a balance of net input (such as crop roots and residues) and net output through soil respiration or soil erosion. Therefore, agricultural management strategies which increase organic matter input and/or decrease decomposition are favorable. Conservation tillage, including no tillage (NT), has been used to achieve this objective (Lal et al., 2004). Many studies have reported positive effects of NT on soil organic C (SOC) sequestration. Compared to conventional tillage (CT), NT decreases soil disturbance and increases SOM storage (Franzluebbers et al., 1994; Jastrow and Miller, 1997). However, the potential of NT to sequester more C is limited since previous studies have reported that SOC concentration reaches a peak, or new equilibrium, after one or more decades of imposition. Summarizing global data, West and Post (2002) reported that with a change from CT to NT, C sequestration rates could be expected to peak in 5 to 10 yr, with SOC reaching a new equilibrium in 15 to 20 yr. Those authors also indicated that following initiation of an enhancement in rotation complexity, SOC may reach a new equilibrium in approximately 40 to 60 yr. Therefore, a relatively sensitive index is necessary to measure short-term changes in SOC resulting from different management strategies.

Compared to SOC, labile pools over shorter time periods are preferentially used to estimate longer-term changes in SOC. Soil microbial biomass (SMB), particulate organic matter (POM) C, mineralizable C, and light fraction C have been researched as possible sensitive indicators of changes in SOC. Microbiological properties such as enzyme activities, respiration, and SMBC responded more quickly to environmental conditions than total SOC (Brookes, 1995; Powlson et al., 1987). On chronosequences of reclamation sites, Insam and Domsch (1988) reported that the proportion of SOC as SMBC could be used as a sensitive indicator to determine whether the system is developing or mature. Sparling (1997) proposed that the sensitivity of SMB is likely due to much faster turnover of this pool than SOC. Using a simple size fractionation, Cambardella and Elliott (1992) suggested that POM-C explained most of changes in SOC due to different management strategies. A comparison between labile C pools was made recently by Mclauchlan and Hobbie (2004). These authors suggested that all labile pools, including SMBC, mineralizable C in a 12-day incubation, hydrolyzable C, and light fraction C were positively correlated with each other and increased with SOC. These relationships among labile pools were studied in the northwestern U.S. However, few studies are available on whether these results also occur in warmer, drier portions of the southern U.S. Thus, for a specific site, it may be necessary to select a distinct indicator to reflect changes in SOC status.

The objectives of this experiment were to: (i) determine the effect of tillage, N fertilization, and cropping sequence on various soil labile C pools; (ii) determine relationships between SMBC, mineralizable C in a 24-day incubation, hydrolyzable C, and POM-C; and (iii) determine which of the above indicators is most sensitive to management strategies in our environment.

MATERIALS AND METHODS

Crop Management and Site Characteristics

A long-term field experiment was initiated in 1982 on the Brazos River floodplain in southcentral Texas ($30^0$32'N, $94^0$26'W). Sorghum [*Sorghum bicolor* (L.) Moench.] was managed under conventional (disk) tillage and NT in continuous sorghum (CSorghum), and rotated wheat (*Triticum aestivum* L.)/soybean [*Glycine max* (L.) Merr.]-sorghum (SWS) cropping sequences. Soybean was managed under conventional (disk) tillage and NT in continuous soybean (CSoybean), continuous wheat/soybean (WS), and SWS cropping sequences. Wheat was managed under conventional (disk) tillage and NT in continuous wheat (CW), WS, and SWS cropping sequences. Crop growing seasons were from early November to late May for wheat, early June to late October for soybean, and late March to late July for sorghum. Continuous crops produced one crop each year, WS produced two crops per year, and SWS produced three crops every two years. Nitrogen fertilizer (NH_4NO_3) was broadcast on wheat at 0 or 6.8 g N m^{-2} during late winter or early spring. Soybean did not receive N fertilizer, while sorghum received 0 or 9 g N m^{-2} banded preplant.

A split-split plot within a randomized complete block design was established with cropping sequence as the main plot, tillage as the sub plot, and N fertilizer rate as the sub-sub plot. Plots measured 4 x 12.2 m, and treatments were replicated four times.

The soil used is classified as a Weswood silty clay loam (fine-silty, mixed, superactive, thermic, Udifluventic Haplustepts) and contains an average of 115, 452, and 433 g kg^{-1} of sand, silt, and clay, respectively. Under cultivation, this soil has a pH of 8.2 (1:2, soil:water) and an organic C content of approximately 8 g C kg^{-1} soil. Annual temperature is 20 ^{0}C and annual rainfall is 978 mm.

Soil Sampling

Soil samples were collected shortly after wheat, sorghum, and soybean harvesting in May, August, and October 2002, respectively. Individual samples consisted of 25 composited cores (19-mm dia.) per plot that were divided into depth increments of 0 to 5, 5 to 15, and 15 to 30 cm. 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 ^{0}C. A portion of the sieved, moist soil was also dried at 60 ^{0}C for 48 h for chemical analysis.

Chemical and Biological Analyses

Dried soil was passed through a 2-mm screen and analyzed for initial NH_4^+-N and NO_3^--N concentrations using an automated salicylic acid modification of the indophenol blue method (Technicon Industrial Systems, 1977a) and cadmium reduction method

(Technicon Industrial Systems, 1977b), respectively, following extraction with 2 *M* KCl (1:4 w:v) by shaking for 30 min.

Mineralizable C and N were estimated using a short-term incubation method (Campbell et al., 1991a) with the following modifications. Fifty-gram oven-dry soil samples were placed in 50-mL beakers, brought to a water potential of approximately 50% field capacity, and incubated in 1-L air-tight glass jars in the presence of 10 mL of 1.0 *M* KOH at 25 ^{0}C. Vials of KOH were removed after 1, 7, 17, and 24 days, with evolved CO_2-C determined by titration (Anderson, 1982). Soil subsamples were dried at 60 ^{0}C for 48 h and ground to pass a 2-mm screen. A 7-g portion was extracted in 28 mL of 2 *M* KCl and the extract analyzed for NH_4^+-N and NO_3^--N using autoanalyzer techniques.

Soil microbial biomass C and N were estimated using the chloroform fumigation-incubation method (Jenkinson and Powlson, 1976) with the following modifications. After a 7-day pre-incubation at 50% field capacity, moist soils were fumigated with alcohol-free chloroform and incubated in 1-L air-tight glass jars in the presence of 10 mL of 1.0 *M* KOH at 25 ^{0}C for 10 days. The quantity of CO_2-C absorbed in the alkali during this period was determined by titration (Anderson, 1982). Soil microbial biomass C was determined by dividing the quantity of CO_2-C evolved over 10 days by 0.41 (Voroney and Paul, 1984).

Soil microbial biomass N was determined from the following equation:

$$\text{SMBN} = [(\text{mg NH}_4^+\text{-N kg}^{-1}\text{ soil 10 day}^{-1})_{\text{fumigated}} - (\text{mg NH}_4^+\text{-N kg}^{-1}\text{ soil})_{\text{initial}}]/k_n \quad (3\text{-}1)$$

where, $k_n = 0.41$ (Carter and Rennie, 1982). No significant increase in NO_3^--N occurred in the fumigated sample.

Specific respiratory activity of soil microbial biomass carbon (SRAC) and specific mineralization activity of soil microbial biomass nitrogen (SMAN) were estimated by dividing the net potential microbial activity (i.e., mineralizable C and N) by the size of the microbial pool (i.e., SMBC and SMBN) (Campbell et al., 1991a).

POM C was determined with depth in wheat systems, but only in surface soils (0 to 5 cm) for sorghum and soybean systems. POM was separated from the <4.8-mm soil following the method of Cambardella and Elliott (1992). Subsamples (50 grams) were dispersed in 100 mL of 0.001 *M* $Na_4P_2O_7$ by shaking for 16 h on a reciprocal shaker. The dispersed soil samples were passed through a 53-µm sieve, and retained materials were rinsed several times with water. Material retained on the sieve and the soil slurry that passed through the sieve containing the mineral-associated and water-soluble C and N were both dried in a forced-air oven at 55 ^{0}C. The dried slurry sample was subsequently ground with a mortar and pestle to pass a 0.2-mm sieve and was analyzed for total organic C and N as previously described in other chapters. The difference between the C and N values for the soil slurry and those obtained from a nondispersed, whole soil sample was considered to be equal to the C or N retained on the sieve.

Acid-hydrolyzable organic C which also represents more labile C pools was determined using the method suggested by Rovira and Vallejo (2002) with the following modifications. One g of oven-dry soil subsample passing through 60 mesh was hydrolyzed in 25 mL of 6 *N* HCl on a digestion tube in an aluminum block digestor at

110 ^{0}C for 18 h, with occasional shaking. After cooling, the unhydrolyzed residue was recovered by centrifuging at 2851x g for 20 min and decanting the liquid. The process of washing with deionized water, centrifugation and decantation was repeated several times until neutral pH was reached. The residue was then transferred to a pre-weighed vial, dried at 60 ^{0}C to constant weight, and analyzed for C as previously described. Thus, acid-hydrolyzable C was quantified by the difference between the C values for the residue and those obtained from a whole soil sample.

Statistical Analysis

Analysis of variance, correlation, and regression were conducted as appropriate (SPSS, 2001). All differences discussed are significant at the $P<0.05$ probability level, unless otherwise stated. Fisher's protected least significant difference was calculated only when the analysis of variance F-test was significant at $P<0.05$.

RESULTS AND DISCUSSION

Soil Microbial Biomass C and N

Soil microbial biomass C was more affected by tillage than by crop intensity or N fertilization in wheat, sorghum, and soybean systems (Fig. 3.1). In wheat systems, SMBC under NT was 18, 25, and 13% greater in CW, SWS, and WS, respectively, than with CT at 0- to 5-cm depth, but was 26, 5, and 10% lower in CW, SWS, and WS, respectively, than with CT at 5- to 15-cm depth. At the 15- to 30-cm depth, however, there was no consistent difference between CT and NT. Little difference was noted

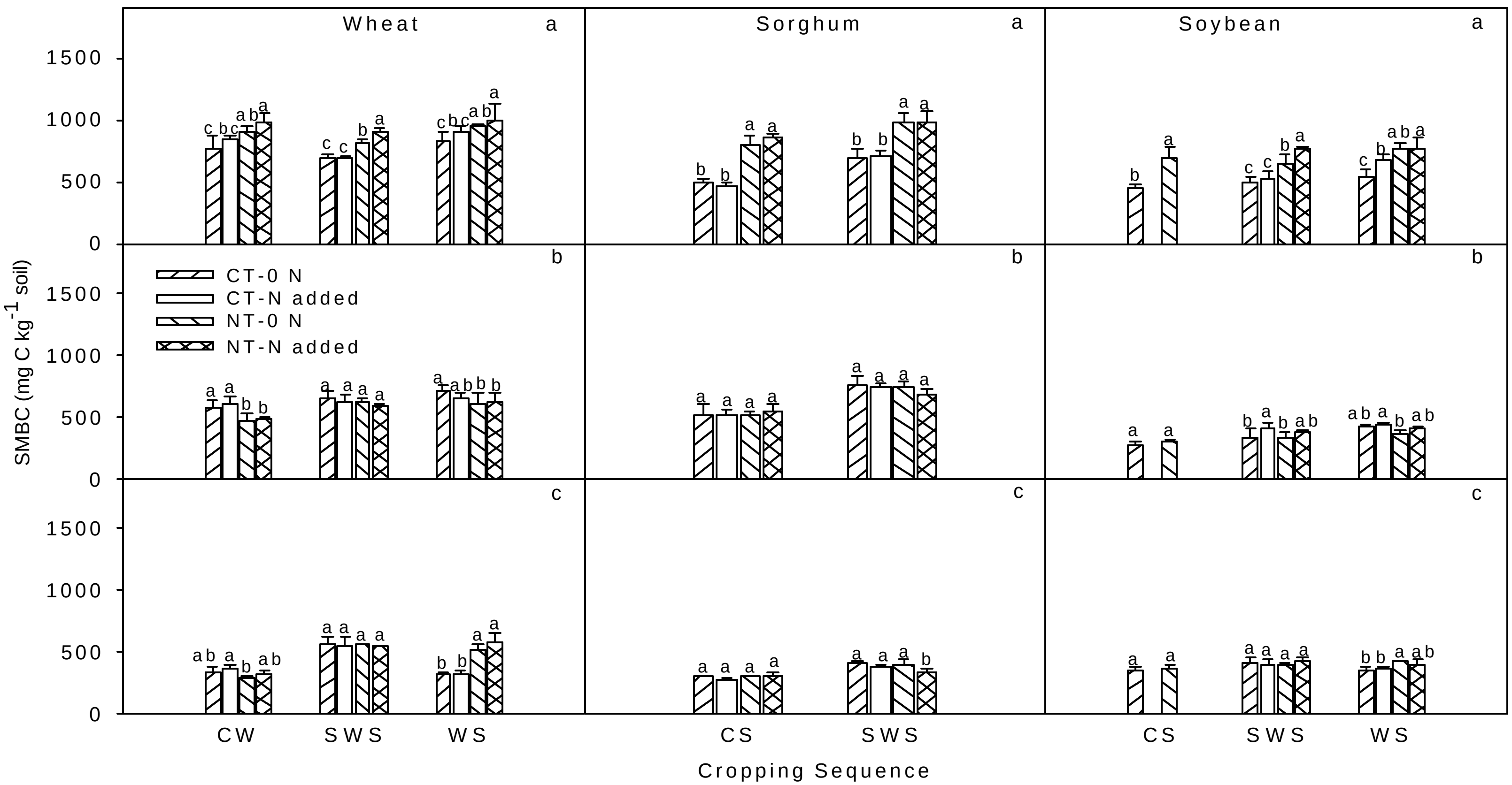

Figure 3.1. Soil microbial biomass C (SMBC) with depth as affected by cropping sequence, tillage, and N fertilization at a) 0- to 5-, b) 5- to 15-, and c) 15- to 30-cm depths. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean samples collected after wheat, sorghum, and soybean harvesting, respectively. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

at this depth, except greater SMBC with NT in WS compared to CT. An important factor may be the effect of surface litter in an NT system. Microbial biomass decreased with soil depth in our study. Balota et al. (2004) suggested that the accumulation of crop residues at the soil surface provides substrate for soil microorganisms, which accounts for the higher SMBC in surface soil under NT.

Nitrogen fertilization also increased SMBC in all crop systems, although the increases generally were not significant. Our results contrasted with those of Ladd et al. (1994), where SMBC was observed to decrease with N fertilization. One indirect reason for this result in their study was due to decreasing soil pH with N fertilization, which may have caused reduced SMBC in acidic soil (Jenkinson and Powlson, 1976). Our soil pH, however, was greater than 7.0.

The effect of tillage and cropping intensity on SMBC also varied with cropping systems. The most significant difference in SMBC between CT and NT was observed in surface soil in sorghum systems, where SMBC under NT was 73 and 40% greater than with CT for CSorghum and SWS, respectively. Increased SMBC with increasing cropping intensity regardless of tillage was also only observed in sorghum systems. Granatstein et al. (1987) observed greater SMBC under CT in an intensive 3-yr rotation with a forage legume than in 1- yr rotations, but no differences were observed among cropping sequences under NT. In contrast, Franzluebbers et al. (1994) reported increases in SMBC with increased crop intensity under both NT and CT in wheat systems. Soil microbial biomass C is generally considered an active pool which is more impacted by factors such as crop and tillage management practices, climate, season, and so on.

Crop species also affected SMBC, especially at 0 to 5 cm (Fig. 3.1). Compared with Csoybean, SMBC under NT without N fertilization was 31 and 15% greater for CW and Csorghum, respectively. Similar results under CT without N fertilization were also observed. Our results of SMBC as affected by crop species were consistent with SOC in surface soils (Chapter II) (Fig. 2.2).

Soil microbial biomass C decreased with depth in all crop systems regardless of treatment (Fig. 3.1). This result was consistent with those reported by Paul and Clark (1989). Using a direct count, these authors reported a linear decrease in SMB with increasing depth in the soil profile.

The effect of tillage on the proportion of SOC as SMBC varied with cropping system (Fig. 3.2). In both wheat and soybean systems, this proportion under CT was greater in all depths than NT except for WS at 15 to 30 cm in wheat system, where this fraction was greater with NT. This result was consistent with that reported by Balota et al. (2004) in a Brazilian Oxisol. Sorghum sequences generally showed little tillage effect on SMBC/SOC. Nitrogen fertilization and cropping intensity did not significantly affect the proportion of SOC as SMBC at any depth in the various cropping systems, except at 15 to 30 cm in the wheat systems.

Unlike SOC or SMBC, the proportion of SOC as SMBC did not change drastically with depth (Fig. 3.2). This proportion in our study was about 5 to 8% for all depths. Our proportions were greater than those reported by several previous studies. Jenkinson and Ladd (1981) reported that with few exceptions, about 2 to 3% of the organic C in European soils was present as SMB in the 10- to 20- cm depth. In tropical soils,

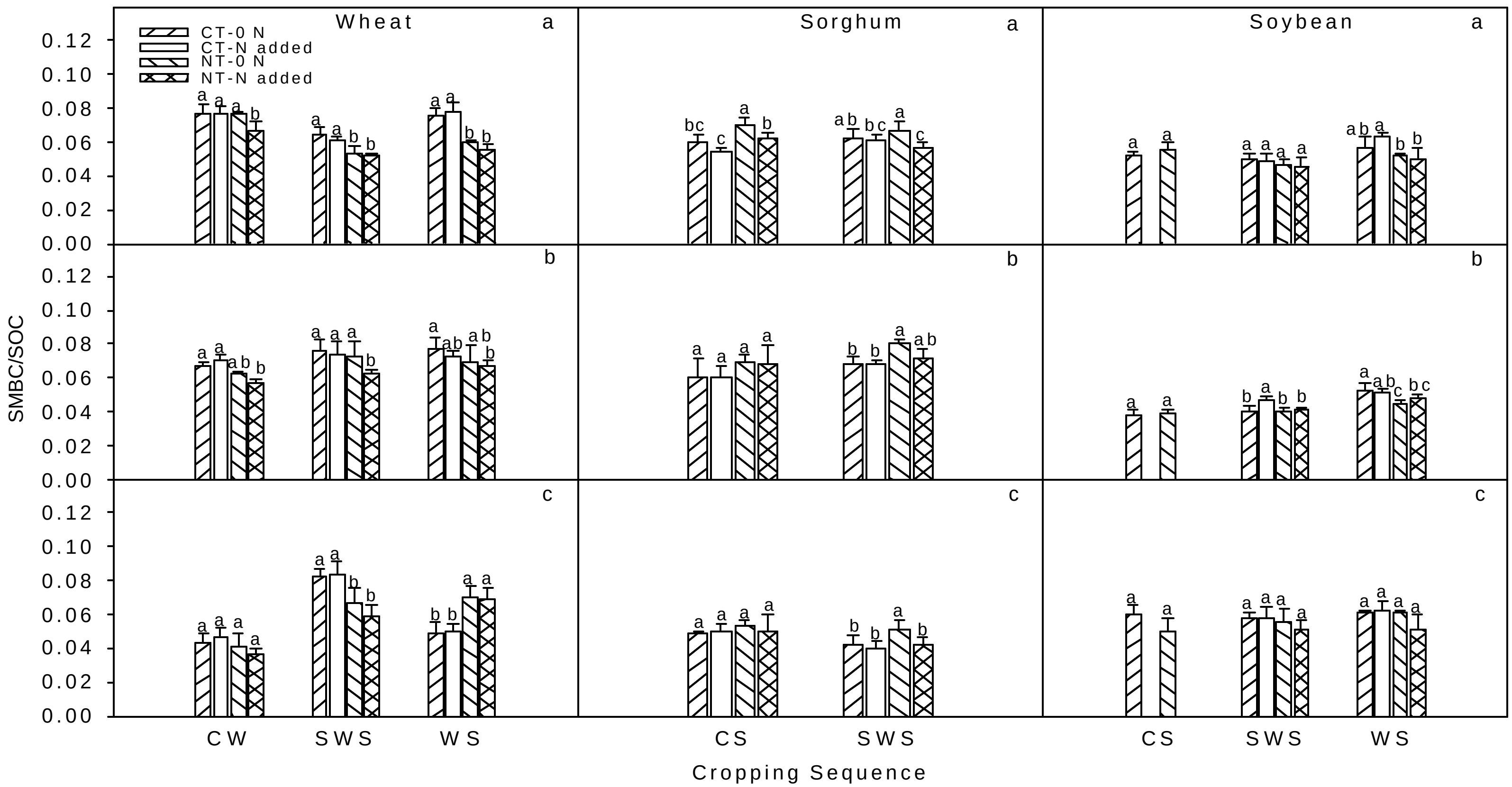

Figure 3.2. The proportion of SOC as soil microbial biomass C (SMBC) with depth as affected by cropping sequence, tillage, and N fertilization at a) 0- to 5-, b) 5- to 15-, and c) 15- to 30-cm depths. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean samples collected after wheat, sorghum, and soybean harvesting, respectively. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

Balota et al. (2004) observed that this ratio was <1.8% in a long-term tillage and crop rotation study. Several possible factors may have resulted in the observed difference. One difference is that previous authors subtracted the control soil respiration from fumigated samples; while we did not. Our calculation also used 0.41 for k_c instead of 0.45 used by other authors. Our results were similar to these reported by Franzluebbers et al. (1994), however, the trends with soil depth were different. No statistical differences were noted among the different soil depths, indicating that similar proportions of SMBC as SOC were present with depth. The activities of soil microbes were different in different depths as will be shown by specific respiratory activity. The proportion of SMBC as mineralizable C decreased with soil depth. In general, situations favoring accumulation of organic matter increase both the amount of SMBC and the proportion on a soil organic matter basis (Jenkinson and Ladd, 1981). In our study, only the former was observed.

SMBN as expected was highly related to SMBC and other SOC pools in wheat, sorghum, and soybean systems (Table 3.1). SMBN was significantly affected by tillage under the three crops. For example in the wheat systems, SMBN under NT was 50, 123, and 108% greater than CT in CW, SWS, and WS at the 0- to 5-cm depth, respectively (Fig. 3.3). Greater differences between NT and CT for SMBN than SOC may reflect that SMBN was more sensitive to management. Across wheat cropping sequences and tillage, N fertilization increased SMBN by 29% compared with the no N control. At the 5- to 15-cm depth, however, NT significantly decreased SMBN compared to CT (Fig. 3.3b).

Table 3.1. Correlation matrix of soil carbon and nitrogen pools at 0 to 5 cm in soil samples following wheat, sorghum, and soybean, respectively (n = 48, 32, and 40 for wheat, sorghum, and soybean, respectively).

		SOC	TN	Soil microbial		Mineralizable		Particulate organic matter C	Hydroly-zable C
				C	N	C	N		
Wheat	SOC†	---	**	**	**	**	**	**	**
	TN‡	0.97	---	**	**	**	**	**	**
	SMBC§	0.66	0.75	---	**	**	**	**	**
	SMBN#	0.96	0.97	0.76	---	**	**	**	**
	Mineralizable C	0.84	0.80	0.59	0.87	---	**	**	**
	Mineralizable N	0.72	0.79	0.80	0.83	0.76	---	**	**
	POMC¶	0.92	0.84	0.52	0.87	0.90	0.65	---	**
	Hydrolyzable C	0.99	0.95	0.64	0.93	0.84	0.69	0.92	---
Sorghum	SOC	---	**	**	**	**	**	**	**
	TN	0.99	---	**	**	**	**	**	**
	SMBC	0.92	0.93	---	**	**	**	**	**
	SMBN	0.99	0.99	0.94	---	**	**	**	**
	Mineralizable C	0.83	0.84	0.85	0.87	---	**	**	**
	Mineralizable N	0.89	0.91	0.84	0.92	0.91	---	**	**
	POMC	0.92	0.93	0.81	0.92	0.83	0.92	---	**
	Hydrolyzable C	0.99	0.99	0.91	0.98	0.86	0.90	0.92	---
Soybean	SOC	---	**	**	**	**	**	**	**
	TN	0.96	---	**	**	**	**	**	**
	SMBC	0.82	0.85	---	**	**	*	**	**
	SMBN	0.70	0.72	0.80	---	**	**	**	**
	Mineralizable C	0.60	0.57	0.42	0.88	---	**	**	**
	Mineralizable N	0.64	0.60	0.37	0.92	0.92	---	**	**
	POMC	0.94	0.88	0.77	0.64	0.60	0.63	---	**
	Hydrolyzable C	0.99	0.94	0.82	0.73	0.55	0.6	0.96	---

†Soil organic carbon.
‡Total nitrogen.
§Soil microbial biomass carbon.
#Soil microbial biomass nitrogen.
¶Particulate organic matter C.
* and ** denote significance at P = 0.05 and 0.01, respectively.

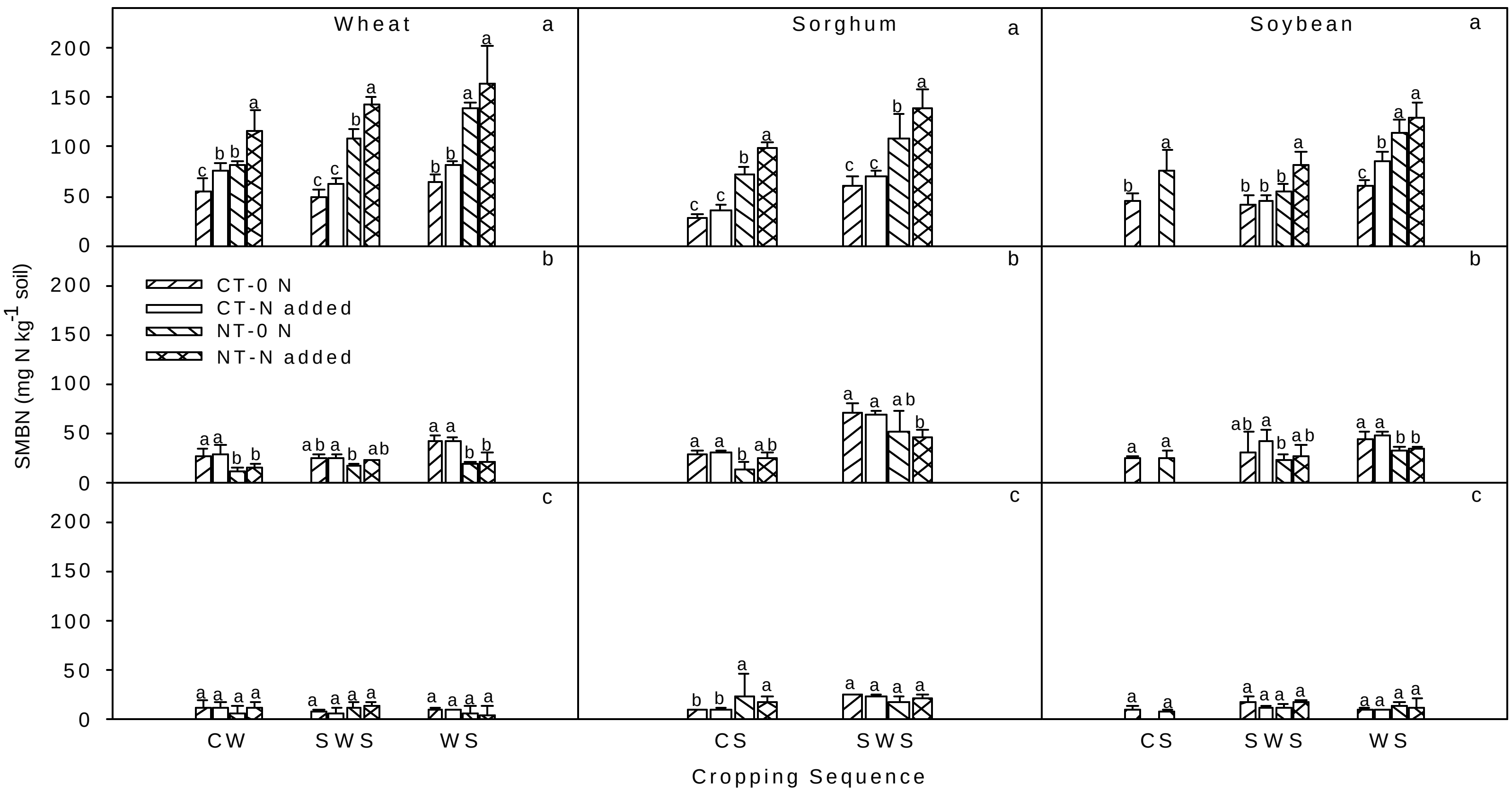

Figure 3.3. Soil microbial biomass N (SMBN) with depth as affected by cropping sequence, tillage, and N fertilization at a) 0- to 5-, b) 5- to 15-, and c) 15- to 30-cm depths. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean samples collected after wheat, sorghum, and soybean harvesting, respectively. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

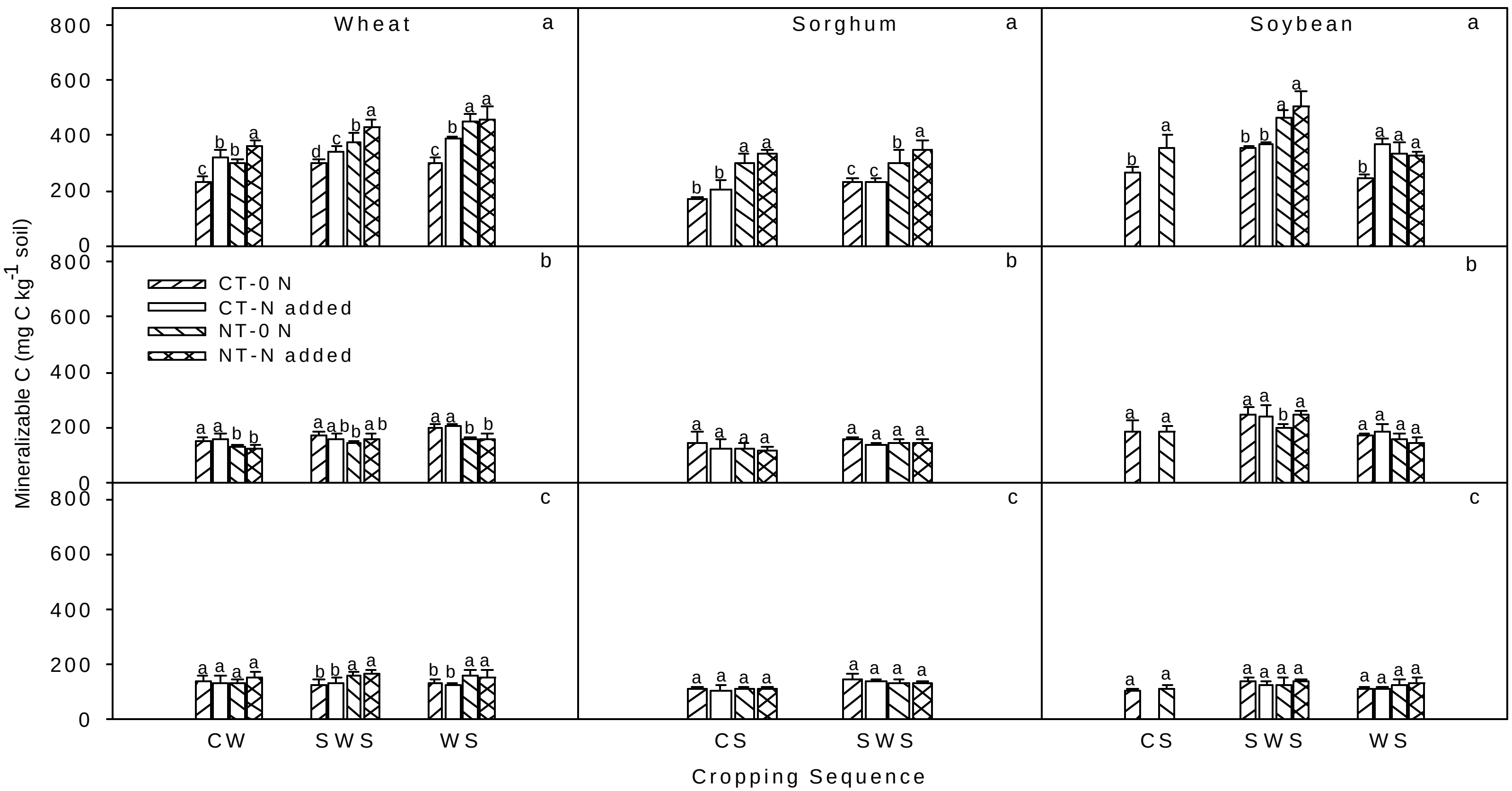

Figure 3.4. Mineralizable C in 24-day incubation with depth as affected by cropping sequence, tillage, and N fertilization at a) 0- to 5-, b) 5- to 15-, and c) 15- to 30-cm depths. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean samples collected after wheat, sorghum, and soybean harvesting, respectively. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

One possible reason may be lower crop residue input with depth under NT compared with CT, where tillage partially added surface crop residue into this depth. No significant differences were observed for depth of 15 to 30 cm. Similar trends were also observed in sorghum and soybean systems.

Unlike SOC or SMBC, soil microbial biomass N decreased faster with depth in all studied crop systems. For example in the wheat systems, SMBN at 5- to 15- and 15- to 30-cm was only 26 and 9% of that in surface soil, respectively. Similar tends were observed in sorghum and soybean systems. The faster decline in SMBN with increasing depth has also beeen observed in other studies (Saffigna et al., 1989; Spedding et al., 2004).

Mineralizable C and N

Mineralizable C was highly correlated with SOC, SMBC, SMBN, POM C, and hydrolyzble C in wheat, sorghum, and soybean systems (Table. 3.1). NT significantly increased mineralizable C in surface soil. Mineralizable C in surface soil increased with increasing cropping intensity under both tillage and N fertilization regimes, except for soybean in WS (Fig. 3.4). This result was consistent with the report of Franzluebbers et al. (1994) who proposed that mineralizable C was sensitive to changes in SOM quantity and quality due to increased crop residue input with increased cropping intensity. This indicated that mineralizable C was more sensitive than SOC or SMBC to management factors. At the 5- to 15-cm depth, mineralizable C was greater under CT than NT in all crop systems. Cropping intensity and N fertilization had little effect on mineralizable C of this depth.

Mineralizable C was also affected by soil depth and crop species. Mineralizable C decreased faster with soil depth than SOC or SMBC. This result was in accordance with the observation that mineralizable C was more sensitive to crop management practices than SOC and SMBC. Further, amount of mineralized C decreased in the order of wheat > soybean > sorghum. The greatest difference in mineralizable C between CT and NT was observed in the sorghum systems at the 0- to 5-cm depth, where NT increased mineralization by 53% compared to CT.

The specific respiratory activity of SMBC (SRAC) was more affected by crop species than tillage or N (Fig. 3.5). Minor differences among tillage, cropping intensity, and N fertilization were observed within sorghum and soybean systems. Greater SRAC under soybean at all depths, especially for SWS, may have been due to differences in crop residue quality. Soybean residues have a narrower C:N ratio than that of wheat or sorghum. Another possible explanation is seasonal changes in SMBC. Soil samples for wheat and sorghum were collected in May and August 2002, respectively, while samples for soybean were taken in October. Compared to May and August, soil was wetter and temperature was milder in October. Therefore, the October condition may have been more suitable for microbial activity. This result is consistent with that observed by Acosta-Martinez et al. (2004). These authors proposed that microbiological properties responded more quickly to environmental conditions than SOC. SRAC also tended to

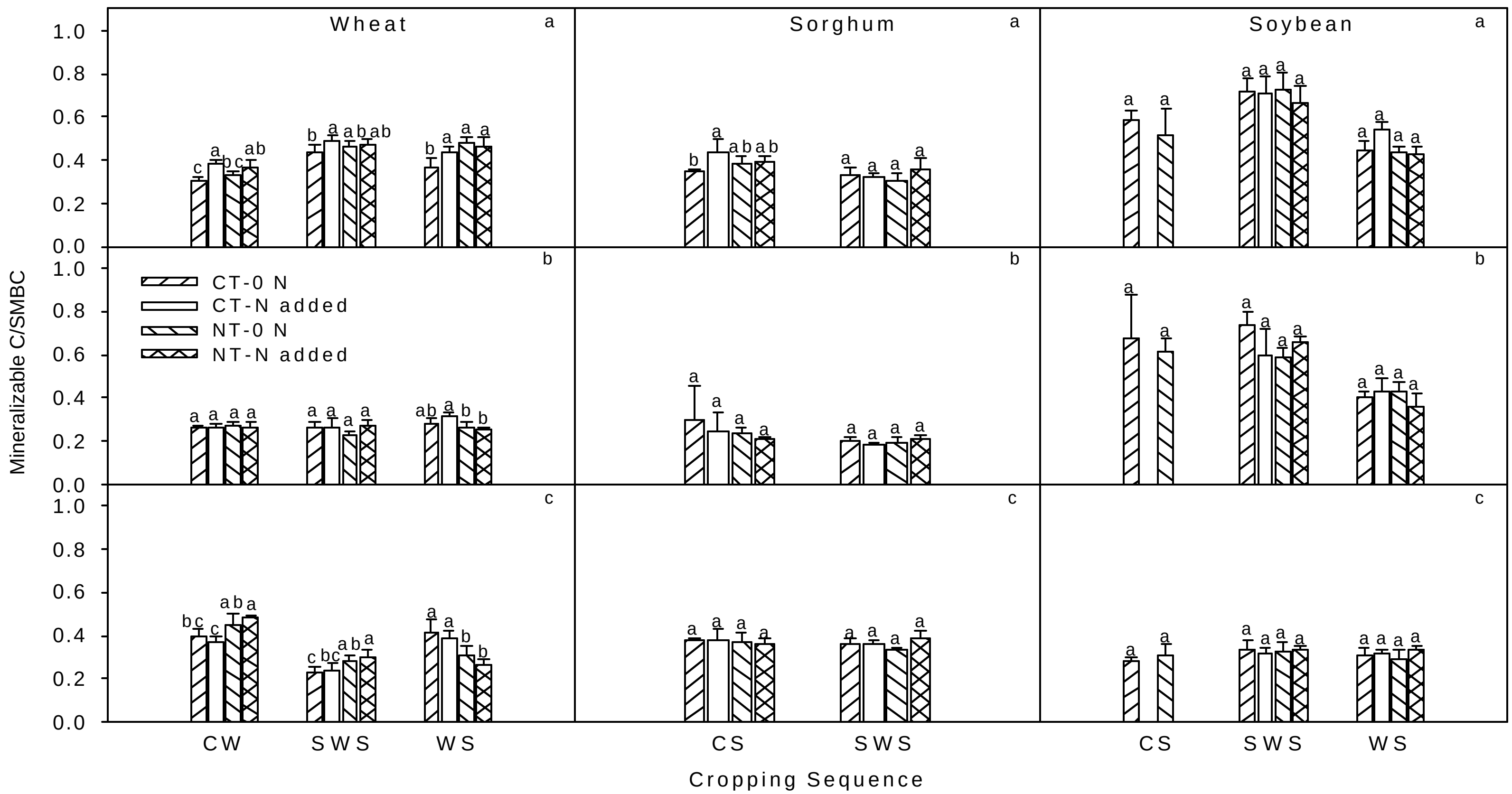

Figure 3.5. Specific respiratory activity of soil microbial biomass C (SMBC) with depth as affected by cropping sequence, tillage, and N fertilization at a) 0- to 5-, b) 5- to 15-, and c) 15- to 30-cm depths. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean samples collected after wheat, sorghum, and soybean harvesting, respectively. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

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decrease with depth in all crop systems. This result contrasted with that reported by Franzluebbers et al. (1994), where an increase with depth was observed. This discrepancy possibly indicates the sensitivity of this index to cropping system, sampling time, and other management practices. Balota et al. (2004) reported lower qCO_2 (an index similar to SRAC) under NT compared with CT in different crop rotations and with depth. However, these authors did not observe a consistent effect of cropping systems on SRAC within either CT or NT.

The pattern of the proportion of SOC as mineralizable C as affected by tillage, cropping intensity, and N fertilization within wheat, sorghum, and soybean systems was similar to that observed for SRAC (Fig. 3.6). The greatest proportion of SOC as mineralizable C was also observed in soybean systems. The difference, however, was smaller among crop systems compared with SRAC. A greater proportion of SOC as mineralizable C in soybean systems may also indicate a difference in SOM quality, as previously mentioned. This parameter was significantly lower under NT than CT for 0 to 5 and 5 to 15 cm samples. This proportion tended to decrease with depth, and may indicate that SOM was less decomposed or SMB was more active in surface soil.

Mineralizable N was highly correlated with mineralizable C in soil from wheat, sorghum, and soybean systems, but was more related to SMBN, indicating that biomass may also serve as a source of labile N (Fig. 3.7, Table 3.1). Since the patterns for N mineralization were similar for all cropping sequences, only results for wheat in SWS were reported. During a 24-day aerobic incubation, most mineralizable N appeared in

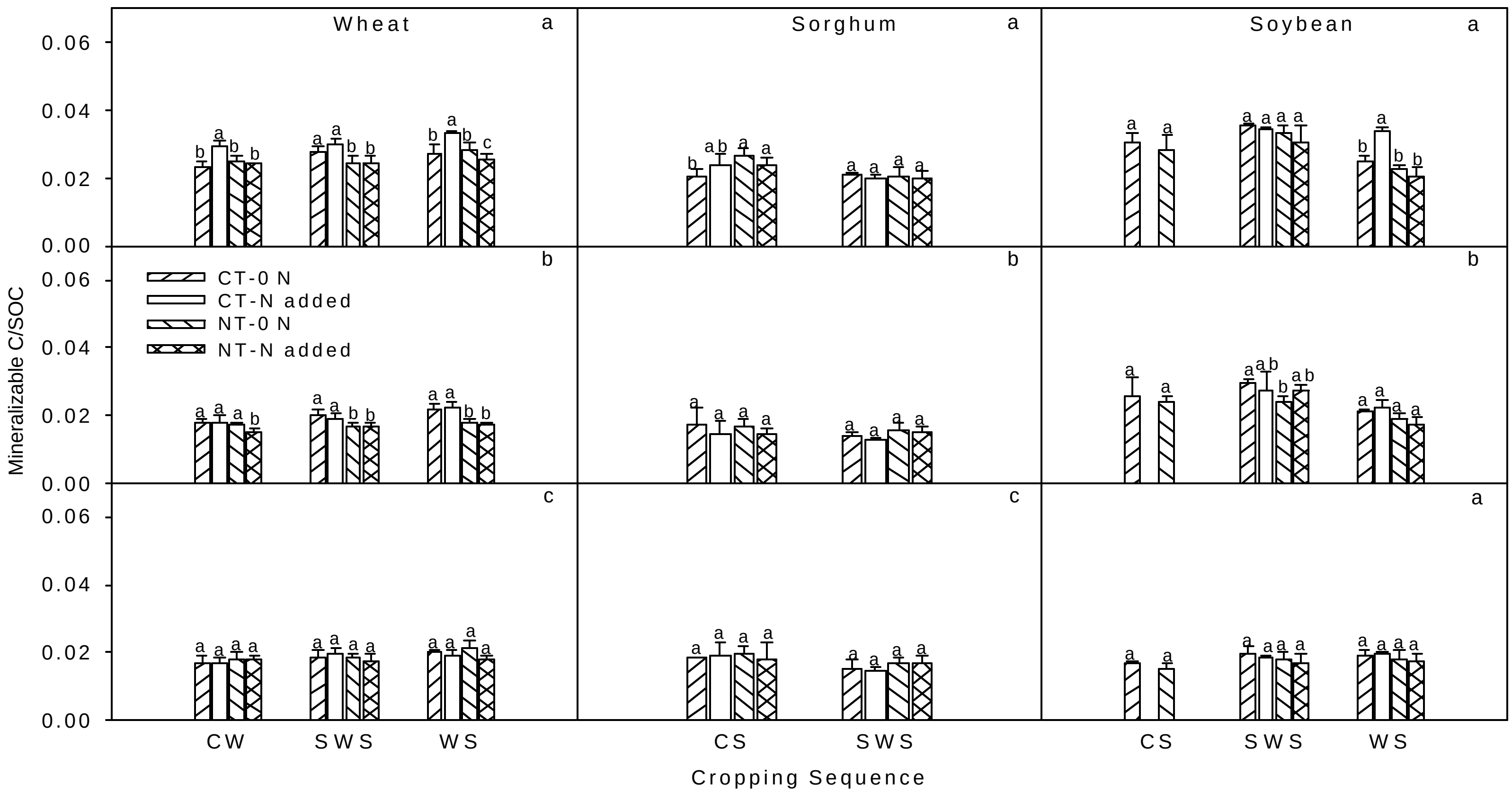

Figure 3.6. The proportion of soil organic C (SOC) as mineralizable C with depth as affected by cropping sequence, tillage, and N fertilization at a) 0-to 5-, b) 5- to 15-, and c) 15- to 30-cm depths. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean samples collected after wheat, sorghum, and soybean harvesting, respectively. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at P<0.05.

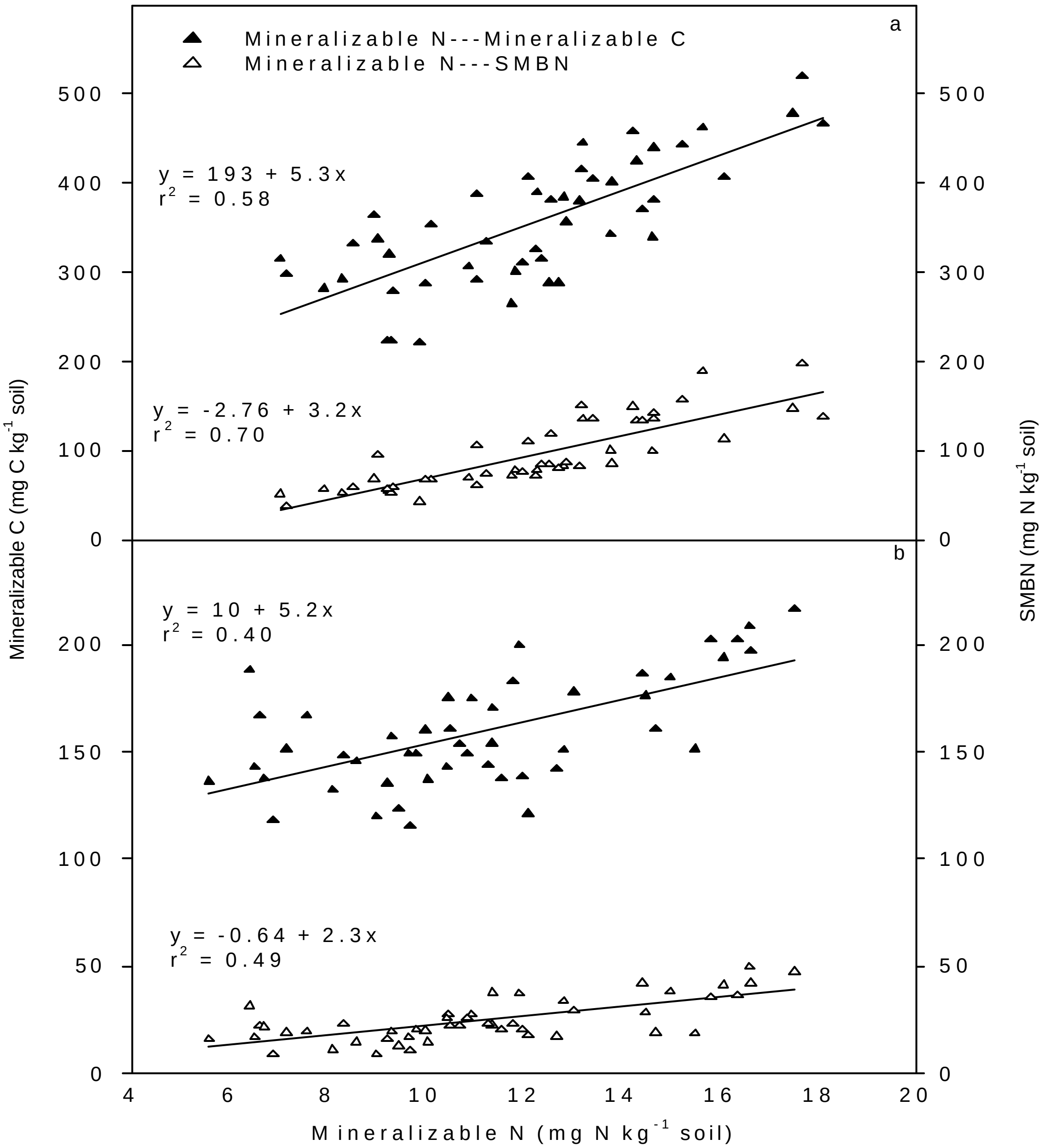

Figure 3.7. Linear regression of soil mineralizable N with mineralizable C or soil microbial biomass N (SMBN) at a) 0- to 5- and b) 5- to 15-cm depths.

the first 7 days, and very slowly increased thereafter (Fig. 3.8). No tillage and added N increased mineralizable N compared to CT and no N fertilization. Differences were more pronounced in the surface soil, but some differences were also observed with depth. The rapid increase in N mineralization after rewetting was consistent with many studies (Orchard and Cook, 1983; Stevenson, 1956). Several sources are responsible for the increased N mineralization after drying and rewetting. One source is killed SMB and another is POM exposed during drying and rewetting cycles that previously was protected by aggregates (Van Gestel et al., 1991; Van Veen et al., 1985).

Particulate Organic Matter C (POM C)

At the 0- to 5-cm depth, no-till significantly increased POM C in wheat, sorghum, and soybean systems compared with CT (Fig. 3.9). For example, POM C under NT in wheat systems was 43, 58, and 92% greater for CW, SWS, and WS, respectively, compared to CT. Compared with CW, POM-C was greater for SWS or WS after wheat regardless of tillage, indicating that soil POM C increased with enhanced cropping intensity. Similar differences were also observed for sorghum and soybean systems. Differences in POM- C caused by N fertilization were minimal except in sorghum systems, where a significant interaction between tillage and N fertilization was observed. POM-C under NT was 39% and 44% greater in Csorghum and SWS, respectively, with than without N fertilization, while no differences due to N were observed with CT.

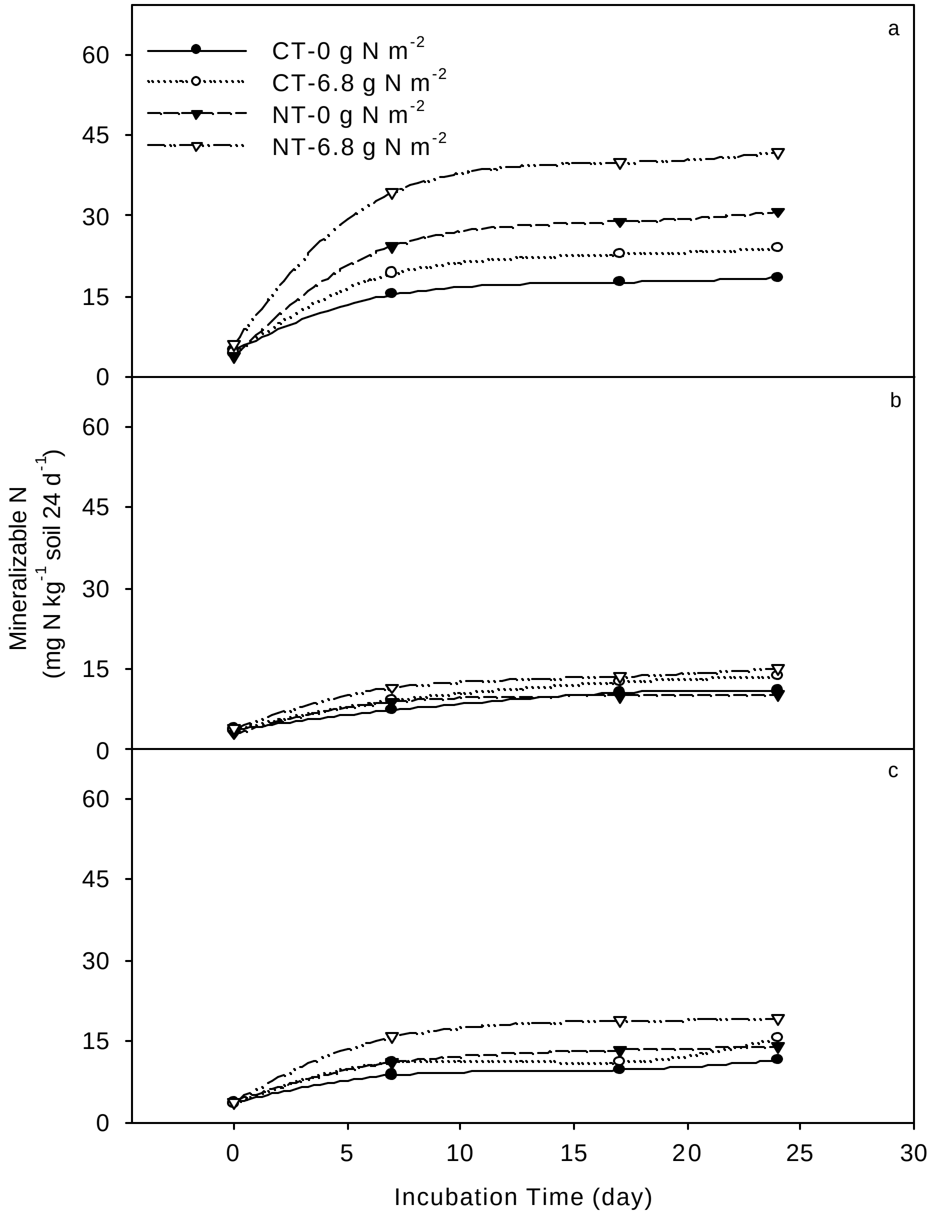

Figure 3.8. Cumulative N mineralized during a 24-day incubation of soil samples from the sorghum-wheat-soybean rotation for depths of a) 0 to 5 cm, b) 5 to 15 cm, and c) 15 to 30 cm as affected by tillage and N fertilization. CT and NT refer to conventional and no tillage. 0 and 6.8 g N m^{-2} represent N addition treatments.

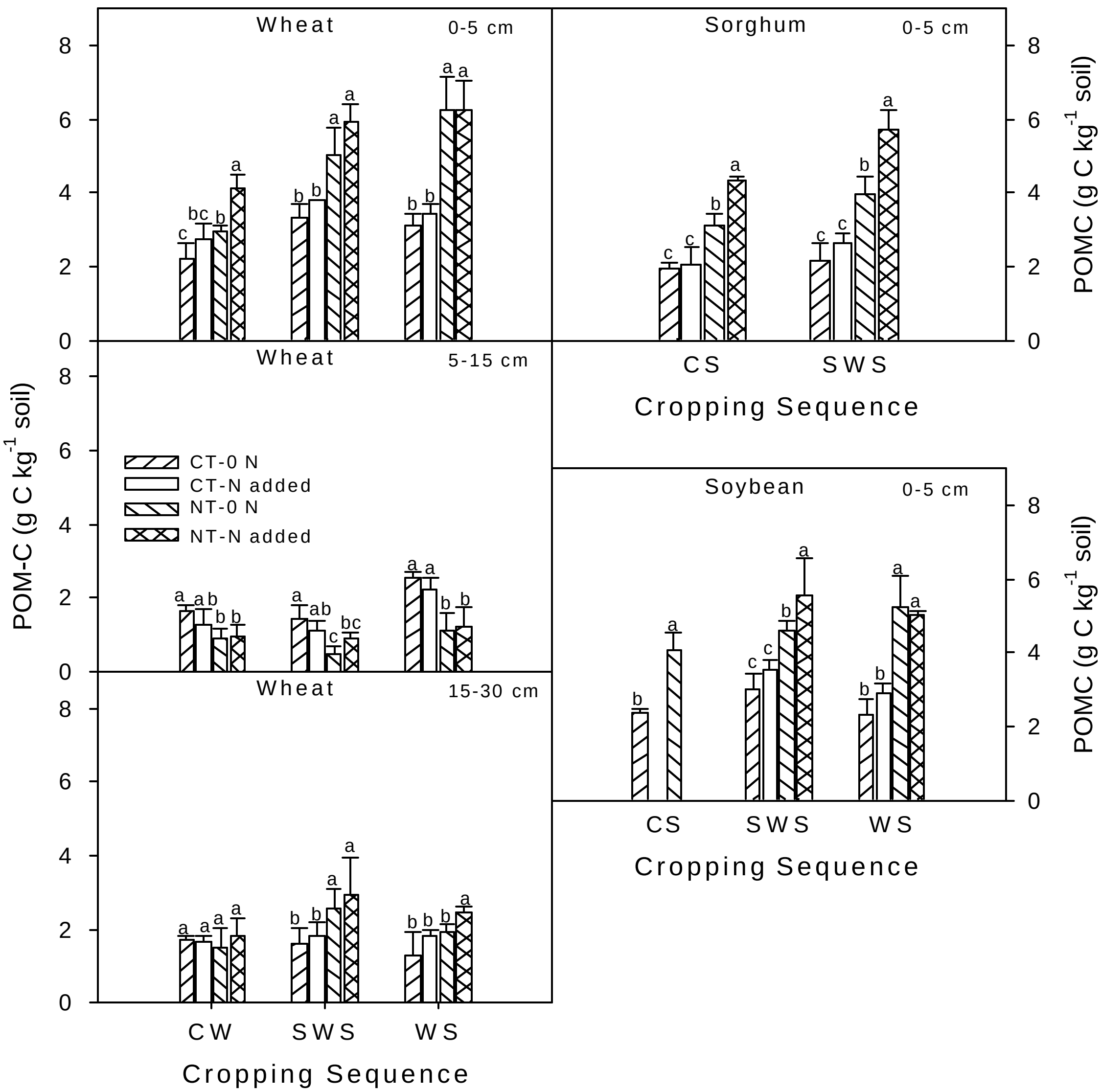

Figure 3.9. Particulate organic matter C (POM-C) with depth as affected by cropping sequence, tillage, and N fertilization. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS represents continuous sorghum and soybean in sorghum and soybean systems, respectively. CT and NT refer to conventional and no tillage. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean refer to soil samples collected after wheat, sorghum, and soybean harvesting, respectively. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at P<0.05.

The relationship between tillage and POM-C in the 5- to 15-cm depth was quite different from that in the surface soil (0 to 5 cm) for wheat. POM-C was 45, 86, and 106% lower for NT compared with CT. POM-C in the deeper layer 15 to 30 cm showed a similar pattern as in the surface soil, although differences were smaller. Chan (1997) also found that NT increased POM-C at the soil surface but decreased it in deeper layers. Increased POM-C with depth with CT partially results from burying plant residue with plowing. Based on the observation that C inputs from crop production were not different between tillage treatments, Cambardella and Elliott (1992) suggested that lower POM under CT was due to more rapid decomposition than with NT. In our study, however, crop residue input was significantly higher with N fertilization than without under both CT and NT (Chapter II), but the difference in POM-C was insignificant between N treatments. For CT, this result could be due to more rapid decomposition. Under NT, however, it may indicate a limited capacity of the soil matrix to protect additional POM. Greater POM-C under NT at 15 to 30 cm may be explained by more anaerobic conditions of deeper soil layers and contributions mainly from crop roots (Franzluebbers and Stuedemann, 2003). After investigating microbial populations and soil water contents, Doran et al. (1998) reported that the biochemical environment of NT soils was less oxidative than under CT, especially at deeper depth.

The proportion of SOC as POM-C followed similar patterns as for POM-C, except that the differences tended to be less distinct (Fig. 3.10). The proportion of SOC as POM-C with wheat averaged 30, 15, and 25% for 0- to 5-, 5- to 15-, and 15- to 30-cm depths, respectively. Compared to CT with wheat, the proportion under NT was 15%

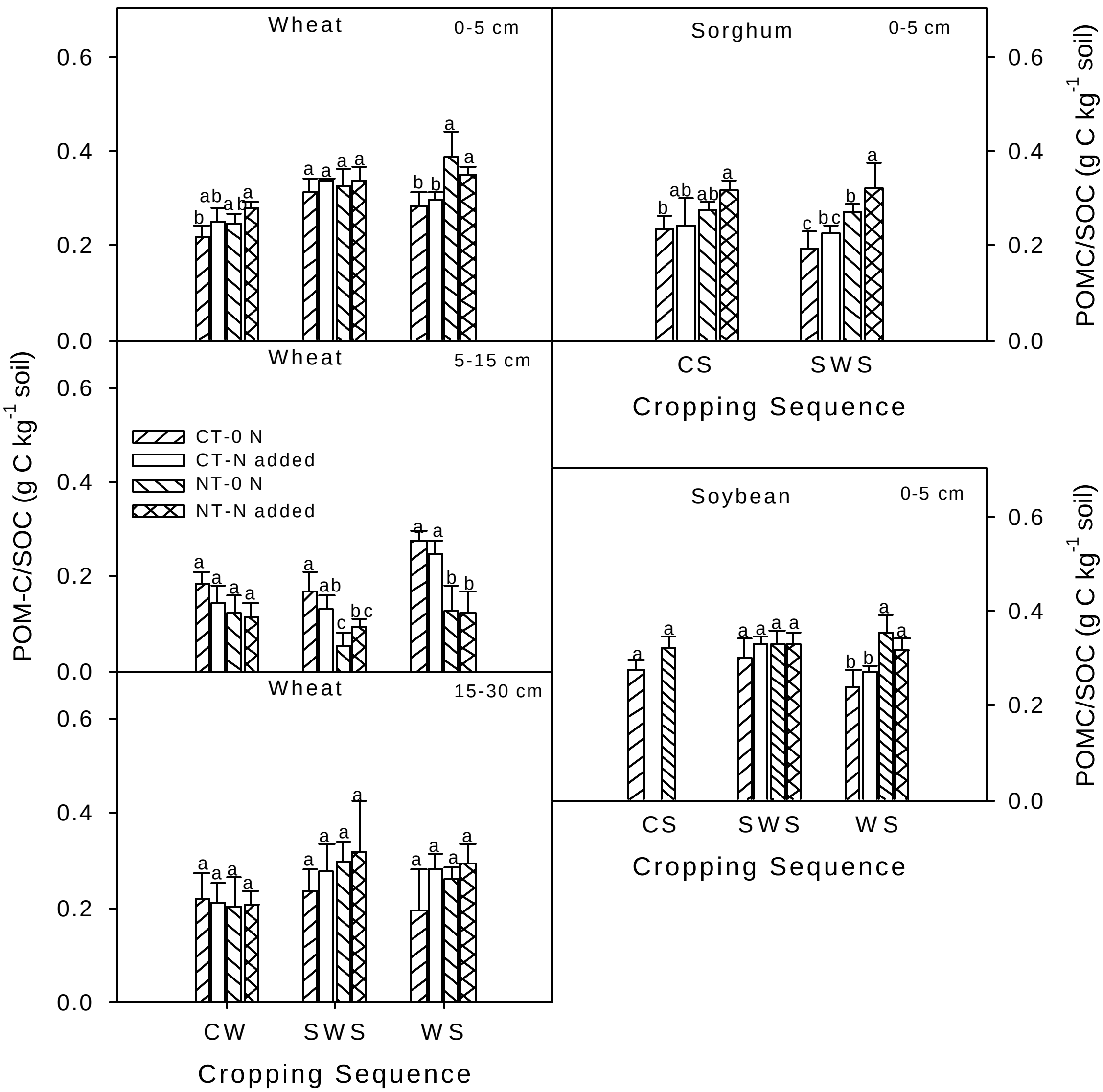

Figure 3.10. The proportion of soil organic C (SOC) as particulate organic matter (POM) C with depth as affected by cropping sequence, tillage, and N fertilization. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS represents continuous sorghum and soybean in sorghum and soybean systems, respectively. CT and NT refer to conventional and no tillage. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean refer to soil samples collected after wheat, sorghum, and soybean harvesting, respectively. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

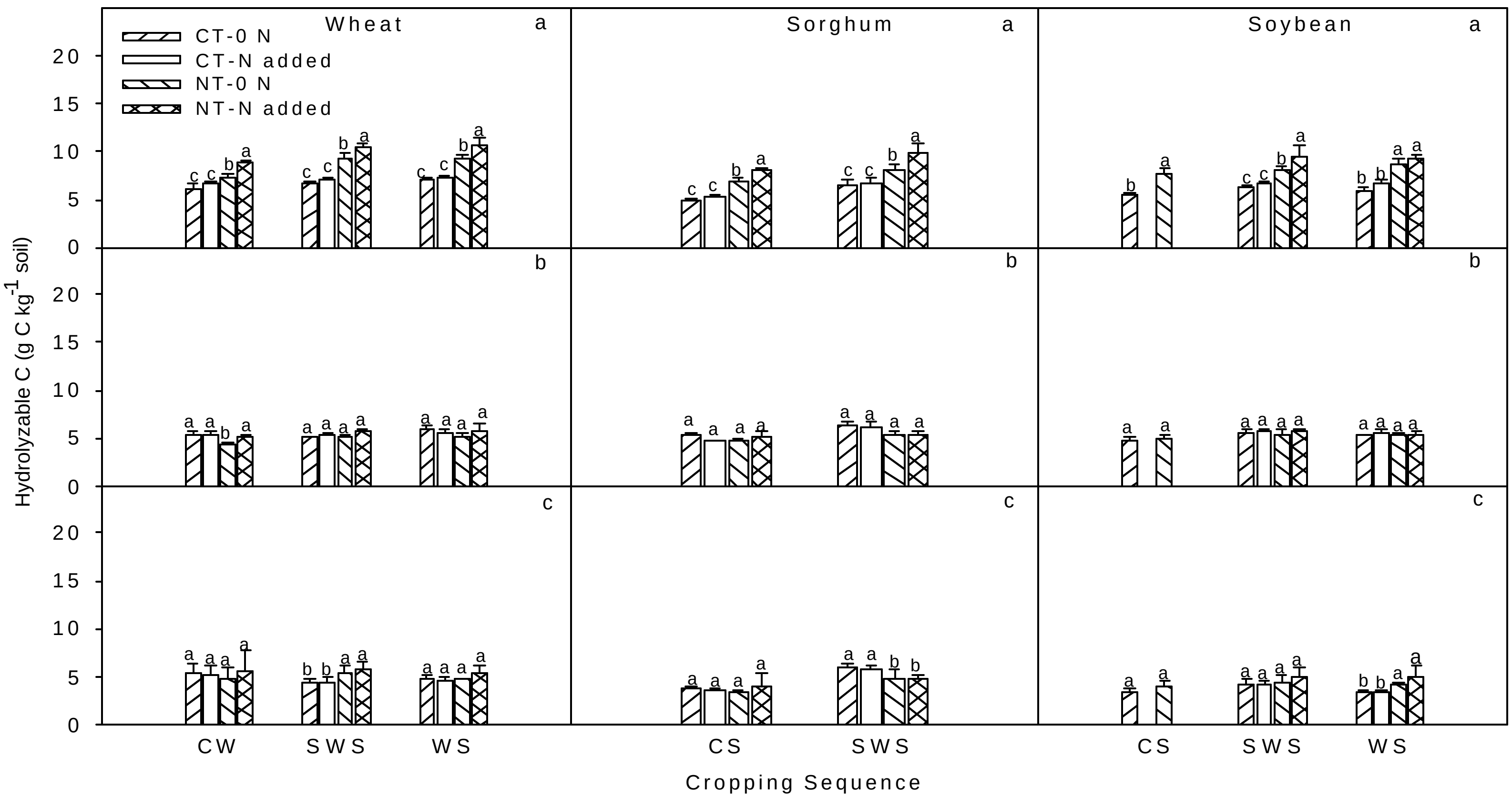

Figure 3.11. Hydrolyzable C with depth as affected by cropping sequence, tillage, and N fertilization at a) 0- to 5-, b) 5- to 15-, and c) 15- to 30-cm depths. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean samples collected after wheat, sorghum, and soybean harvesting, respectively. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

greater in surface soil, but was 80% lower at 5 to 15 cm. No significant tillage difference was observed for this characteristic at 15 to 30 cm.

Particulate organic matter N (POM-N) was highly related to POM-C, and decreased significantly with depth in all crop systems (data not shown). Trends for POM-N in wheat systems were very similar to these of POM-C, especially in the first two depths. No tillage and N fertilization increased POM-N at 0 to 5 cm, but NT decreased this characteristic at 5 to 15 cm compared with CT. The C:N ratios of POM under wheat averaged 15, 8, and 19 for 0- to 5-, 5- to 15-, and 15- to 30-cm depths, respectively.

Acid-hydrolyzable C

Hydrolyzable C in surface soil was more affected by tillage, cropping intensity, and N fertilization than in deeper soils under all three crops (Fig. 3.11). A significant tillage by N fertilization interaction was observed for this parameter (Fig. 3.11a). Hydrolyzable C in NT soils (0 to 5 cm) following sorghum was 16 and 22% greater for CSorghum and SWS, respectively, with than without N fertilization, while no fertilization effect was noted with CT. Similar results were also observed for wheat and soybean.The significant role of N addition was attributed to more crop residue return compared to no N controls. Under CT, however, tillage accelerates the decomposition of added crop residue. Thus, increased crop residue input enlarged the hydrolyzable C pool under NT, but not CT.

Increased cropping intensity increased the hydrolyzable C pool with both CT and NT (Fig. 3.11). Hydrolyzable C in SWS following sorghum was 30% greater than in Csorghum with CT. However, hydrolyzable C under NT was only 19% greater with increased cropping intensity. Greater proportional increase of hydrolyzable C with CT than NT may result from possible differences in SOC saturation of the two systems. Christensen (1996) proposed that SOM associated with soil minerals impedes stabilization of additional SOM on those portions of soil mineral surfaces that are already covered in SOM. We assumed that soil mineral properties are similar for CT and NT. Differences in quantities and qualities of residues in SWS vs. CSorghum may also contribute to the observed differences.

In general, hydrolyzable C decreased with increasing soil depth. No significant effect of tillage, N addition, and cropping intensity on hydrolyzable C was observed at 5 to 15 cm (Fig. 3.11b). At 15 to 30 cm, hydrolyzable C was inconsistently affected by tillage in different crop systems. These results may also reflect differences in residue quantities and qualities.

The proportion of SOC as hydrolyzable C varied with tillage, cropping intensity, and soil depth, but was little affected by N fertilization following the three crop species (Fig. 3.12). A significant interaction of tillage by cropping intensity on this parameter was observed in surface soil (Fig. 3.12a). This proportion was significantly greater with CT than with NT in SWS or WS, but no tillage effect was observed in monocultures. Minimal differences between treatments occurred with depth. Averaged over all main factors, the proportion of SOC as hydrolyzable C increased slightly with soil depth. This result is consistent with the report by Collins et al. (2000). These authors observed that the proportion of SOC as hydrolyzable C increased with soil depth.

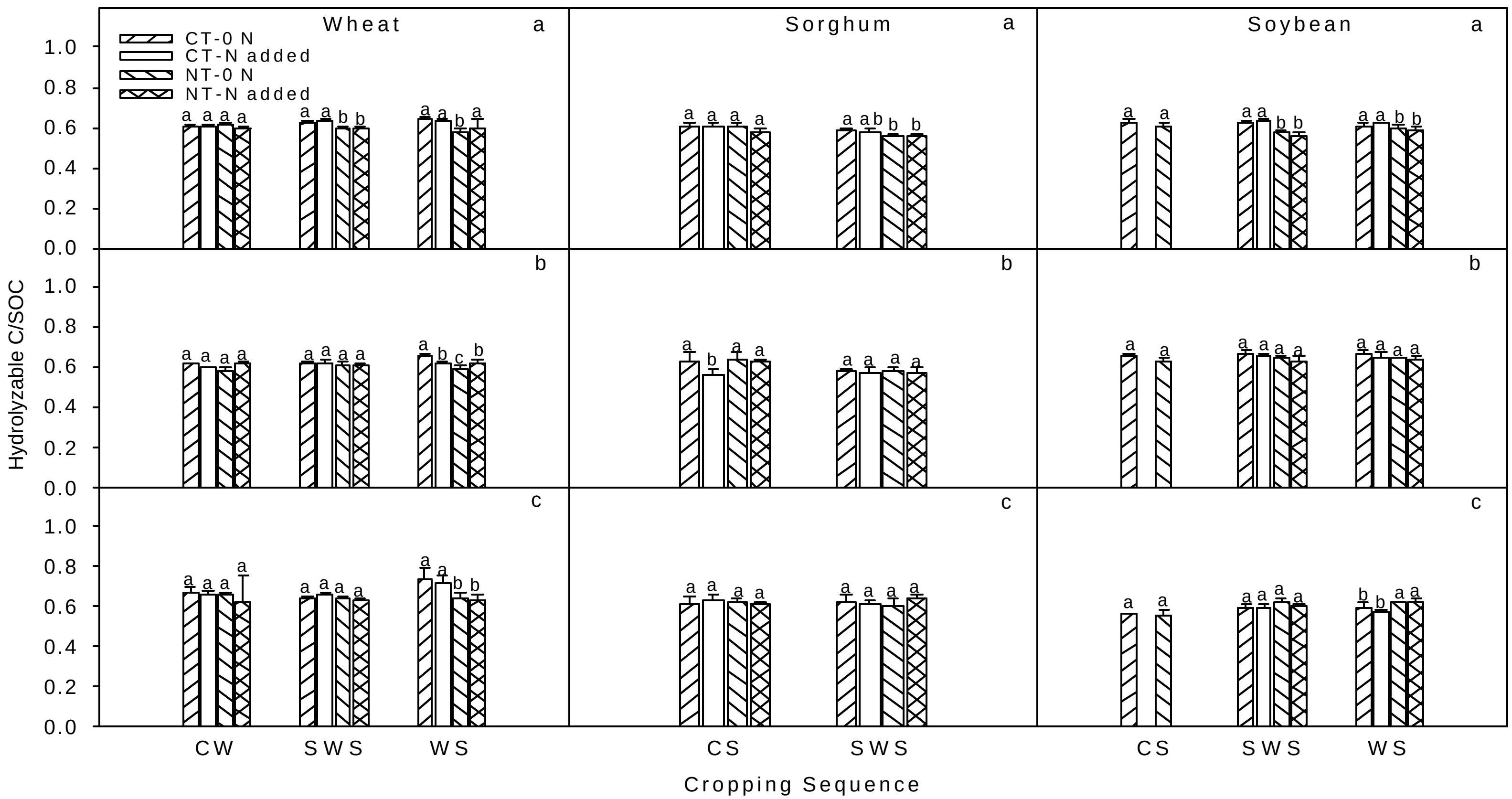

Figure 3.12. The proportion of soil organic C (SOC) as hydrolyzable C with depth as affected by cropping sequence, tillage, and N fertilization at a) 0- to 5-, b) 5- to 15-, and c) 15- to 30-cm depths. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean samples collected after wheat, sorghum, and soybean harvesting, respectively. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at P<0.05.

Comparison of Labile Organic C Pools

Because the various labile C pools showed similar patterns for sorghum, soybean, and wheat systems, only results for sorghum will be discussed. Labile pools in surface soil such as SMBC, mineralizable C, POM-C, and hydrolyzable C were significantly ($P < 0.01$) and positively correlated with SOC (Table 3.1). These results were consistent with those reported by Mclauchlan and Hobbie (2004), who observed SMBC, acid-hydrolyzable C, light fraction C, mineralizable C in a 12-day incubation, and SOC were all positively correlated with each other. On average, SMBC was 5% of SOC, mineralizable C in a 24-day incubation was 3% of SOC, POM-C was 35% of SOC, and hydrolyzable C was 45% of SOC in our study. Of the C pools, hydrolyzable C was most correlated with SOC, followed by POMC and SMBC, and then mineralizable C.

Although labile SOC pools increased as SOC increased, they exhibited significant differences in rates of increase (Table 3.2). After adjusting all values to the same units (mg C kg^{-1} soil), the slope for mineralizable C during a 24-day incubation was lowest, and that for hydrolyzable C was the highest. The slopes for both POM-C and hydrolyzable C were several times those for mineralizable C or SMBC. Our results contrasted with these observed by Mclauchlan and Hobbie (2004). These authors did not observe a significant difference between the slopes for mineralizable C andSMBC. One reason may be that they used soil samples with different soil series treated with management practices. In addition, they sampled from 0 to 10 cm.

Conventional tillage increased SMBC per unit of SOC compared to NT, while the opposite effect was noted for POMC.

Table 3.2. Estimates of slopes and their confidence intervals (a = 0.05) from linear regression of soil organic carbon (g C kg^{-1} soil) and labile pools (mg C kg^{-1} soil) at 0 to 5 cm (n = 16).

Method	Tillage†	Slope	Lower confidence limit	Upper confidence limit
Mineralizable C	CT	15.19	8.16	22.23
	NT	6.73	-0.39	13.85
Soil microbial biomass C	CT	71.29	56.15	86.43
	NT	32.60	18.42	46.77
Particulate organic matter C	CT	155.88	34.20	277.55
	NT	427.54	306.44	548.63
Hydrolyzable C	CT	535.28	474.63	595.93
	NT	460.50	412.88	508.12

† CT and NT denote conventional and no tillage.

Selected measurements of four labile SOC pools were also significantly ($P<0.01$) correlated with each other: mineralizable C, SMBC, POM-C, and hydrolyzable C (Table 3.1). Hydrolyzable C was highly correlated with mineralizable C ($r = 0.863$), SMBC ($r = 0.914$), and POM-C ($r = 0.916$). Mineralizable C was positively correlated with both SMBC ($r = 0.846$) and POM-C ($r = 0.832$). SMBC correlated positively with POM-C ($r = 0.812$). These results were consistent with those of previous studies (Doran et al., 1998; Fliebbach and Mader, 2000; Franzluebbers et al., 1995a). However, a higher correlation of the hydrolyzable C with other labile C was observed in our study. One reason may be different volumes of acid solution used. We used 25 ml of 6 *M* hydrochloric acid added to one-gram of soil instead of 10 ml. Thus, more complete hydrolysis of the SOC pool may have occurred in our study. According to the three-pool SOC model, hydrolyzable C is the sum of active and slow pools. In other words, all labile SOC should be contained in this pool. Therefore, it was not surprising to observe high correlations between hydrolyzable C and other labile pools.

Sensitivity of different labile pools to changes in SOC varied with CT or NT (Fig. 3.13). Compared with NT, the slopes for regressions between SOC and SMBC and hydrolyzable C were significantly greater with CT. This result indicated that these labile pools were more affected by CT than NT. Under NT, the slope between SOC and POMC was significantly greater than that with CT, increasing almost three times more per unit of SOC. Our results indicate that the ability of different labile pools to predict changes in SOC may vary with tillage treatment.

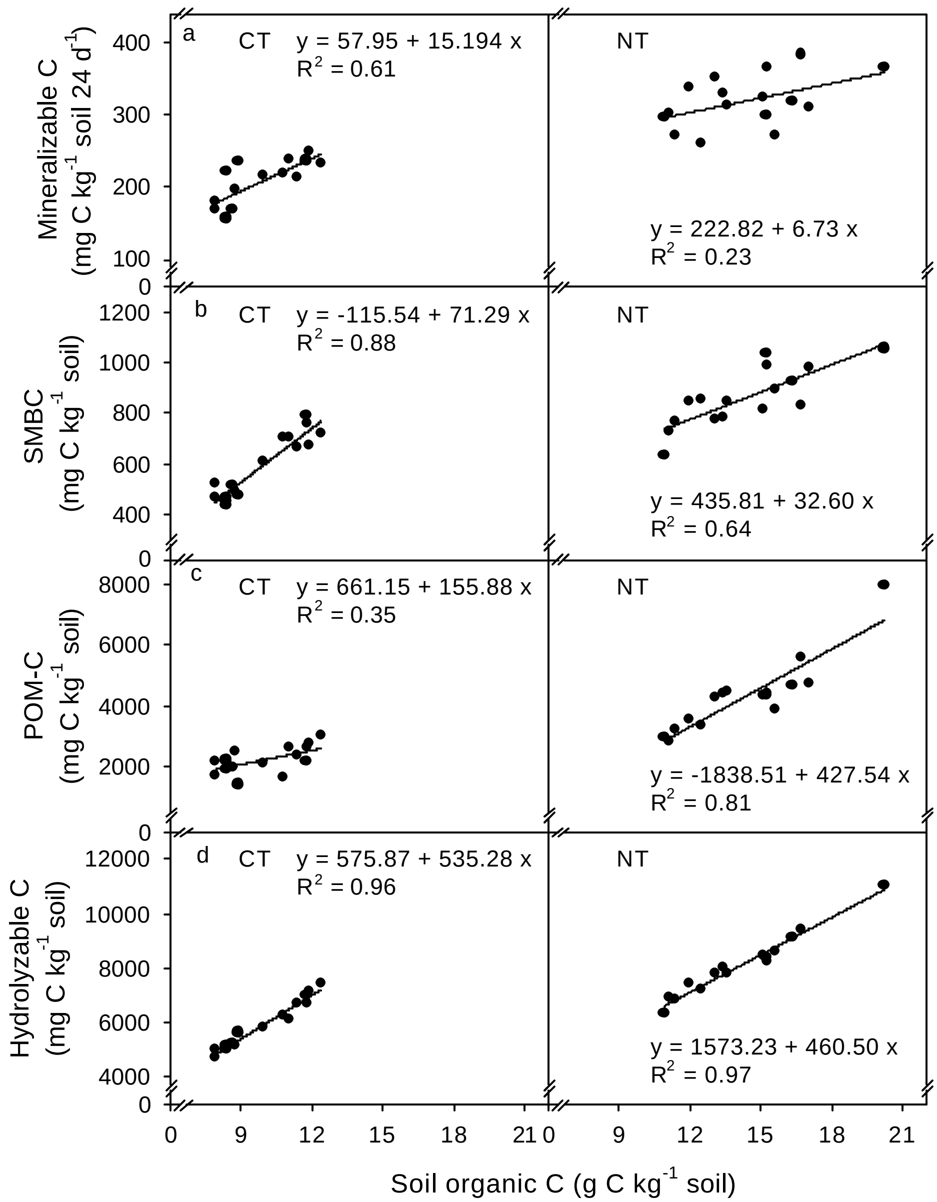

Figure. 3.13. Linear regression of soil organic C (SOC) and results of four methods for estimating labile SOC under conventional tillage (CT) and no tillage (NT) in surface soil (0 to 5 cm). Regressions include SOC vs. a) C mineralizable during a 24-day incubation, b) soil microbial biomass (SMB) C, c) particulate organic matter (POM) C, and d) hydrolyzable C.

CONCLUSIONS

The majority of significant effects of management practices on labile C pools were observed in surface soil. SMB, mineralizable C and N, POM, and hydrolyzable C were all significantly greater with NT than CT at 0 to 5 cm. The size of labile C pools and the significance of management effects decreased with depth. Tillage exerted the greatest influence on these pools, but enhanced cropping intensity and N fertilization also increased these labile pools, though not always significantly. In addition, different crop species affected labile pool sizes, partially due to differences in sampling season and/or crop residue quality and quantity.

SMB, mineralizable C and N, POM, and hydrolyzable C were highly correlated with each other and SOC, but their slopes were significantly different, being lowest for mineralizable C and highest for hydrolyzable C, suggesting that different methods included different fractions of total SOC. The labile C pools exhibited varying sensitivity to soil tillage regime. Compared with NT, SMBC, mineralizable C, and hydrolyzable C exhibited greater slopes with increasing SOC under CT.

CHAPTER IV

SLOW AND RESISTANT ORGANIC CARBON POOLS AS AFFECTED BY TILLAGE, CROPPING SEQUENCE, AND NITROGEN FERTILIZATION

INTRODUCTION

Agricultural management practices influence not only the amount of soil organic C (SOC), but also the rate of C cycling. Furthermore, concerns about the effect of increasing concentrations of greenhouse gases in the atmosphere on global climate have increased interest in the soil C cycle, with a focus on the potential for increasing soil C sequestration (Donigan et al., 1997; Lal et al., 1999). Conventional tillage (CT) has caused reductions in organic C contents of agricultural soils through increased decomposition rates and redistribution of C (Christensen, 1996). These reductions can be mitigated by incorporation of sustainable management practices such as reduced tillage, decreased bare fallow, increased residue input, and conversion to perennial vegetation (Paustian et al., 1997). No-till (NT) has been reported to enhance SOC sequestration in many studies (Bayer et al., 2001; Campbell et al., 1991b; Franzluebbers et al., 1994; Six et al., 2002b). Increased cropping intensity has also been observed to enhance C sequestration (Campbell et al., 1998). Further, N addition normally increases plant residue input, and thus may also increase C sequestration (Christensen, 1996). However, studies have shown that the relative increases in soil organic matter (SOM) occurs more in coarser size fractions compared to finer fraction, indicating that the sequestered C is present in more labile pools (Cambardella and Elliott, 1994; Jastrow and Miller, 1997). If this is the case, then this C may be sequestered more for the short- rather than the long-term.

Stabilization of SOM is a key process determining soil quality and whether a soil is a sink or source of C to the atmosphere (Six et al., 2002c). Christensen (1996) proposed three mechanisms responsible for stabilization of SOM: chemical recalcitrance of the organic matter, chemical stabilization of otherwise decomposable compounds by chemical interaction of substrates with the mineral part of the soil, and physical protection of otherwise decomposable substrates within micro- or macroaggregates by physical barriers created between substrates and decomposers.

Various physical and chemical fractionation methods have been used to separate C pools with different turnover rates. By the ^{13}C natural abundance labeling technique, Balesdent and Mariotti (1996) and Christensen (1996) showed that particle-size fractions represent different pools of SOC in terms of turnover time. Organic compounds in sand-sized fractions [particulate organic matter (POM)] are turned over rather rapidly, within several years or less, whereas SOC associated with fractions <50 µm show markedly slower turnover times, thus being involved in the mid- and long-term dynamics of SOC. Using acid hydrolysis and ^{14}C dating, Anderson and Paul (1984) and Paul et al. (1997) related OC not hydrolyzed in acid to resistant organic C (ROC), with higher turnover times of > 1000 years. Little information, however, is available on the effect of different long-term agricultural management practices on slow and ROC pools in the southern USA. A direct determination of effects on these two C pools, coupled with a knowledge of the relationship between them developed by physical and chemical separation methods, can add to our understanding of SOM processes.

The objectives of this research were to assess the effect of tillage [conventional (CT) vs. NT], cropping intensity (monoculture vs. enhanced cropping systems), and N fertilization (no N vs. N addition) on mineral-associated and ROC pools.

MATERIALS AND METHODS

Crop Management and Site Characteristics

A long-term field experiment was initiated in 1982 on the Brazos River floodplain in south-central Texas ($30^0$32'N, $94^0$26'W). Sorghum [*Sorghum bicolor* (L.) Moench.] was managed under CT (disk) and NT in continuous sorghum (CSorghum), and rotated wheat (*Triticum aestivum* L.)/soybean [*Glycine max* (L.) Merr.]-sorghum (SWS) cropping sequences. Soybean was managed under CT (disk) and NT in continuous soybean (CSoybean), continuous wheat/soybean (WS), and rotated wheat/soybean-sorghum (SWS) cropping sequences. Wheat was managed under CT (disk) and NT in continuous wheat (CW), continuous wheat/soybean (WS), and rotated wheat/soybean-sorghum (SWS) cropping sequences. Crop growing seasons were from early November to late May for wheat, early June to late October for soybean, and late March to late July for sorghum. Continuous crops produced one crop each year, WS produced two crops per year, and SWS produced three crops every two years. Nitrogen fertilizer (NH_4NO_3) was broadcast on wheat at 0 or 6.8 g N m^{-2} during late winter or early spring. Soybean did not receive N fertilizer, while sorghum received 0 or 9 g N m^{-2} banded preplant. Nitrogen treatments in soybean refer to N fertilization of the previous crop.

A split-split plot within a randomized complete block design was established with cropping sequence as the main plot, tillage as the sub plot, and N fertilizer rate as the sub-sub

plot. Plots measured 4 x 12.2 m, and treatments were replicated four times.

The soil used is classified as a Weswood silty clay loam (fine-silty, mixed, superactive, thermic, Udifluventic Haplustepts) and contains an average of 115, 452, and 433 g kg^{-1} of sand, silt, and clay, respectively. Under cultivation, this soil has a pH of 8.2 (1:2, soil:water) and an organic C content of approximately 8 g C kg^{-1} soil. Annual temperature is 20 ^{0}C and rainfall is 978 mm.

Soil Sampling

Soil samples were collected shortly after wheat, sorghum, and soybean harvesting in May, August, and October 2002, respectively. Individual samples consisted of 25 composited cores (19-mm dia.) per plot that were divided into depth increments of 0 to 5, 5 to 15, and 15 to 30 cm. All samples taken after wheat were analyzed, but only 0 to 5 cm sample after sorghum and soybean were analyzed. Soil was sieved to pass a 2-mm screen (visible pieces of crop residues and roots removed) and oven-dried at 60 ^{0}C for 48 h for physical and chemical analysis.

Mineral-associated Organic C (MAC) and N (MAN)

Mineral-associated SOC was separated from the < 2-mm soil following the method of Cambardella and Elliott (1992). Fifty-g subsamples were dispersed in 100 mL of 0.001 *M* $Na_4P_2O_7$ by shaking for 16 h on a reciprocal shaker. The dispersed soil samples were poured through a 53-μm sieve, and the soil slurries that contained the mineral-associated and water-soluble C and N that passed through the sieve were dried in a forced-air oven at 55 ^{0}C. Dried slurry samples were subsequently ground with a mortar and pestle to pass a 200-μm sieve and were analyzed for total organic C and N as previously described (Chapter II).

Resistant Organic C

Resistant organic C was determined using the method suggested by Rovira and Vallejo (2002) with the following modifications. One g of oven-dry soil subsample passing through a 200-um sieve was hydrolyzed with 25 mL of 6 *N* HCl in a digestion tube in an aluminum block digestor at 110 ^{0}C for 18 h, with occasional shaking. After cooling, the unhydrolyzed residue was recovered by centrifuging at 3500 rpm for 20 minutes and decanting the liquid. The process of washing with deionized water, centrifugation and decantation was repeated several times until neutral pH was reached. The residue was then transferred to a pre-weighed vial, dried at 60 ^{0}C to constant weight, and analyzed for C as previously described.

Statistical Analysis

Analysis of variance and regression were conducted as appropriate (SPSS, 2001). All differences discussed are significant at the $P<0.05$ probability level, unless otherwise stated. Fisher's protected least significant differences were calculated only when the analysis of variance F-test was significant at $P<0.05$.

RESULTS AND DISCUSSION

Mineral-associated Organic C and N

In surface soil (0 to 5 cm), a significant interaction of tillage by nitrogen fertilization for MAC was observed following wheat, sorghum, and soybean (Fig. 4.1). Nitrogen addition under NT significantly increased MAC in all three crops in surface soil (0 to 5 cm). However, no significant increase was observed due to N addition under CT. Under NT following wheat, MAC was 19, 12, and 19% greater for CW, SWS, and WS, respectively, than under CT. Similar results were also observed following sorghum and soybean. Greater SOC in this fraction under NT with N addition may be partially due to more plant residue input as we observed previously (Chapter II). Enhanced cropping intensity also increased MAC. Greatest differences were observed following sorghum, where this fraction was 40 and 27% greater under CT and NT, respectively, with increased cropping intensity. A similar effect of enhanced cropping intensity on MAC was observed following wheat and soybean. Crop species also affected MAC, with CW containing the highest concentration of this fraction, regardless of tillage. Similar results were previously discussed for SOC and TN.

Overall, MAC decreased with depth. Our result is consistent with the work by Franzluebbers and Arshad (1997). In soils below 5 cm following wheat, MAC under NT was greater than with CT, although variation increased with depth (Fig. 4.1). Enhanced cropping intensity increased MAC a 5 to 15 cm, but not 15 to 30 cm. Nitrogen addition also slightly increased MAC in below surface soil depths.

The mineral-associated organic C pool is organic C associated with silt- and clay-sized particles as well as soluble organic C and probably represents more stabilized and

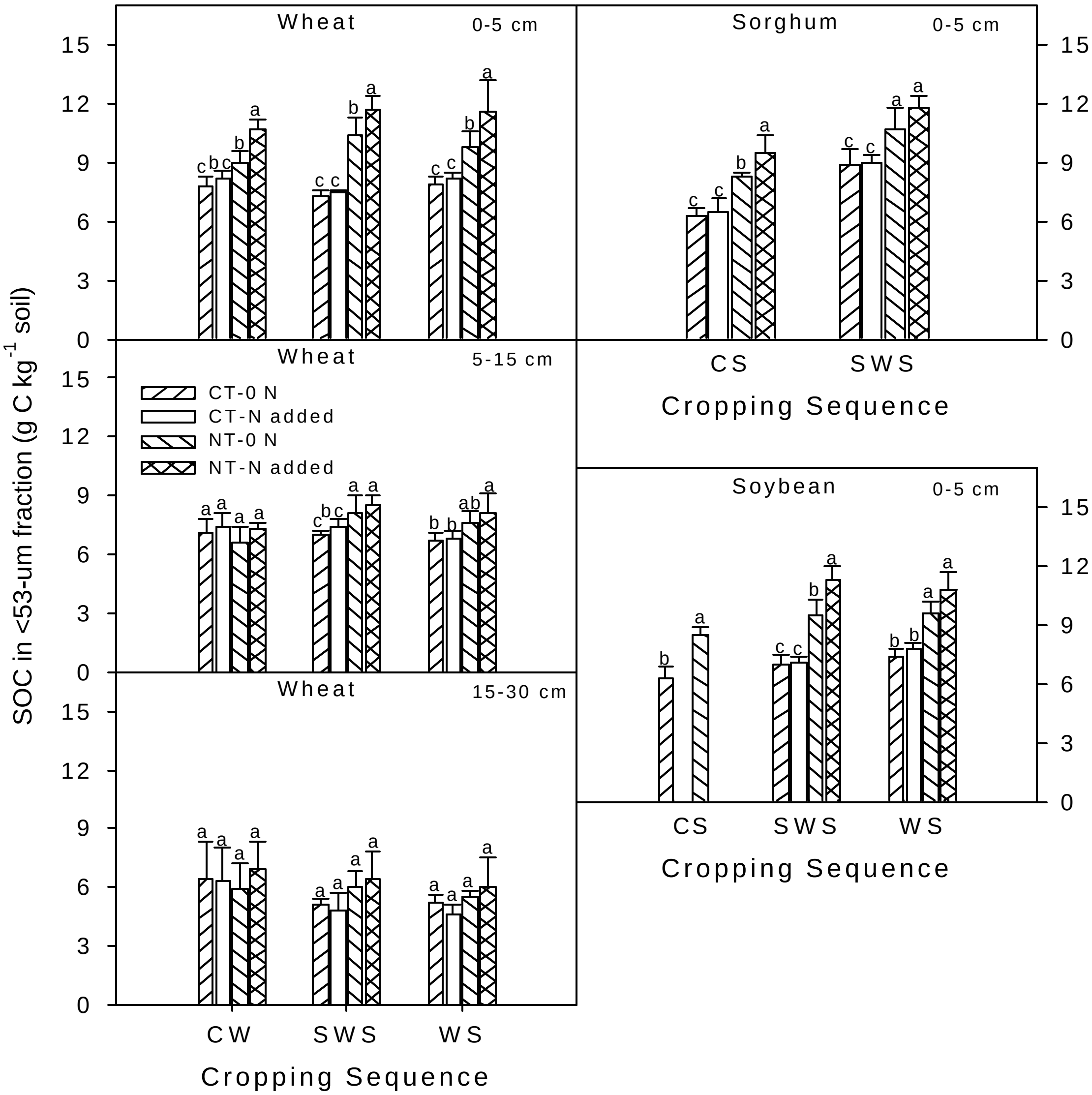

Figure 4.1. Mineral-associated organic C (MAC) as affected by cropping sequence, tillage, and N fertilization. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean legends refer to soil samples collected after wheat, sorghum, and soybean harvesting. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

humified organic C with a slower turnover rate than particulate organic C (Cambardella and Elliott, 1992; Christensen, 1996; Hassink, 1995). One might expect no or minimal fluctuation in MAC as affected by tillage, N addition, and enhanced cropping intensity. Significantly greater C associated with this pool under NT, however, indicated increased C stabilization compared to CT (Fig. 4.1). Nitrogen addition significantly increasing MAC only under NT may suggest more physical protection of added C. According to a recent hypothesis by Six et al. (2000a), the turnover rate of organic matter under NT was slower than under CT due to less disturbance. Therefore, more SOC was likely stabilized by microaggregates. Enhanced cropping intensity may have a similar role in increasing C stabilization. Decreasing MAC with depth may reflect a lower input of plant residue into subsurface soil compared to surface soil. In general, the amount of plant residue input in subsurface soil under CT is greater than NT due to mixing by tillage.

The proportion of SOC as MAC under NT was lower than under CT in surface soil, but difference were not always significant for all cropping sequences (Fig. 4.2). Both N addition and cropping intensity minimally affected this proportion. Similar results were also observed at a depth of 15 to 30 cm. The greatest proportion of SOC as MAC was observed at 5 to 15 cm, where this proportion under NT was greater compared to CT. The proportion of SOC as MAC increased with soil depth below 5 cm. This result agreed with work reported by Franzluebbers and Arshad (1997), who found this proportion increased with depth from 0.44to 0.88 in cultivated soil. Similar trends were also observed in vertisols under pasture (Chan, 1997).

The proportion of SOC as MAC decreasing with depth likely reflects differences in plant residue input. Most of aboveground residue is returned to surface soil, and crop

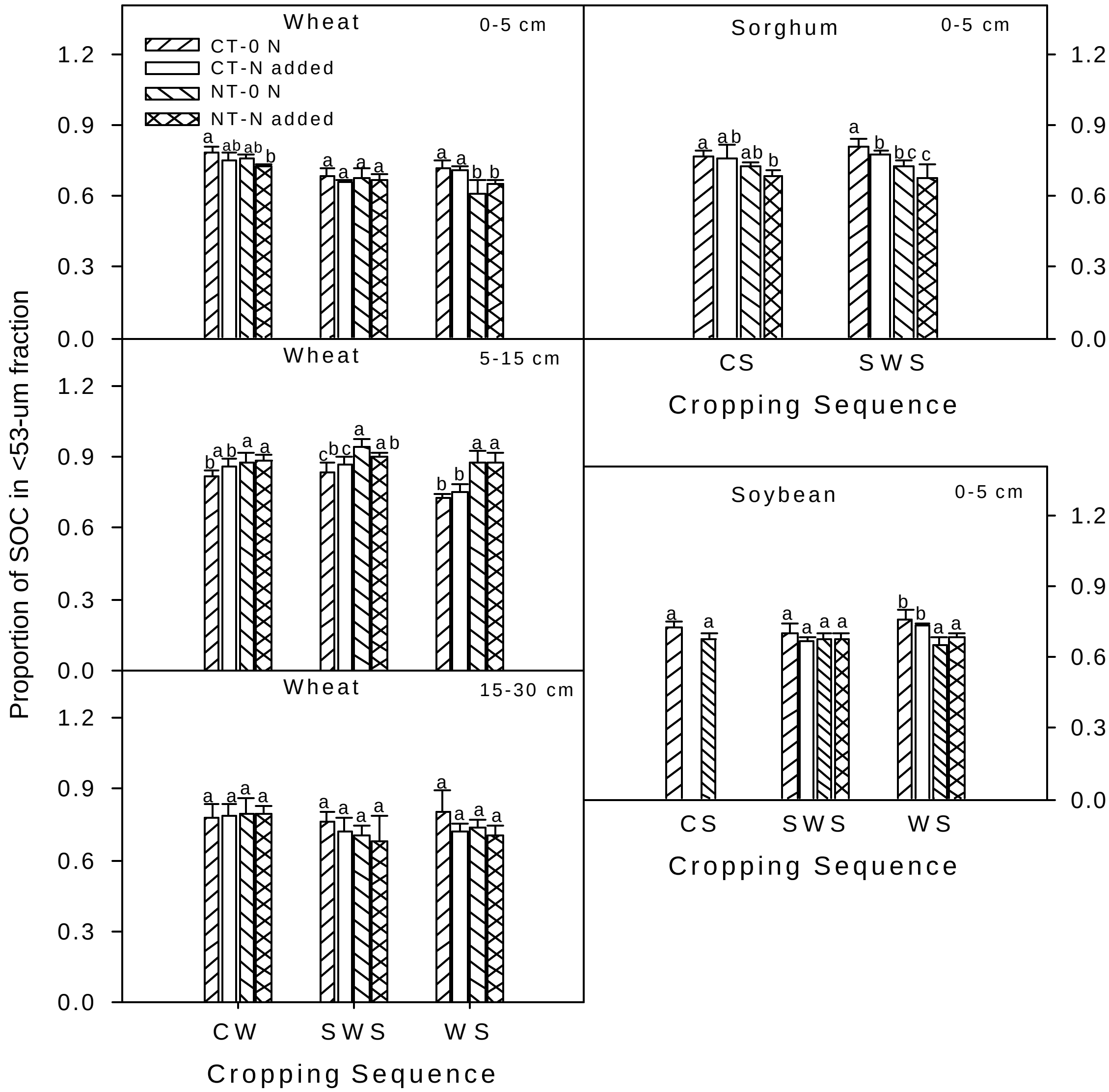

Figure 4.2. The proportion of soil organic C (SOC) as mineral-associated organic C as affected by cropping sequence, tillage, and N fertilization. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean legends refer to soil samples collected after wheat, sorghum, and soybean harvesting. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at P<0.05.

root density is also higher in surface soil (Moroke, 2002). The greatest proportion of SOC as MAC at a depth of 5 to 15 cm from wheat (Fig. 4.2) may indicate a combined differential effect of residue input and decomposition. Compared to deeper soil, plant residue input is greater at this depth, but decomposition may also be greater compared to colder and wetter soil at a depth of 15 to 30 cm. Since NT resulted in proportionally greater SOC as MAC at 5 to 15 cm than CT, roots may be a greater contribution to this result than surface residues.

Total soil N accumulated with the < 53-µm mineral fraction generally mirrored patterns observed for organic C in three crop studied (Fig. 4.3). In surface soil, MAN in this fraction ranged from 613 to 1024, and 890 to 1344 mg N kg soil^{-1} for CT and NT, respectively, across crop and N treatments. Nitrogen addition increased MAN under NT, but not with CT. Increased cropping intensity showed a similar interaction with tillage. The C:N ratio for SOM in the < 53-µm fraction was not significantly affected by tillage, N addition, or cropping intensity (data not shown). Cambardella et al (1992) also reported that this ratio was not affected by tillage or cropping system.

Resistant Organic C

After 20 yr, most effects of tillage, N fertilization, and cropping intensity on ROC were observed in surface soil of all cropping systems (Fig. 4.4). A similar significant interaction of tillage and N addition existed for ROC as was observed for SOC and other C pools. In wheat systems, ROC under NT was 30, 15, and 8% greater for CW, SWS, and WS, respectively, with than without N addition. Minimal differences were observed under CT for N treatment. Similar results were also observed in sorghum and soybean

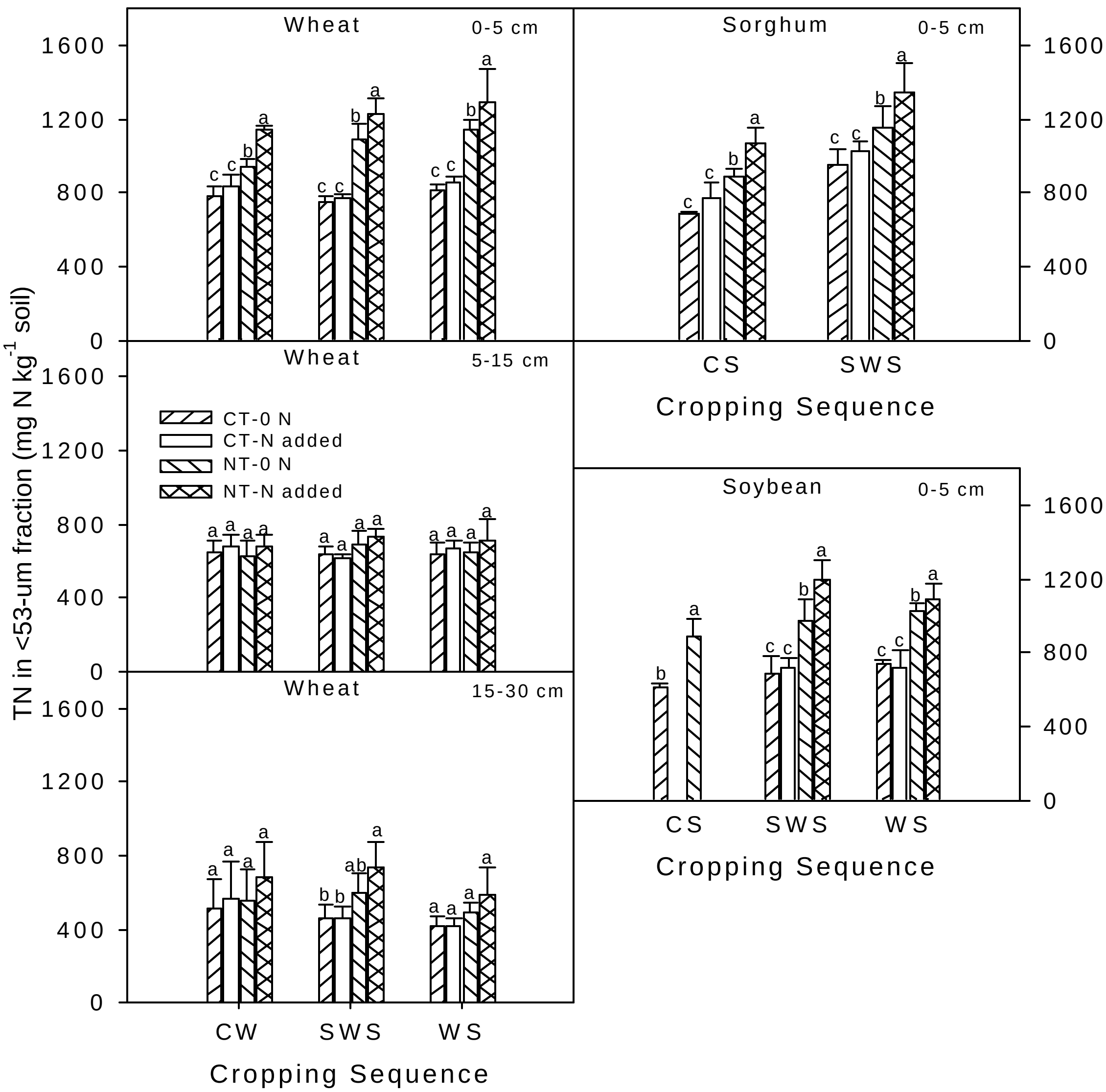

Figure 4.3. Mineral-associated total N (TN) as affected by cropping sequence, tillage, and N fertilization. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean legends refer to soil samples collected after wheat, sorghum, and soybean harvesting. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

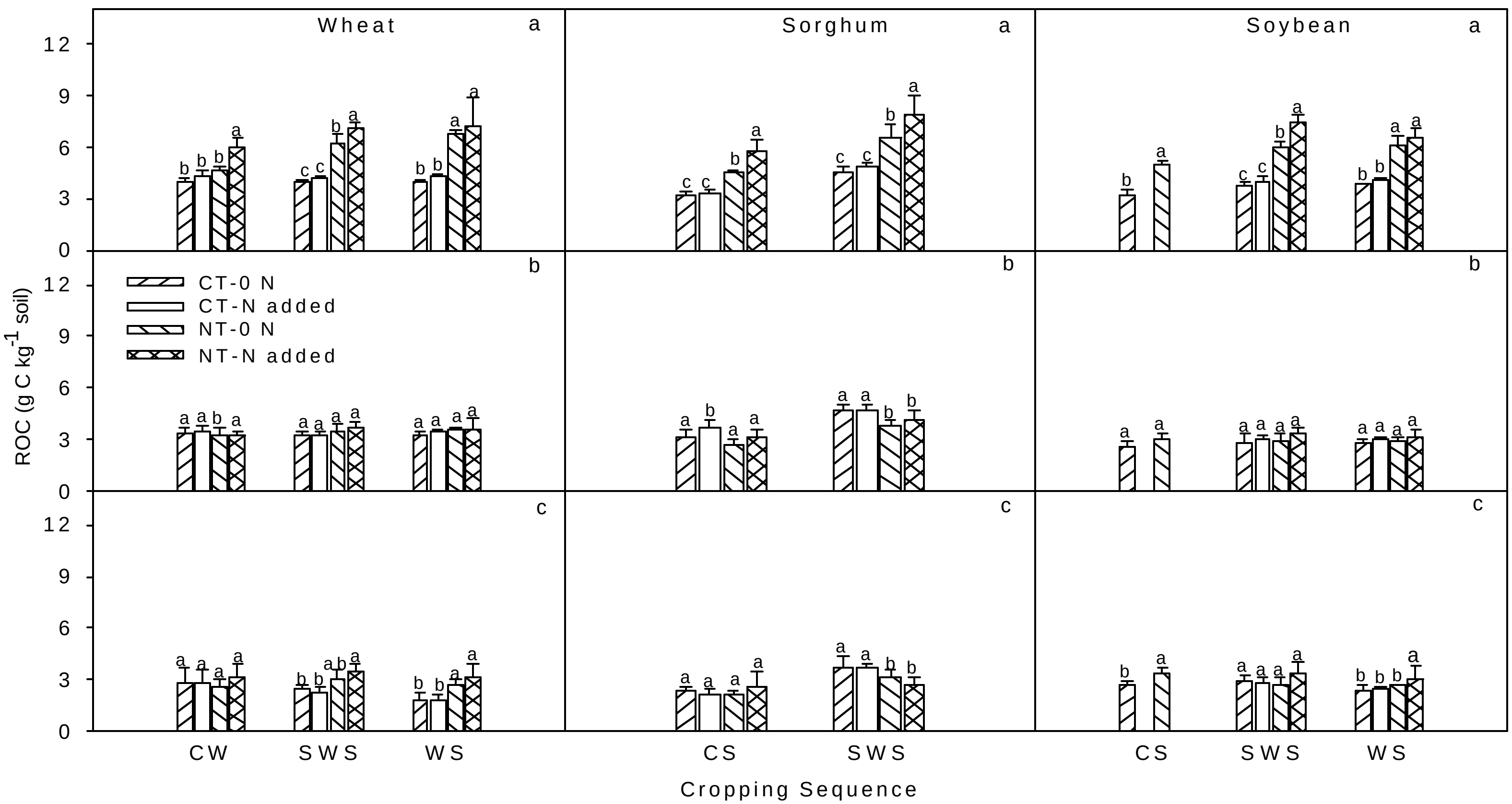

Figure 4.4. Resistant organic C (ROC) in soil with depth as affected by cropping sequence, tillage, and N fertilization at a) 0- to 5-, b) 5- to 15-, and c) 15- to 30-cm depths. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean legends refer to soil samples collected after wheat, sorghum, and soybean harvesting. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

systems. These results indicated that cultivation may speed decomposition of returned residues and also decrease the formation of ROC. Since the main components of ROC are lignin, its decomposed moieties, and plant celluloses, our study is consistent with the hypothesis of Amelung et al. (1999) that lignin does not appear to be stabilized by CT. Significantly greater ROC under NT was observed compared to CT. The greater results under NT in our study are consistent with the work by Collins et al. (2000), that reported 21 to 40% greater ROC under NT than CT at the 0- to 20-cm depth. ROC also increased with enhanced cropping intensity in all cropping systems with one exception; the concentration of ROC under CT was equivalent for CW, SWS, and WS in wheat systems.

In addition, crop species affected ROC (Fig. 4.4a). ROC averaged over N addition and tillage decreased in the order of continuous wheat > continuous sorghum > continuous soybean, indicating possible effects of crop residue quality on ROC. After hydrolysis with 6*N* HCl, Follet et al. (1997) found that 42% of the C of wheat straw and 34% of the C of maize (*Zea mays* L.) residues were not dissolved.

Concentrations of ROC decreased with soil depth (Fig. 4.4), and averaged 5.2, 3.4, and 2.7 g C kg^{-1} soil at 0- to 5-, 5- to 15-, and 15- to 30-cm in wheat systems, respectively. Comparative values were also observed in sorghum and soybean systems. Collins et al. (2000) reported wider values of ROC ranging from 4.3 to 10.0 g C kg^{-1} at a depth of 0 to 20 cm. The lower value of ROC in our study may partially be explained by differences in climates. Higher temperature in the southern USA accelerates decomposition of residues as well as more resistant materials (Couteautx et al., 1995). At deeper depth, no consistent difference in ROC was observed for tillage, N fertilization, or cropping sequence, except in the sorghum system, where CT tended to increase ROC compared to NT (Fig. 4.4b, c).

The proportion of SOC to ROC generally was increased by NT in surface soil for most cropping systems compared to CT, though difference were small (Fig. 4.5). This proportion decreased slightly with depth. Decreasing this proportion with depth was consistent with other studies (Collins et al., 2000; Paul et al., 1997). Follet et al. (1997) reported that this fraction was 59, 54, and 49% for 0- to 10-, 10- to 20-, and 20- to 30-cm depths, respectively, in a wheat-fallow system. No tillage increased the proportion of SOC to ROC compared to CT at depths of 0 to 15 cm. By contrast, these authors reported a more rapid decrease in this proportion in a native soil with depth compared to cultivated soil. One possible explanation may be due to changes in lignin content. Highest lignin contents were observed in coarser soil fractions, and lignin contents gradually decreased with decreasing particle size (Amelung et al., 1999).

Relationship of Slow and Resistant Organic C

Mineral-associated and ROC were highly correlated with each other ($P < 0.01$) across all cropping and tillage systems (Fig. 4.6). The coefficients of determination were 0.87, 0.90, and 0.95, respectively, in surface soils for wheat, sorghum, and soybean. This result suggests that MAC may be a quantitative indicator of change in the ROC pool. Intercepts and slopes of all linear regressions in the 0- to 5-cm depth were very similar for the different cropping systems. Slopes were 0.77, 0.79, and 0.80, respectively, for wheat, sorghum, and soybean systems and corresponding intercepts ranged from –1.90 to –1.84.

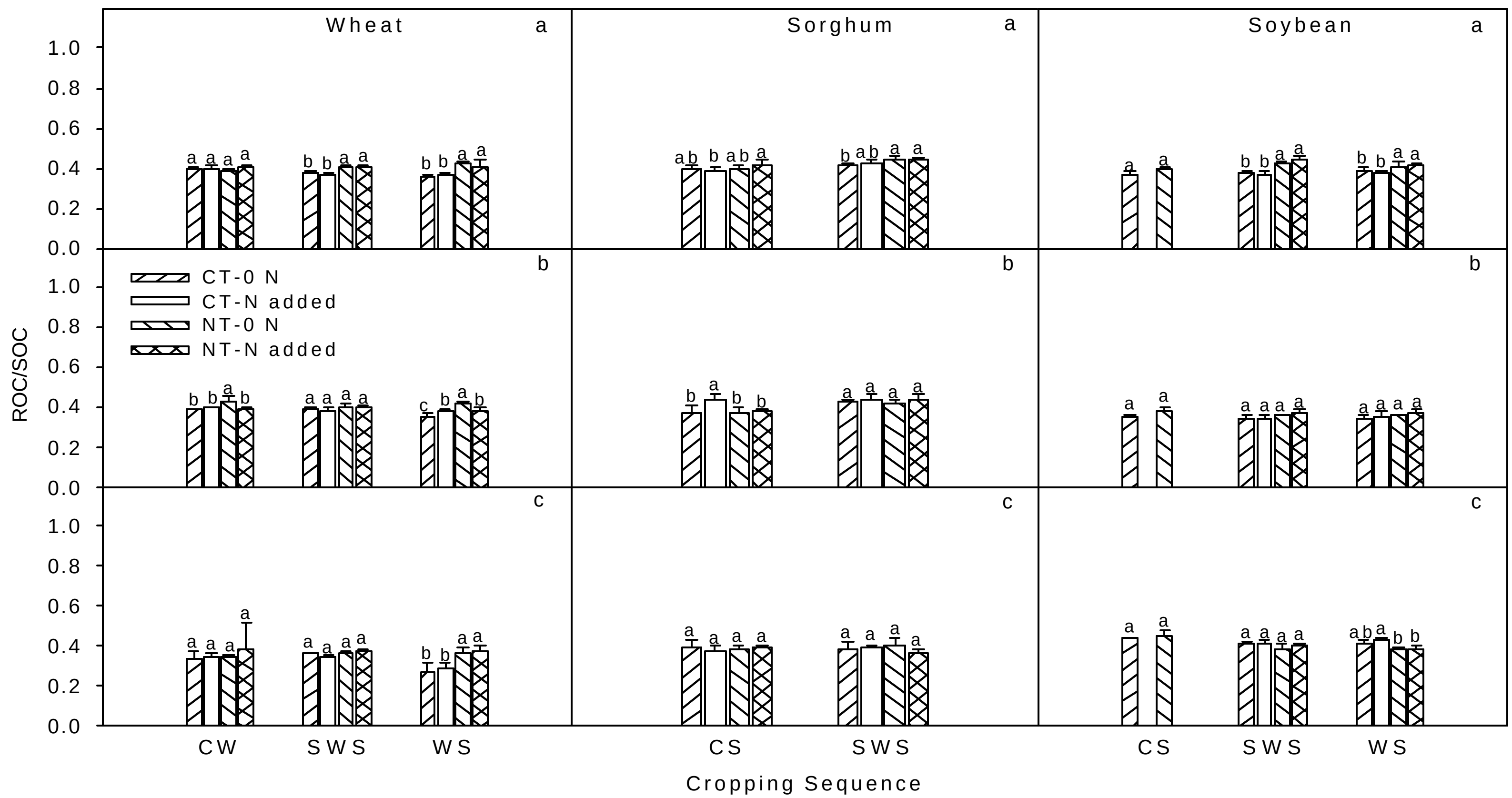

Figure 4.5. The proportion of soil organic C (SOC) as resistant organic C (ROC) with depth as affected by cropping sequence, tillage, and N fertilization at a) 0- to 5-, b) 5- to 15-, and c) 15- to 30-cm depths. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat-soybean, respectively. CS in sorghum and soybean refers to continuous sorghum and soybean, respectively. CT and NT refer to conventional and no tillage. Nitrogen fertilization in soybean systems refers to the previous crop that received N fertilization. Wheat, sorghum, and soybean legends refer to soil samples collected after wheat, sorghum, and soybean harvesting. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

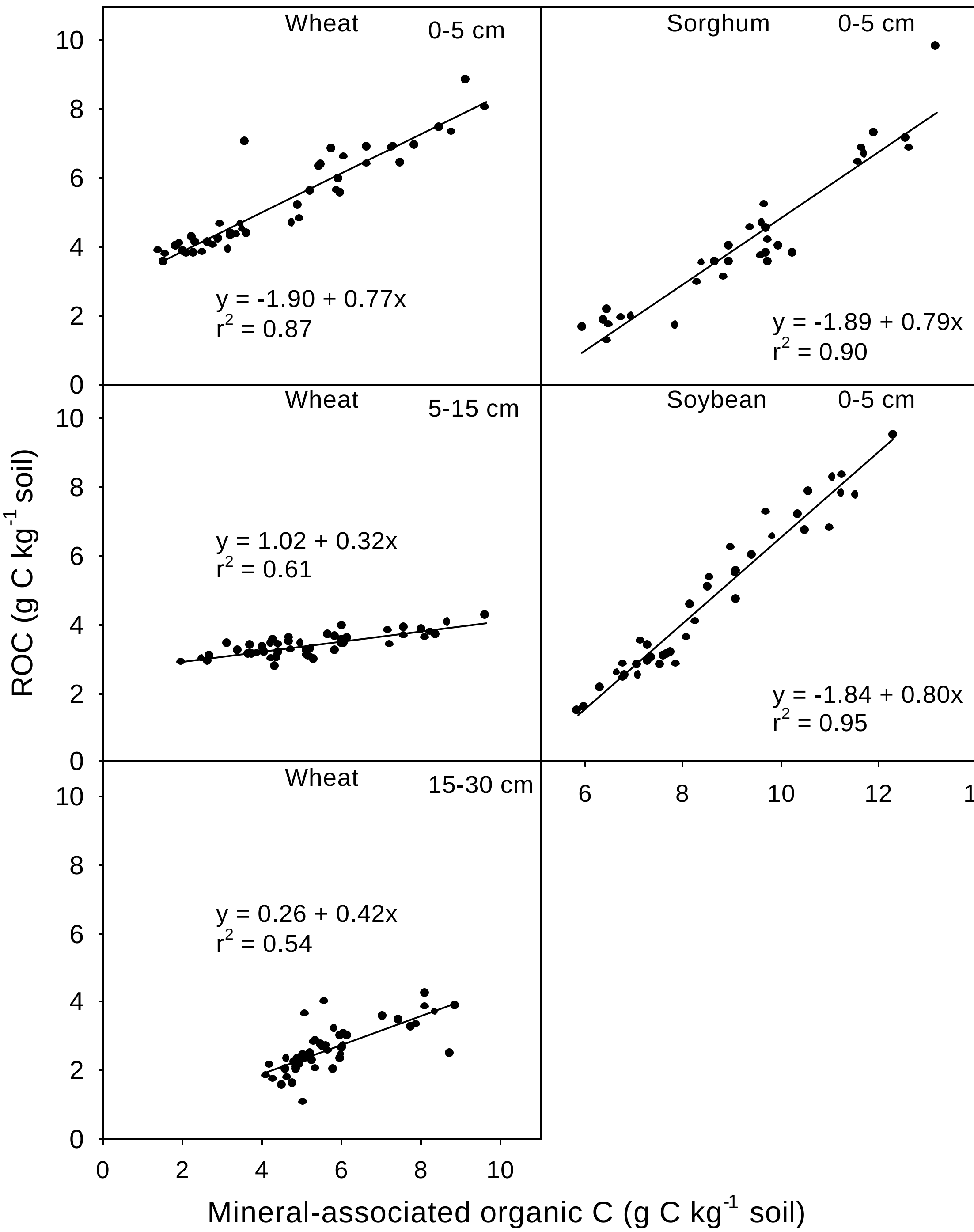

Figure 4.6. Linear regression of mineral-associated organic C and resistant organic C (ROC) in wheat, sorghum, and soybean cropping systems.

These results indicated that a high association between MAC and ROC, and that the relationship was independent of cropping system. Compared to the determination of MAC, measuring ROC presents several disadvantages. First, separation of ROC requires special reflux equipment and a fume hood. Second, removing acid from the insoluble residue is time consuming. Third, due to low yield of residue after acid hydrolysis, greater variations were observed for the ROC data. Therefore, it may be desirable to estimate the ROC pool using simple linear regression models of MAC vs. ROC.

In the wheat systems, the coefficients of determination of MAC vs. ROC decreased with soil depth from 0.87 to 0.54, although regressions were still significant. Furthermore, the slopes of the linear regressions also decreased from 0.77 to 0.32. Decreasing slope with depth indicated a lower ROC content in the MAC, which is consistent with our previous results. Using physical fractionation and CuO oxidation methods, Kiem and Kogel-Knabner (2003) proposed that polysaccharides (a slow C pool), mainly those of microbial origin, contributed to the fine refractory C pool, and CuO-oxidized lignin was associated with coarse fractions.

CONCLUSIONS

After 20-yr of treatment imposition, MAC and ROC were affected by tillage, N addition, and cropping intensity. Most significant effects occurred at a depth of 0 to 5 cm. Significant interactions of tillage by N addition for these parameters were observed in all cropping systems in surface soil. Mineral-associated C and N under NT were significantly greater with than without N addition. However, minimal difference was observed under CT. These results indicated that greater protection by soil minerals under

NT resulted in greater C storage. Enhanced cropping intensity also increased C and N sequestration in the mineral-associated < 53-µm pool, which suggests that greater plant residue input with increased cropping intensity benefited C sequestration, too. In addition, crop species also exhibited different impacts on MAC in the decreasing order: continuous wheat > sorghum > soybean. This order may reflect a difference in quality of residue input, since wheat and sorghum had essentially equivalent return of stover. The proportion of SOC as MAC ranged from 0.61 to 0.95, and increased with soil depth, indicating that more C stabilization with depth. High correlations ($r^2 \geq 0.85$) were observed between MAC and ROC pools for all crop species. The proportion of SOC as ROC, however, decreased with depth. High correlation between these two pools suggested that MAC might be a quantitative indicator of ROC across diverse factors.

CHAPTER V

STORAGE OF CARBON AND NITROGEN IN PHYSICAL FRACTIONS RESPONSE TO TILLAGE, CROPPING SEQUENCE, AND NITROGEN FERTILIZATION

INTRODUCTION

Organo-mineral interactions not only influence the formation and stabilization of soil aggregates (Tisdall and Oades, 1982; Tisdall, 1996; Christensen, 2001), but also contribute to the dynamics of soil organic matter (SOM) (Christensen, 1996; Haynes and Beare, 1996). Compared to macroaggregates, microaggregates are more stable (Tisdall, 1996) and less affected by agricultural practices (Christensen, 2001). The basic structure of microaggregates consists of primary particles bonded by particulate organic matter (POM). Golchin et al. (1994) separated two light fractions of POM with different chemical characteristics. One was the free light fraction consisting mainly of undecomposed plant fragments of 50- to 200-μm diameter, and some larger particles of plant origin encrusted with clay. The other was the occluded light fraction, now called protected POM, with a smaller diameter of 10 to 20 μm. Compared to the former, the occluded light fraction contained more alkyl C and aromatic C, and less O-alkyl C which is thought to originate from plants.

Physical fractionation has recently been widely used to investigate the dynamics of C and N associated with different SOM pools (Christensen, 2001). Various chemical fractionation and characterization methods have not proven particularly useful in following the dynamics of organic materials in soils and in identifying specific SOM pools that diminish upon intensive management (Stevenson et al., 1989). Compared to chemical fractionation, physical techniques are considered chemically less destructive, and the results obtained from physical soil fractions relate more directly to the structure and function of SOM in situ (Christensen, 1992).

Different agricultural management practices can affect the amount and turnover of SOM (Angers and Carter, 1996). Compared to conventional tillage (CT), conservation tillage or no tillage (NT) not only altered the distribution of plant residue input, but also the dynamics of soil aggregates and environmental conditions (Franzluebbers et al., 1995a). Cultivation disturbs soil structure by destroying soil macroaggregates and exposing protected organic matter to decomposers (Cambardella and Elliott, 1993). Tillage incorporates aboveground plant residues into the soil matrix, which accelerates decomposition. In contrast, NT leaves more plant residue on the soil surface, and reduces gas and energy exchange between the soil surface and the atmosphere. These reductions decrease soil temperature and increase soil water content, thereby, favoring C accumulation (Franzluebbers et al., 1995b; Grant et al., 1997). In addition, greater fungal growth, which contributes to the formation and stabilization of macroaggregates, has been reported under NT (Tisdall and Oades, 1982; Holland and Coleman, 1987). Moreover, Six et al. (2000b) observed that the rate of macroaggregate formation and degradation (i.e., aggregate turnover) was reduced under NT compared to CT and led to a formation of stable microaggregates in which C was sequestered long term.

Cropping sequence and nitrogen fertilization can also affect plant residue distribution and production (Franzluebbers et al., 1994; Haynes and Beare, 1996). West and Post (2002) examined a total of 67 global, long-term agricultural experiment sites consisting of 276 paired treatments. In this analysis, enhancement of rotation complexity referred to a change from monoculture to continuous rotation cropping, a change from crop-fallow systems to continuous monoculture or rotation cropping, and an increase in the number of crops used in a rotation cropping system. These authors indicated that enhancing rotation complexity could sequester an average extra 20 $\pm$ 12 g C m^{-2} yr^{-1}, excluding a change from continuous corn (*Zea mays* L.) to corn-soybean which may not result in a significant accumulation of SOC. In addition, Dick, (1992) suggested that crop rotation promotes crop productivity by suppressing deleterious microorganisms that flourish under monocultures.

In this study, a long-term experiment with tillage, cropping sequence, and nitrogen fertilization treatments was initiated to explore changes in soil C and N associated with i) silt and clay fractions, ii) in microaggregates, and iii) in POM.

MATERIALS AND METHODS

Crop Management and Site Characteristics

A long-term field experiment was initiated in 1982 in the Brazos River floodplain in southcentral Texas ($30^0$32'N, $94^0$26'W). Wheat (*Triticum aestivum* L.) was managed under conventional (disk) tillage and no tillage in continuous wheat (CW), continuous wheat/soybean [*Glycine max* (L.) Merr.] (WS), and rotated wheat/soybean-sorghum [*Sorghum bicolor* (L.) Moench.] (SWS) cropping sequences. Crop growing seasons were from early November to late May for wheat, early June to late October for soybean, and late March to late July for sorghum. Continuous wheat produced one crop each year, WS produced two crops each year, and SWS produced three crops every two years. Cropping intensity was defined as the fraction of the year when a crop was growing, and was 0.5 for CW, 0.65 for SWS, and 0.88 for WS. Nitrogen fertilizer (NH_4NO_3) was broadcast on wheat at 0 or 6.8 g N m^{-2} during late winter or early spring. Soybean did not receive N fertilizer, while sorghum received 0 or 9 g N m^{-2} banded preplant. Soybean received 0 or 1.5 g P m^{-2}.

A split-split plot within a randomized complete block design was established with cropping sequence as the main plot, tillage as the sub plot, and N fertilizer rate as the sub-sub plot. Split, split plots measured 4 x 12.2 m and treatments were replicated four times.

The soil is classified as a Weswood silty clay loam (fine-silty, mixed, superactive, thermic Udifluventic, Haplustepts) and contains an average of 115, 452, and 433 g kg^{-1} of sand, silt, and clay, respectively. The soil has a pH of 8.2 (1:2, soil:water) and an organic C content of approximately 8 g C kg^{-1} soil. Annual temperature is 20 ^{0}C and rainfall is 978 mm.

Soil Sampling

Soil samples were collected shortly after wheat harvest in May 2002. Individual samples consisted of 25 composited cores (19-mm dia.) per split-split plot that were divided into depth increments of 0 to 5, 5 to 15, and 15 to 30 cm. Only results for the 0- to 5-cm depth are reported. Soil was sieved to pass a 4.8-mm screen (visible pieces of crop residues and roots removed) and oven-dried for 24 h at 40 ^{0}C. A portion of the sieved, moist soil was also dried at 60 ^{0}C for 48 h for chemical and physical analysis.

Size and Density Fractionation

Size and density fractionation was conducted on soil samples to isolate the SOC fractions described in the conceptual model of Six et al. (2002a). Soil (20 g) from the 0-to 5-cm soil layer was immersed in deionized water on top of a 250-µm mesh screen and shaken with 50 glass beads (dia. = 4 mm). A continuous and steady water flow through the screen was used to ensure that microaggregates were immediately flushed onto a 53-µm sieve and not exposed to any further disruption by the beads. After all macroaggregates were broken, the material on the 53-µm sieve was washed to ensure that the isolated microaggregates were water-stable. The inter-microaggregate POM retained together with the microaggregates on the sieve was isolated by density flotation in 1.85 g cm^{-3} sodium polytungstate (Six et al., 2000b). This procedure resulted in the following fractions: (i) coarse, non-protected POM (>250 µm), (ii) fine, non-protected POM (53 to 250 µm), (iii) protected POM (53 to 250 µm), (iv) protected <53-µm fraction, (v) non-protected <53-µm fraction, (vi) resistant organic C (ROC) in the non-protected <53-µm fraction, and (vii) ROC in the protected <53-µm fraction. A schematic of the fractionation scheme is described in Fig. 5.1.

Chemical Analysis

SOC was determined using the modified Mebius method (Nelson and Sommers, 1982) and soil total nitrogen (TN) was determined following the procedure of (Gallaher et al., 1976), with analysis by an automated salicylic acid modification of the indophenol blue method (Technicon, 1977a).

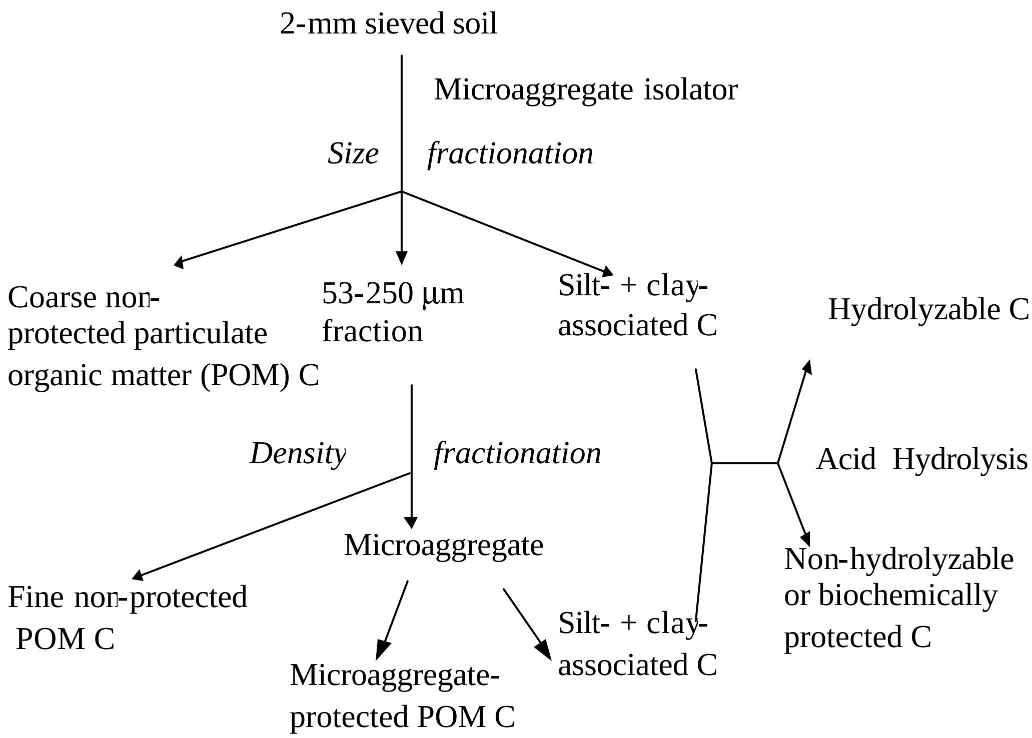

Figure 5.1. Fractionation scheme to isolate soil organic C (SOC) fractions using a conceptual model (Six et al., 2000a)

Resistant organic C and N in free form in the fraction <53 µm within microaggregates was determined using the method suggested by Rovira and Vallejo (2002) with the following modifications. One g of oven-dry <53-µm sample passing through 60 mesh was hydrolyzed with 25 mL of 6 *N* HCl in a digestion tube in an aluminum block digester at 110 ^{0}C for 18 h, with occasional shaking. After cooling, the unhydrolyzed residue was recovered by centrifuging at 2851 x g and decanting the liquid. The process of centrifugation and decantation was repeated several times with deionized water until neutral pH was reached. The residue was then transferred to a pre-weighed vial, dried at 60 ^{0}C to constant weight, and determined with an elemental analyzer (Carlo

Erba EA-1108, Lakewood, NJ, USA) interfaced with a Delta Plus isotope ratio mass spectrometer (ThermoFinnigan, Bremen, Germany) operating in continuous flow mode.

Carbon and N in coarse unprotected and protected POM were finely ground to pass a 150-μm sieve and analyzed following Harris et al. (2001). Briefly, samples were weighed into silver capsules and treated with 12 *M* HCl to remove inorganic carbon ($CaCO_3$), and then analyzed as previously described for ROC.

Statistical Analysis

Analysis of variance and regression were conducted (SPSS, 2001). All differences discussed are significant at the P<0.05 probability level, unless otherwise stated. Fisher's protected least significant difference was calculated only when the analysis of variance F-test was significant at P<0.05.

RESULTS AND DISCUSSION

Size Distribution of Soil

About 99% of the soil was distributed into the two smaller size fractions (<53 and 53 to 250 μm) (Fig. 5.2). There was no significant difference between the amount of the <53 and 53- to 250-μm fractions. The amount of soil in the <53-μm fraction, however, was 8% greater than that in the 53- to 250-μm fraction when averaged across all treatments. The amount of soil in the <53-μm fraction was greater under CT than NT with N applied except in CW but it was insignificant. In contrast to the smallest fraction, soil distribution

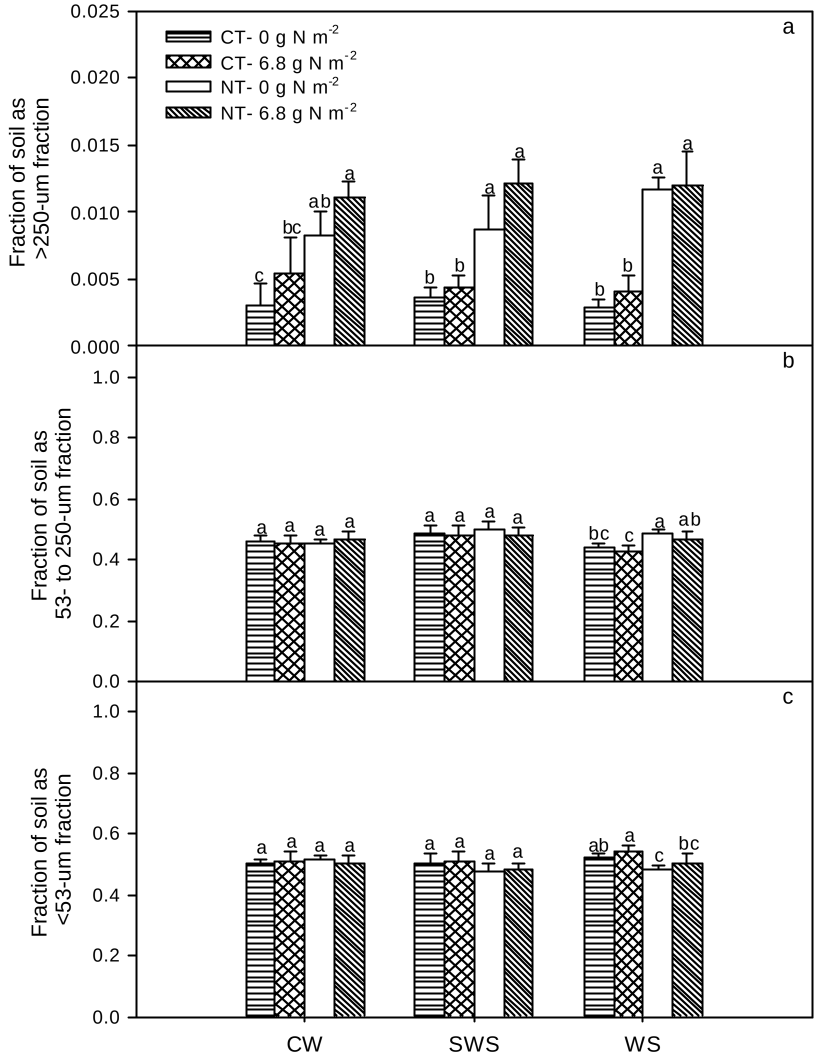

Figure 5.2. Soil distribution into size fractions (a) >250 µm, b) 53 to 250 µm, c) <53 µm) as affected by cropping sequence, tillage, and N fertilization. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat soybean sequences. CT and NT indicate conventional and no tillage. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

into the 53- to 250-μm fraction was greater under NT than CT for all cropping sequences but only significant in WS. Similar results were observed for the >250-μm fraction. No tillage increased soil in the >250-μm fraction compared to CT. Nitrogen application also increased the quantity of soil found in this fraction, partially because of increased residue production.

Organic C and N in Unprotected and Protected <53-μm Fractions

No tillage significantly increased the concentration of organic C in the <53-μm fraction compared to CT for all cropping sequences (Fig. 5.3a). The non-protected organic C concentration in this size fraction under NT was 15%, 29%, 31% greater in CW, SWS, and WS than with CT, respectively (Fig. 5.3a). The protected organic C concentration in the size fraction under NT was 16, 37, and 38% greater in CW, SWS, and WS than CT, respectively (Fig. 5.4a). Unlike NT, no differences in organic C among cropping sequences was observed under CT (Figs. 5.3a and 5.4a). Those results indicated that the organic C concentration of this size fraction increased with crop intensity only under NT.

Nitrogen application increased organic C in this size fraction with NT, but results were only significant for CW (Figs. 5.3a, 5.4a, and 5.4b). Nitrogen addition did not influence organic C under CT.

In contrast, Cambardella and Elliott (1992) did not observe any difference in organic C between NT and CT in the <53μm fraction. One explanation for this observation may be the difference in cropping intensity. The cropping sequences for our study were CW,

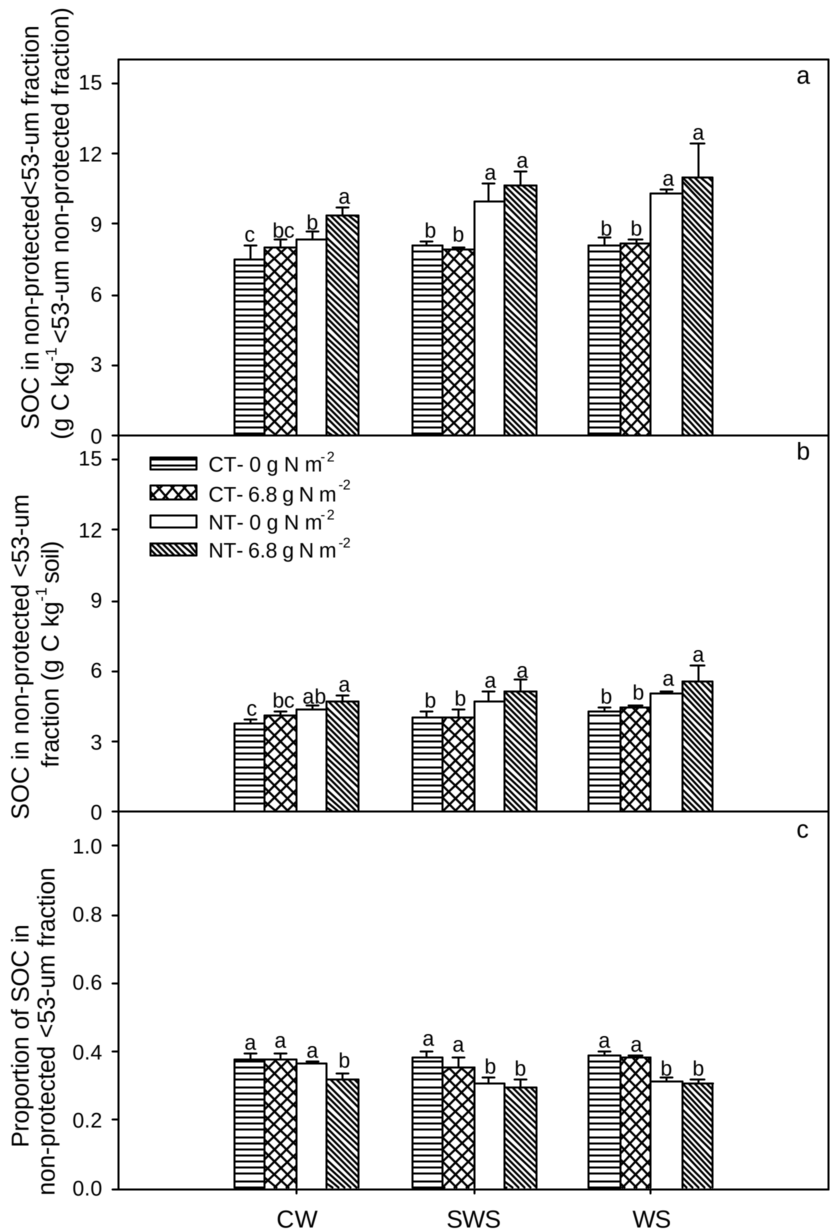

Figure 5.3. Concentration of non-protected organic C in the <53-μm fraction (a), calculated on a whole-soil basis (b), and as a proportion of SOC in the <53-μm fraction (c) as affected by cropping sequences, tillage, and N fertilization. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat soybean sequences. CT and NT indicate conventional and no tillage. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

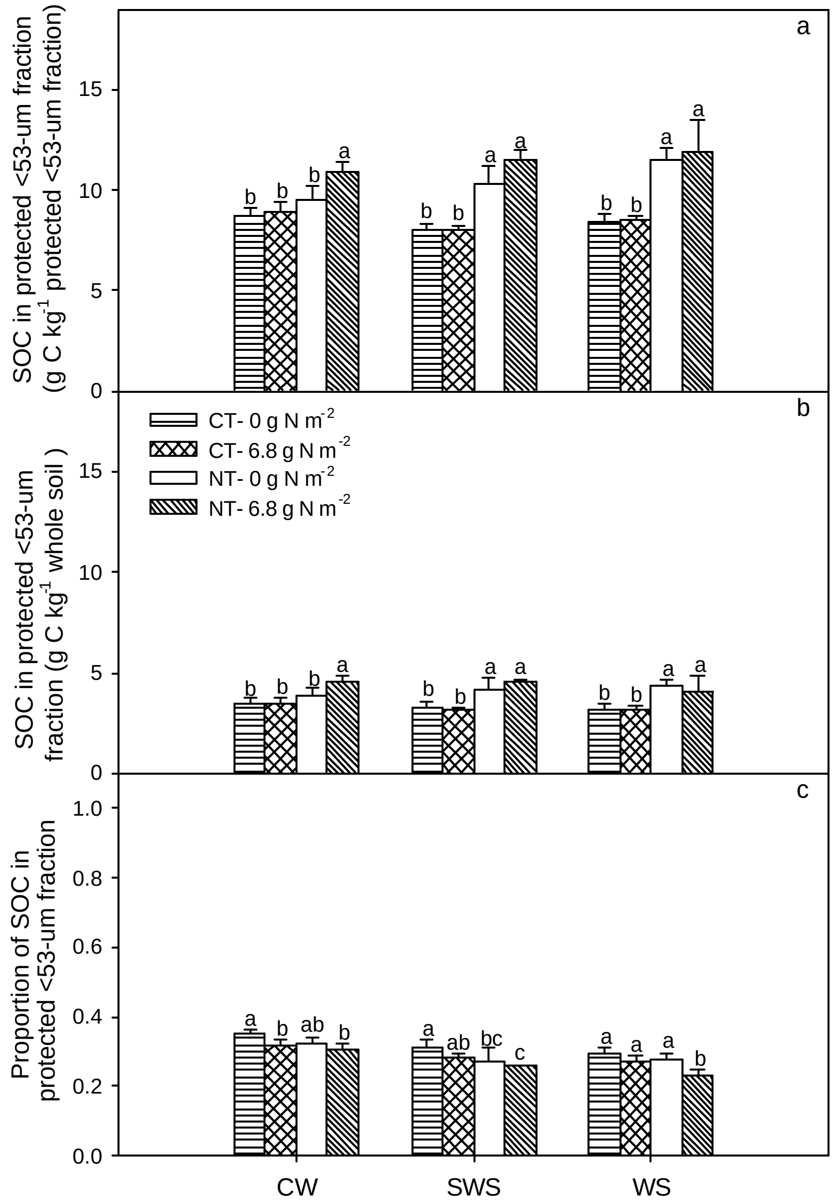

Figure 5. 4. Concentration of protected organic C in the <53-μm fraction (a), calculated on a whole-soil basis (b), and as a proportion of SOC in the <53-μm fraction (c) as affected by cropping sequences, tillage, and N fertilization. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat soybean sequences. CT and NT indicate conventional and no tillage. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

SWS, and WS instead of a wheat-fallow system. A tendency similar to that of the previous authors was observed for CW, especially, with no N fertilization. Another factor might be the difference in sampling depth. We used the surface (0-50 mm) soil, while Cambardella and Elliott (1992) used 0 to 20 cm. We also found no significant difference for SOC in the <53-μm fraction between NT and CT at the 50- to 15-cm depth (data not shown). The concentration of OC reported by Cambardella and Elliott (1992) was three times larger. This result might be explained by differences in climate and soil texture (Christensen, 1996).

The proportion of SOC in the <53-μm fraction was greater under CT than NT (Figs. 5.3c and 5.4c), with proportions ranging from 23 to 39%. Cropping sequence had little impact on the proportions, while N addition tended to decrease it.

Physical protection by microaggregates affected organic C concentration of the <53-μm fraction and the proportion of SOC as OC in the <53-μm fraction. Compared to the non-protected <53-μm fractions, greater C concentration was observed for the protected <53-μm fractions. In addition, the difference between NT and CT for C concentration of the protected <53-μm fraction was greater than the non-protected <53-μm fraction. In contrast to the C concentration, the difference in the proportions of SOC in the non-protected <53-μm fraction between NT and CT was greater compared to the protected <53-μm fraction.

Total nitrogen in this fraction was highly related with organic C (Table 5.1). C:N of the non-protected or protected <53-μm fraction was approximately 10:1 and there was no significant difference between NT and CT. Nitrogen application did not affect C:N ratio.

Table 5.1. Correlation matrix of soil carbon and nitrogen pools in physical fractions at 0 to 5 cm in soil samples following wheat (n = 48).

		<53-um fraction		Microaggregate		PPOM†		Resistant organic matter	
		OC‡	ON§	OC	ON	OC	ON	OC	ON
< 53-um fraction	OC	---	**	**	**	**	**	**	**
	ON	0.812	---	**	**	**	**	**	**
Microaggregate	OC	0.914	0.86	---	**	**	**	**	**
	ON	0.847	0.836	0.965	---	**	**	**	**
PPOM	OC	0.77	0.681	0.866	0.899	---	**	**	**
	ON	0.831	0.732	0.917	0.925	0.986	---	**	**
Resistant organic matter	OC	0.857	0.807	0.934	0.938	0.836	0.856	---	**
	ON	0.675	0.588	0.685	0.664	0.628	0.627	0.744	---

†Protected particulate organic matter.
‡Organic carbon.
§Organic nitrogen.
** denotes significance at P = 0.01.

The <53-μm fractions consist of primary particles such as clay and silt minerals, small microaggregates, as well as small-size particulate organic matter (SPOM) (Shang and Tiessen, 2000). Using a size and density fractionation method, Hassink et al., (1997) observed a positive relationship between the proportion of particles <20 μm in a soil and the amount of C that becomes associated with this fraction in both surface grassland and arable soils. In our case, if we assume both soils under NT and CT have the same proportion of particles <53 μm, then the difference between NT and CT could be explained by several factors. First, organic C in this fraction does not reach a maximum. Second, it was due to the difference in sieve size. We included larger particles into this fraction. They also observed the increased C for the larger size fractions. However, if we consider the effect caused by fraction source, it appears that free fraction still does not reach a maximum, compared to C concentration under NT from microaggregate.

The basic model proposed for organo-mineral is Clay-P-OM, where, P represents polyvalent cations such as Ca^{2+}, et c. (Edwards and Bremner, 1967). It can further form microaggregates such as $(\text{Clay-P-OM})_x$ or $(\text{OM-P-OM})_x$ (x represents integer). It is suggested that C associated with this fraction is very stable, and is not easily disrupted by agricultural practices (Turchenck and Oades, 1978). However, our results contrasted with those observations. It might indicate that microaggregates provide extra physical protection from microbial decomposers, compared to the free fraction.

Resistant Organic C and N

Resistant organic C (ROC) was highly related with organic C of the <53-μm fraction either non-protected or protected by microaggregate (Table 5.1). Concentrations of ROC on both size fraction and whole soil basis were significantly greater under NT than CT ($P<0.05$), except for NT in CW without N application (Figs. 5.5 and 5.6). Based on residue weights after acid hydrolysis, ROC in the non-protected <53-µm fraction under NT was 12%, 29%, and 46% greater for CW, SWS, and WS (Fig. 5.5a). ROC in the protected <53-µm fraction under NT was 18, 58, and 52% greater for CW, SWS, and WS, respectively than with CT (Fig. 5.6a). Nitrogen applications did not consistently affect ROC under either NT or CT. Cropping sequences increased ROC in the order CW< WS< SWS, but difference was insignificant (Figs. 5.5a, 5.6a). Similar tendencies were observed for ROC after conversion to a whole soil basis (Figs. 5.5b and 5.6b).

The percentage of SOC as ROC in the protected <53-µm fraction was significantly higher than that for the unprotected faction (Figs. 5.5c and 5.6c). This result indicated that OC in the non-protected fraction tended to be more labile than that associated with microaggregates.

Resistant N was highly related with ROC in the <53-µm fraction non-protected or protected by microaggregates (Table 5.1). The C:N ratio of resistant organic matter for the protected <53-µm fraction under NT was significantly larger than CT except for CW (Fig. 5.7). Average values of C:N ratio were 8:1 and 10:1 for CT and NT, respectively. The lower C:N under CT indicated that organic matter was more decomposed than under NT. In contrast, Six et al. (2002b) reported a consistently lower C:N of 6 to 8 for the non-hydrolyzable fraction, and suggested that it is a microbially derived SOC pool. Senesi and Loffredo (1999) suggested that resistant organic matter consists of materials from plants such as lignin, suberin, and those from microorganisms such as melanins and

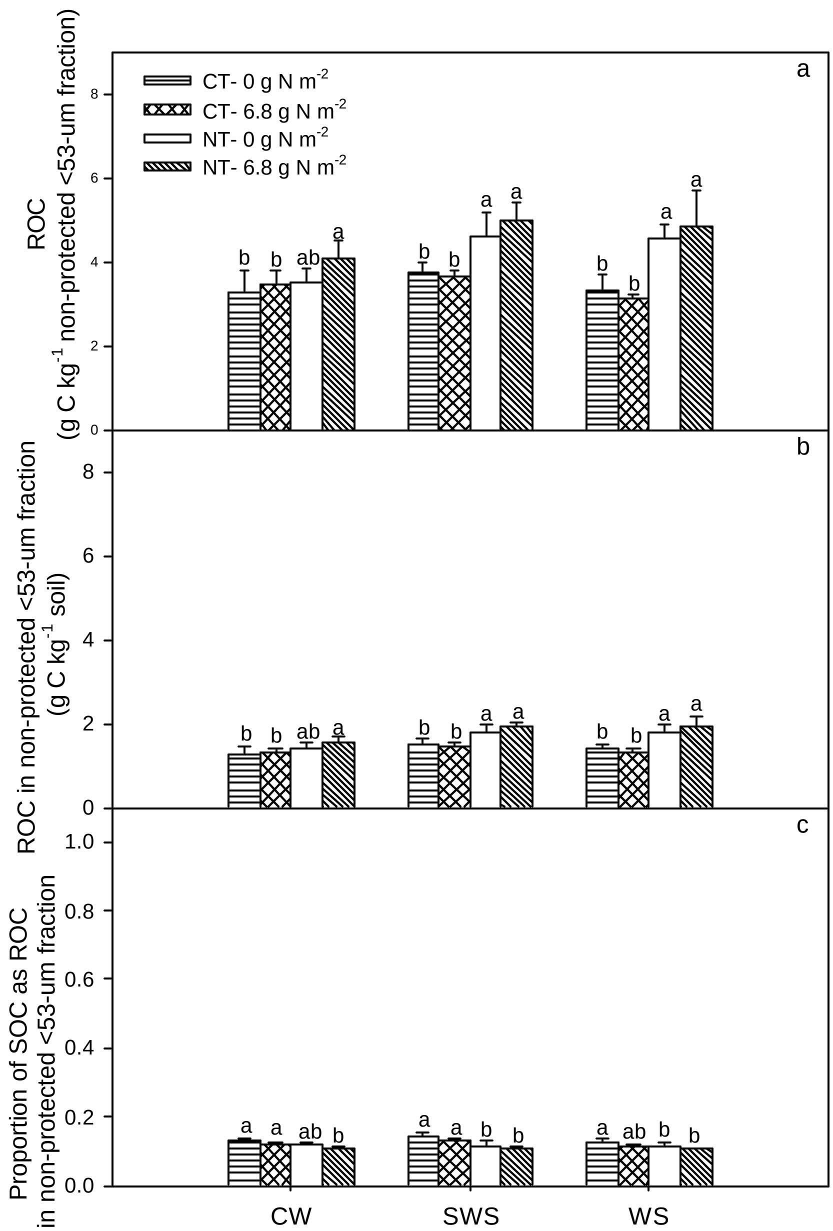

Figure 5.5. Concentration of non-protected resistant organic (ROC) C in the <53-µm fraction (a), calculated on a whole-soil basis (b), and as a proportion of SOC in the <53-µm fraction (c) as affected by cropping sequences, tillage, and N fertilization. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat soybean sequences. CT and NT indicate conventional and no tillage. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

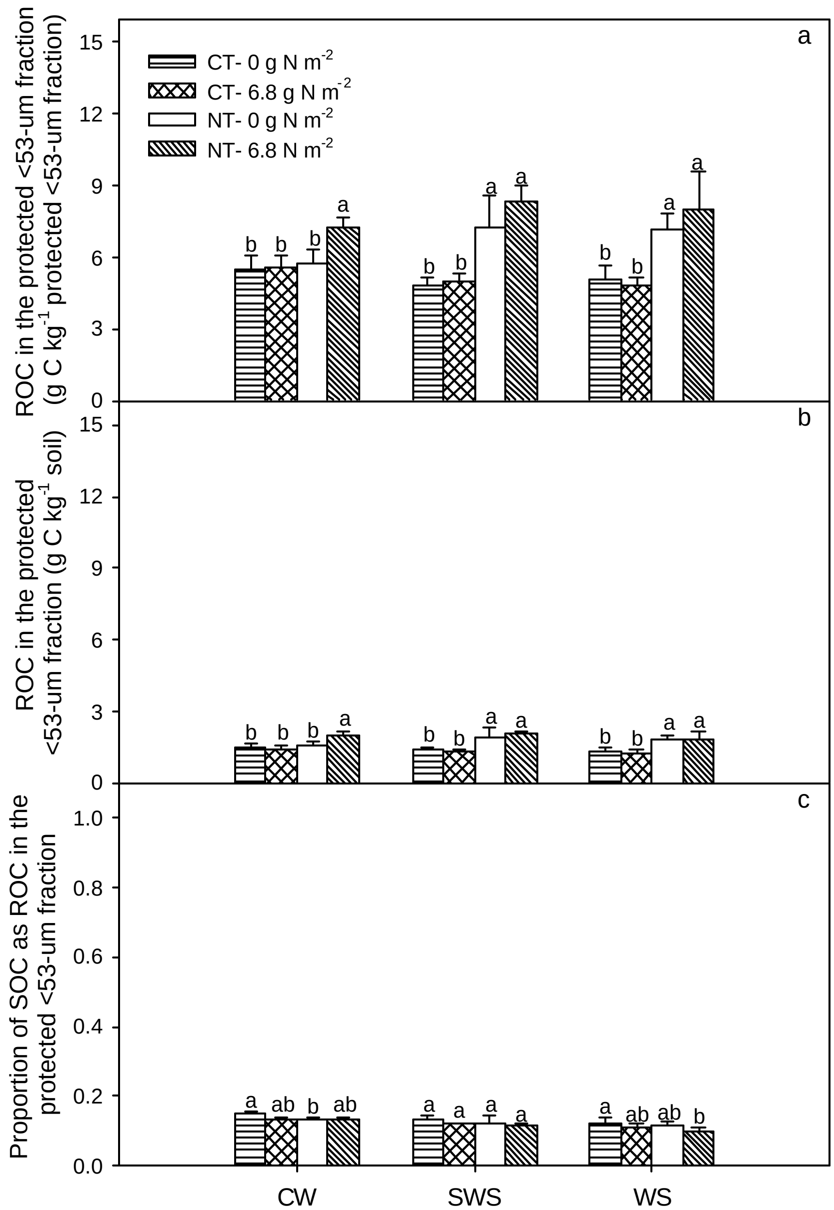

Figure 5. 6. Concentration of protected resistant organic (ROC) C in the <53-μm fraction (a), calculated on a whole soil basis (b), and as a proportion of SOC in the <53-μm fraction (c) as affected by cropping sequences, tillage, and N fertilization. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat soybean sequences. CT and NT indicate conventional and no tillage. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at P<0.05.

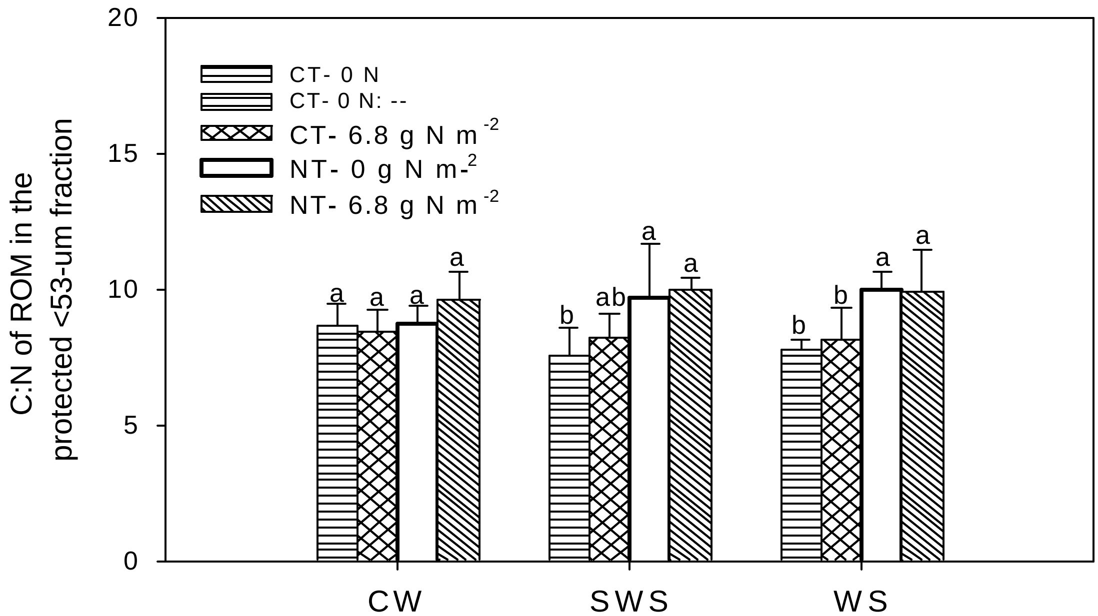

Figure 5.7. C:N of protected resistant organic matter (ROM) in the <53-μm fraction as affected by cropping sequences, tillage, and N fertilization. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat soybean sequences. CT and NT indicate conventional and no tillage. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at P<0.05.

paraffinic macromolecules. No tillage likely exerts more protection for SOC than CT, and thus affected the C:N ratio of resistant organic matter.

Microaggregate Associated C and N

A significant interaction between tillage and N application was observed (P<0.05) for the organic C concentration of microaggregates (53 to 250 μm) calculated on fractional and whole soil bases (Fig. 5.8a, b). Organic C of microaggregates was significantly greater with than without N only under NT. Organic C of microaggregates under NT was 42, 88, and 85% greater in CW, SWS, and WS, respectively, than with CT

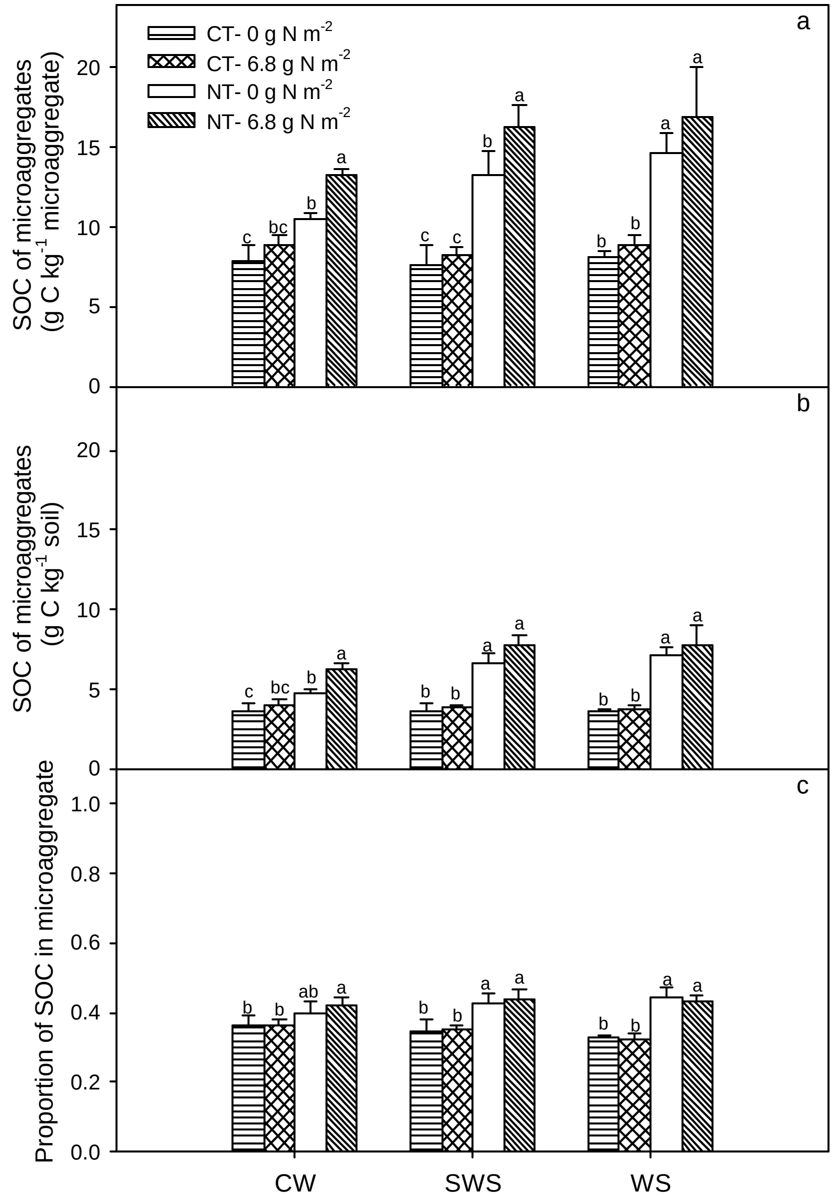

Figure 5.8. Concentration of microaggregate organic C in the 53- to 250-µm fraction (a), calculated on a whole-soil basis (b), and as a proportion of SOC in the 53- to 250-µm fraction (c) as affected by cropping sequences, tillage, and N fertilization. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat soybean sequences. CT and NT indicate conventional and no tillage. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

when averaged across N fertilization. Due to the higher yield of microaggregates under NT than CT, those differences were enhanced when determined on a whole soil basis.

Compared with organic C in the non-protected <53-μm faction, the proportion of SOC in microaggregates under NT was 12, 25, and 35% greater for CW, SWS, and WS, respectively, than with CT (Fig. 5.8c), and indicated that more organic C was physically protected under NT.

Total nitrogen in microaggregates was highly related with organic C (Table 5.1). Greater variation in N was observed than with C. The C:N ratio (8.8 $\pm$ 0.7) of this fraction was less than that (10.7 $\pm$ 0.7) of whole soil (Fig. 5.9).

Tisdall and Oades (1982) suggested that microaggregates consist mainly of <20-μm particles cemented together by plant and fungal debris encrusted with inorganic materials, crystalline oxides and highly disordered aluminosilicates.

Particulate Organic C and N

A significant interaction (P = 0.012) between tillage and N fertilization was observed for protected particulate organic matter C (Figs. 5.10a, b). Protected POM C (PPOM C) was significantly greater with N fertilization than for no N controls under NT, except in WS. Protected POM C for CT calculated on a size-fraction basis (Fig. 5.10a) was not affected by N fertilization except for CW where N addition increased PPOM C. Averaged across N treatments, PPOM C under NT was 115, 150, and 85% greater in CW, SWS, and WS, respectively than with CT. Similar results were observed after converting to a

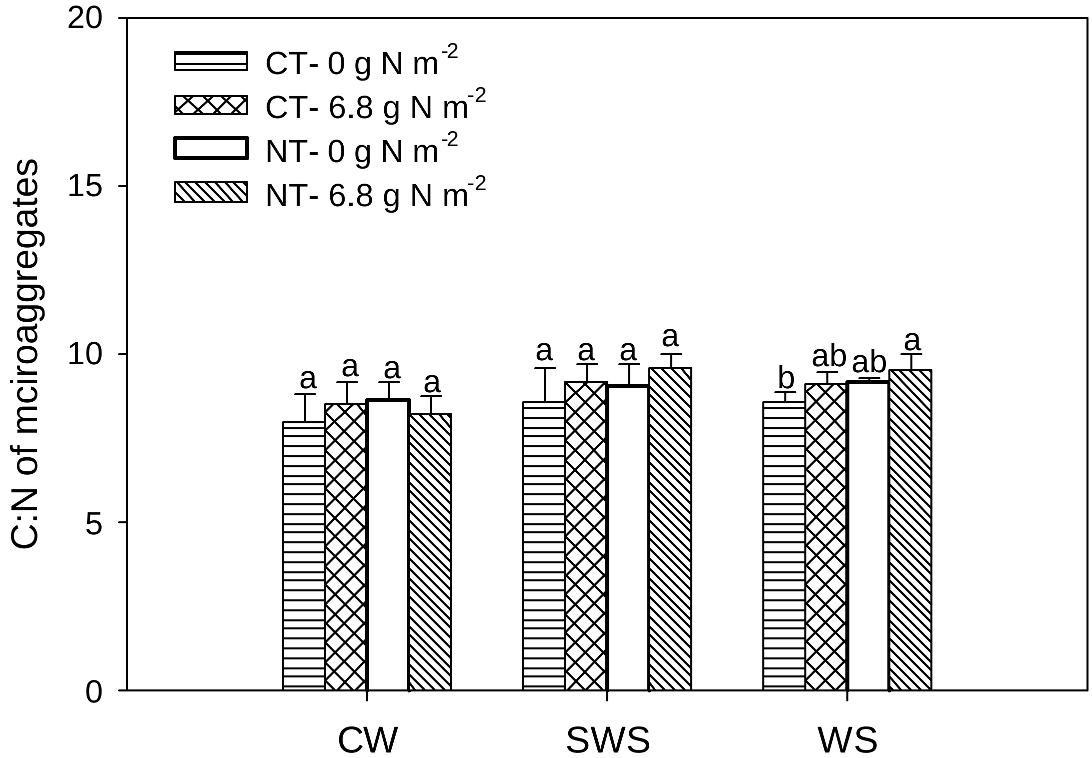

Figure 5.9. C:N ratio of microaggregates (53 to 250 µm) as affected by cropping sequences, tillage, and N fertilization. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat soybean sequences. CT and NT indicate conventional and no tillage. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at P<0.05.

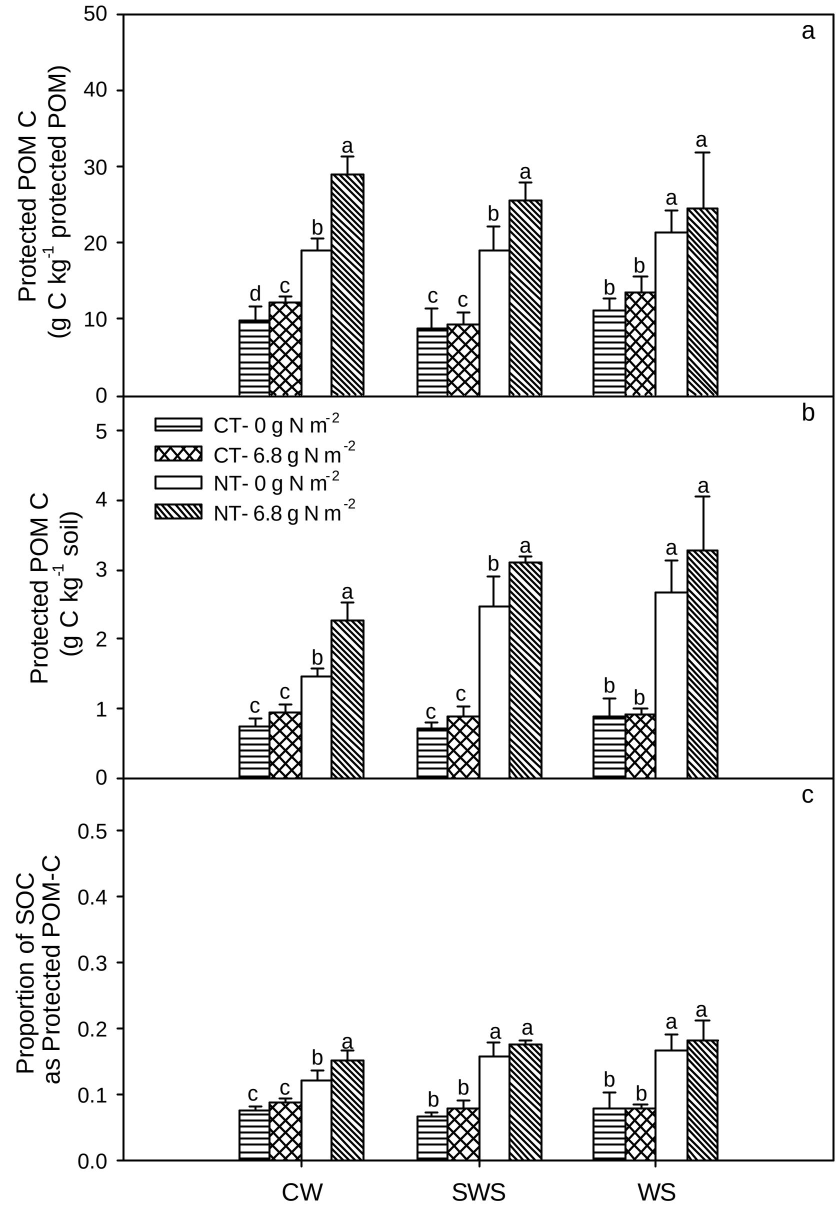

Figure 5.10. Concentration of protected particulate organic matter (POM) C in the 53- to 250-µm fraction (a), calculated on a whole-soil basis (b), and as a proportion of soil organic C (SOC) in the 53- to 250-µm fraction (c) as affected by cropping sequences, tillage, and N fertilization. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat soybean sequences. CT and NT indicate conventional and no tillage. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

whole-soil basis, except that PPOM C in CW decreased compared to the other cropping sequences (Fig. 5.10b). The concentration of PPOM C was significantly lower in CW compared to the other cropping sequences.

The proportion of SOC as PPOM C was significantly greater under NT than CT (Fig. 5.10c). Nitrogen application also tended to increase this proportion, but was significant only for NT with CW. Cropping sequence also had little effect on this proportion. Cambardella and Elliott (1992) observed similar results for tillage treatments. The proportion of SOC as PPOM C tended to be more sensitive to changes in management compared to the proportion of SOC as C within the protected <53-μm fraction (Fig. 5.4c).

Protected particulate organic N generally mirrored the results observed for C (Table 5.1). The overall C:N ratio of coarse, unprotected POM was greater than that of mineral-associated , or protected, POM (Figs. 5.11a, b). The C:N ratio of POM under NT was lower than CT across cropping and N treatments. Based on the morphology and chemical structure of organic materials contained in occluded POM forming the core of microaggregates, Golchin et al. (1995) proposed that the types and amounts of occluded organic materials are dependent upon the nature of organic matter input to soil and the microenvironment within soil aggregates. Furthermore, Golchin et al. (1997) suggested that occluded organic materials are more recalcitrant and have higher alkyl and aromatic C contents than free organic materials. In addition, fungi tend to be more dominant under NT than CT (Frey et al., 1999). Since fungi require more C per unit of N consumed than bacteria, this could result in the lower C:N of POM under NT.

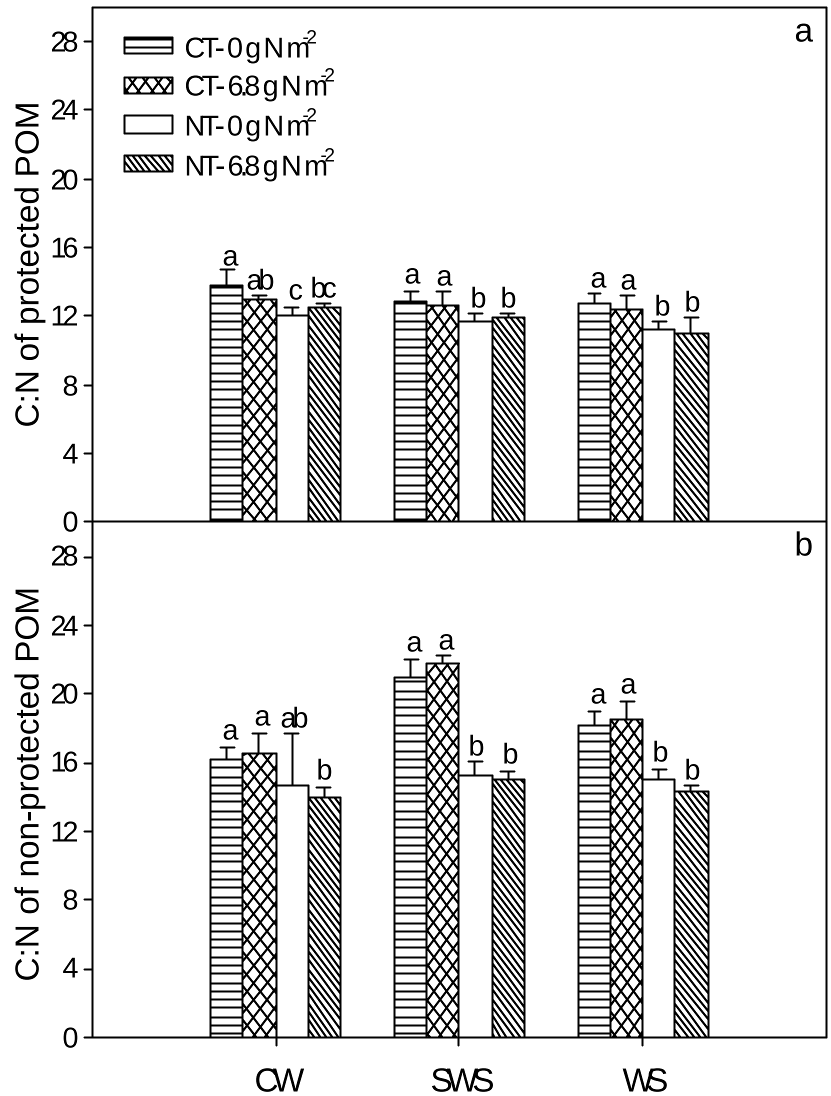

Figure 5.11. C:N ratios of a) protected particulate organic matter (POM) (53- to 250-µm), and b) coarse unprotected particulate organic matter (>250 µm) as affected by cropping sequence, tillage, and N fertilization in surface soil. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat soybean. CT and NT indicate conventional and no tillage. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at P<0.05.

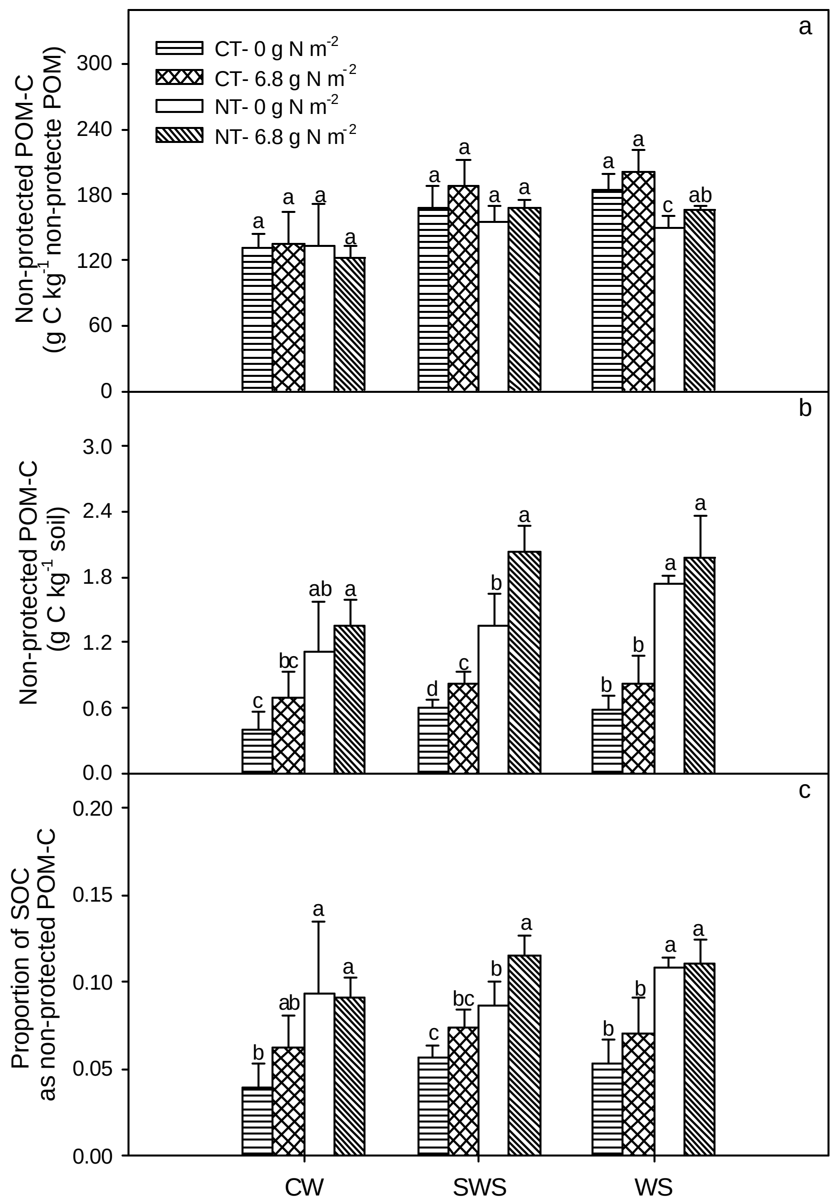

Figure 5.12. Concentration of non-protected particulate organic matter C (POM-C) (>250 µm) in the >250-µm fraction (a), calculated on a whole-soil basis (b), and as a proportion of soil organic C (SOC) in the >250-µm fraction (c) as affected by cropping sequences, tillage, and N fertilization. CW, SWS, and WS indicate continuous wheat, sorghum-wheat-soybean, and wheat soybean sequences. CT and NT indicate conventional and no tillage. Error bars represent standard deviation. Comparisons for tillage and N fertilization are within cropping sequences. Means followed by the same letter are not different at $P<0.05$.

Compared to protected POM, coarse free POMC was not significantly affected by tillage when calculated on a size-fraction basis (Fig. 5.12a). On a whole-soil basis, however, NT increased coarse, unprotected POM C compared to CT (Fig. 5.12b). Nitrogen addition also tended to increase the concentration of this fraction calculated on a whole-soil basis, but was only significant for SWS. Coarse, unprotected POM N was highly related to N (Table 5.1). The proportion of SOC as coarse, unprotected POM followed similar tends as the C concentration of this fraction on a whole-soil basis, with the proportion being greater with NT and being significantly increased by N addition to SWS (Fig. 5.12c).

CONCLUSIONS

Distribution of SOC and N in different pools was affected by tillage, cropping sequence, and nitrogen fertilization. Compared to crop intensity and nitrogen fertilization, tillage exerted a greater effect. Organic C and N under NT increased in all poolscompared with CT. Within enlarged pools, soil microaggregate pools contributed most to increased C sequestration. No tillage affected not only the quantity of SOC and N sequestered, but also the quality. Greater quantities of resistant organic C were found under NT than CT in the <53-µm fractions unprotected or protected by microaggregates. The C:N ratio of the 53- to 250-µm pool which contained the most C and N was lower under NT than CT. All C pools were significantly related with each other, as well as the N in each pool. No tillage plus intensive cropping and N application caneffectively increase soil C and N sequestration.

CHAPTER VI

SOIL CARBON POOL-SIZE ESTIMATIONS AND ^{13}C CHANGES IN PHYSICAL FRACTIONS AS AFFECTED BY TILLAGE, CROPPING SEQUENCE, AND NITROGEN FERTILIZATION

INTRODUCTION

Soil carbon sequestration contributes not only to diminishing the greenhouse gas effect, but also affects soil structure, nutrient cycling, and soil fertility (Lal et al., 2004). Management factors for increasing C sequestration involve enhancing crop residue inputs and decreasing soil organic matter (SOM) decomposition in agricultural ecosystems (Gregorich and Janzen, 1996). Many studies show that conservation tillage, especially no tillage (NT), along with increased cropping intensity, can increase soil C sequestration (Angers et al., 1997; Cambardella and Elliott, 1992; West and Post, 2002). The mechanisms of these management strategies for increasing soil organic C (SOC) have been extensively studied (Jastrow, 1996a; Six et al., 2000b; Tisdall, 1996; Tisdall and Oades, 1982). It is generally accepted that increased cropping intensity increases plant residue input, and positive relationships have been reported between plant residue inputs and SOC (Hassink et al., 1997). Franzluebbers et al. (1994), however, observed no significant difference in SOC under conventional tillage (CT) with increasing crop residue input. Soil organic C increased, though, with increased cropping intensity under NT. Therefore, one infer that SOC under those specific conditions was in a new equilibrium or saturated. Some studies have shown that measurement of SOC is not sensitive enough to respond to short-term changes in management compared to labile pools such as soil particulate organic matter (POM) C or soil microbial biomass (SMB) (Franzluebbers et al., 1994). More information is needed to better understand SOC dynamics under different crop management strategies.

Physical fractionation of soil has recently been used to investigate the dynamics of SOC and N (Christensen, 1992). The concept behind physical fractionation of soil emphasizes the role of soil minerals in SOM stabilization and turnover. Using a physical fractionation scheme, Six et al. (2000b) proposed a model of SOC stabilization under NT in agricultural soils.

The use of ^{13}C natural abundance techniques, coupled with physical and/or chemical fractionation, has provided additional insight into SOM turnover (Balesdent and Mariotti, 1996; Gerzabek et al., 2001; Shang and Tiessen, 2000) for cases where an appropriate change in vegetation from C_3 to C_4 or vice versa has occurred (Balesdent et al., 1987). It is normally assumed that differences in the ^{13}C composition of different parts of C_3- or C_4-derived plants are relatively trivial, and the discrimination of ^{13}C by the microbial community during decomposition is also minor. Thus, the ^{13}C distribution of SOM mirrors the turnover of plant residue, in addition to management and environmental effects.

Long-term incubation has been proposed as a useful tool to distinguish SOC pools with different turnover rates (Collins et al., 2000; Paul et al., 2001; Swanston et al., 2002). Using this tool with stable isotopic tracers, Collins et al. (2000) concluded that SOC mineralized in early stages of organic matter decomposition is from newly added crop residue, while CO_2 released later reflected the slow C pool. Since SOM is a continuum which consists of pools with different turnover rates, we hypothesized that labile pools separated by physical fractionation, active pools determined from long-term incubation, and newly added plant residues should be highly related. However, little information is available using these combined methods to characterize SOC dynamics. The aim of this study was to explore: (i) sizes of the active and slow SOC pools, (ii) mean residence time of the active and slow SOC pools, (iii) the relationship between active, slow, and physically-separated SOC pools, and (iv) changes in ^{13}C of physically-separated SOC pools due to management.

MATERIALS AND METHODS

Crop Management and Site Characteristics

A long-term field experiment was initiated in 1982 in the Brazos River floodplain in south-central Texas ($30^0$32'N, $94^0$26'W). Wheat (*Triticum aestivum* L.) was managed under conventional (disk) tillage and NT in continuous wheat (CW), continuous wheat/soybean [*Glycine max* (L.) Merr.] (WS), and rotated wheat/soybean-grain sorghum [*Sorghum bicolor* (L.) Moench.] (SWS) cropping sequences. Sorghum was managed under conventional (disk) tillage and NT in continuous sorghum (CS), and rotated SWS cropping sequences. Crop growing seasons were from early November to late May for wheat, early June to late October for soybean, and late March to late July for sorghum. Continuous wheat or sorghum produced one crop each year, WS produced two crops each year, and SWS produced three crops every two years. Cropping intensity was defined as the fraction of the year when a crop was growing, and was 0.5 for CW, 0.65 for SWS, and 0.88 for WS. Nitrogen fertilizer (NH_4NO_3) was broadcast on wheat at 0 or

6.8 g N m^{-2} during late winter or early spring. Soybean did not receive N fertilizer, while sorghum received 0 or 9 g N m^{-2} banded preplant.

A split-split plot within a randomized complete block design was established with cropping sequence as the main plot, tillage as the sub plot, and N fertilizer rate as the sub-sub plot in 1982. Plots measured 4 x 12.2 m, and treatments were replicated four times.

The soil used is classified as a Weswood silty clay loam (fine-silty, mixed, superactive, thermic, Udifluventic Haplustepts) and contains an average of 115, 452, and 433 g kg^{-1} of sand, silt, and clay, respectively. Under cultivation, this soil has a pH of 8.2 (1:2, soil:water) and an organic C content of approximately 8 g C kg^{-1} soil. Annual temperature is 20 ^{0}C and annual rainfall is 978 mm.

Soil Sampling

Soil samples were collected shortly after wheat harvesting in May and sorghum harvesting in August 2002. Individual samples consisted of 25 composited cores (19-mm dia.) per plot that were divided into depth increments of 0 to 5, 5 to 15, and 15 to 30-cm. Only results for the 0- to 5-cm depth are reported. 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 ^{0}C. A portion of the sieved, moist soil was also dried at 60 ^{0}C for 48 h for chemical and physical analysis.

Long-term Incubation of Surface Soil

Long-term incubation was by the method proposed by Robertson et al. (1999) with the following modifications. Mineralizable soil C was measured during extended laboratory incubation of initially oven-dry samples. One hundred-g soil samples of each field replicate were adjusted to 55% water holding capacity and incubated in sealed 1000-mL bottles in the dark at 25^0C. Water holding capacity was estimated by the method suggested by Paul et al. (2001). Vials containing 10 mL of water were placed in bottles to maintain humidity, while evolved CO_2 was trapped in vials containing 10 mL of 1 *M* KOH. Control jars contained no soil. The trapped CO_2 was precipitated as $SrCO_3$ using 2 *M* $SrCl_2$ (Harris et al., 1997). The quantity of CO_2 evolved was measured by titration of residual KOH to pH 7.0 with 0.1 *M* HCl. Carbon dioxide evolved was determined at intervals of 1, 7, 17, 24, 52, 80, 108, 136, 164, 192, 220, 248, 276, and 304 days. A constrained two-pool, first-order model was used to estimate pool size and turnover rates of each pool (Paul et al., 2001):

$$C_t = C_a e^{-kat} + C_s e^{-kst} \qquad (6\text{-}1)$$

where C_t is total organic C in soil at time t excluding resistant organic C determined at the beginning of the experiment, C_a and k_a represent size and mean residence time (MRT) of the active pool, respectively, and C_s and k_s represent size and MRT of the slow pool, respectively. The parameters C_a, k_a, C_s, and k_s were estimated using exponential decay (Sigmaplot, 2002).

Size and Density Fractionation

Size and density fractionations were conducted following the same procedures in previous chapter V.

Chemical and Stable Isotopic Tracer Analysis

Resistant organic matter in the protected or non-protected <53-µm fraction was extracted using the method suggested by Rovira and Vallejo (2002) with the following modifications. One g of oven-dried soil of the protected or non-protected <53-µm fraction was hydrolyzed with 25 mL of 6 *N* HCl in a digestion tube in an aluminum block digester at 110 ^{0}C for 18 h with occasional shaking. After cooling, the unhydrolyzed residue was recovered by centrifugation at 2500 rpm and decanting the liquid. The process of centrifugation and decantation was repeated several times with deionized water until neutral pH was reached. The residue was then transferred to a pre-weighed vial, and dried at 60 ^{0}C to constant weight.

Carbon, N, and d^{13}C were analyzed using the method by Harris et al. (2001). Briefly, oven-dried samples were ground and homogenized in a commercial blender to pass through a 150-µm sieve. Four or 40-mg soil samples, depending on the C concentration, were weighed into silver capsules and inorganic carbonate removed by exposure to HCl atmosphere in a desiccator. d^{13}C, %C, and % N were determined using an elemental analyzer (Carlo Erba EA-1108, Lakewood, NJ, USA) interfaced with a Delta Plus isotope ratio mass spectrometer (ThermoFinnigan, Bremen, Germany) operating in the continuous flow mode. Precision for the d^{13}C measurements was < 0.1‰. d^{13}C values were expressed relative to the V-PDB standard (Coplen, 1995).

Statistical Analysis

Analysis of variance and correlationship were conducted using SPSS 11.0 (SPSS, 2001). All differences discussed are significant at the P<0.05 probability level unless otherwise stated. Fisher's protected LSD was calculated only when the ANOVA was significant at $P < 0.05$.

RESULTS AND DISCUSSION

Carbon Mineralization and Curve Fitting

Accumulated C mineralized in 304 days was significantly affected by tillage in both wheat and sorghum systems (Fig. 6.1). Compared to CT, mineralized C with no-till was 40, 40, and 46% greater for CW, SWS, and WS sampled after wheat, and 128 and 70% greater for CS and SWS sampled after sorghum, respectively. Nitrogen fertilization affected C mineralization in wheat and sorghum systems in different ways. In wheat systems, cumulative C mineralized under both CT and NT was significantly greater with than without N fertilization. However, in sorghum systems, N fertilization only increased cumulative C under NT. Increased cropping intensity only increased C mineralization in wheat systems, and the reverse trend was observed for sorghum systems.

The quantity of C mineralized during the 304-day incubation at 25 ^{0}C was about 10% of initial SOC. This amount is greater than that reported by Collins et al. (2000), who used deeper samples (0 to 20 cm) and a shorter incubation (260 days).

Constrained, two-pool exponential decay models adequately described the experimental results (Fig. 6.2). All fitted curves were highly related, regardless of

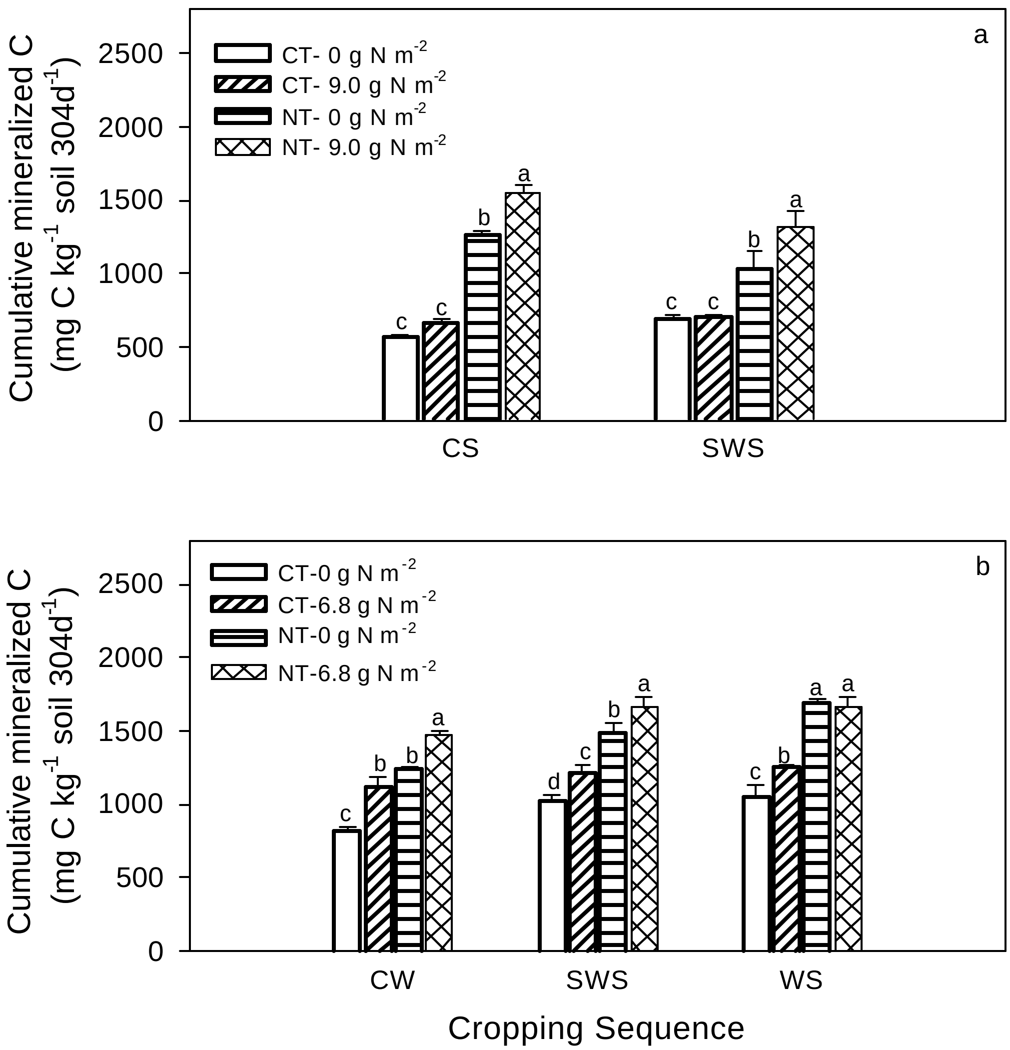

Figure 6.1. Carbon mineralized in 304 days as affected by cropping sequence, N fertilization and tillage for a) soil sampled after sorghum, and b) soil sampled after wheat. CS, CW, SWS, and WS refer to continuous sorghum, continuous wheat, sorghum-wheat-soybean rotation, and wheat-soybean, respectively. CT and NT indicate conventional and no tillage.

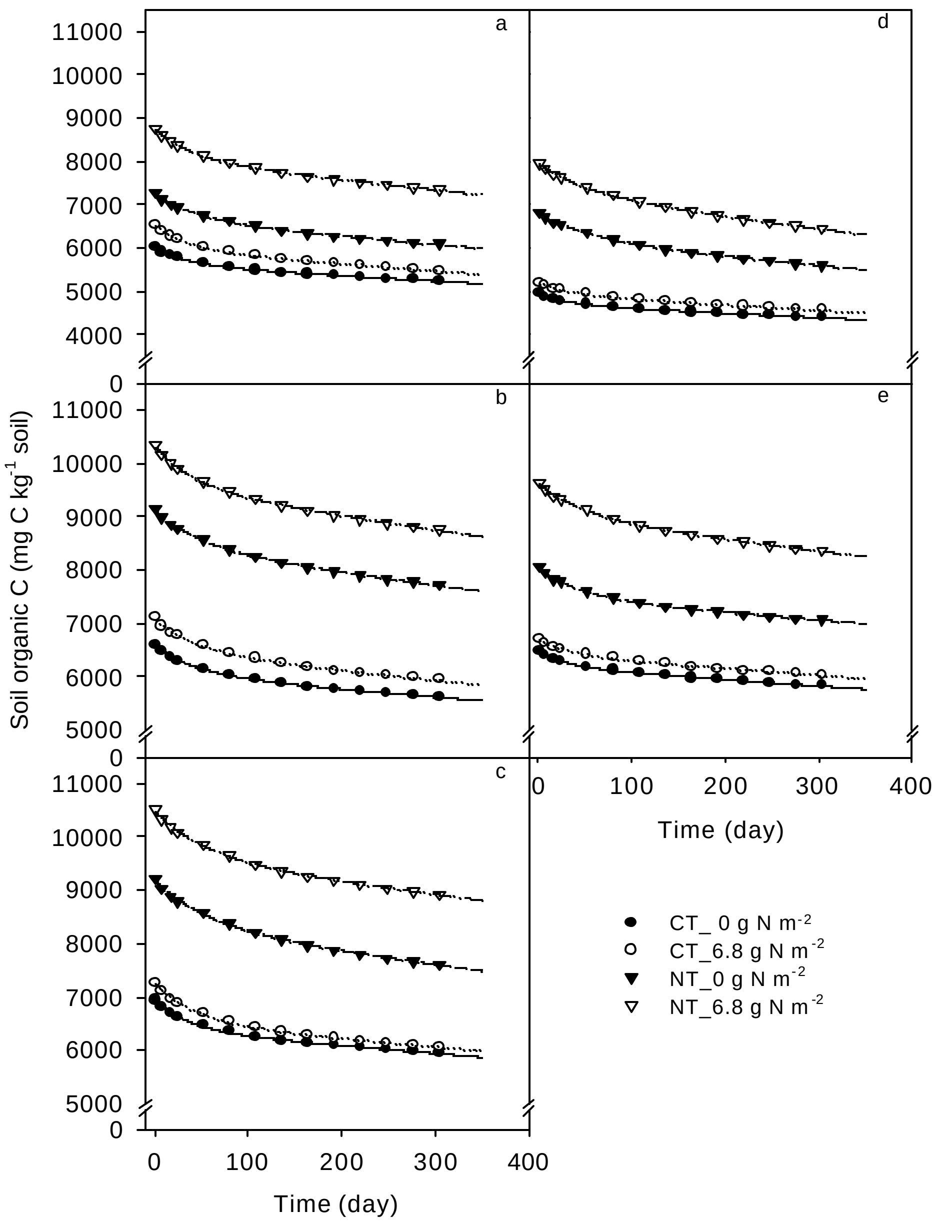

Figure 6.2. Measured (symbols) and modeled (lines) carbon mineralization with time as affected by N fertilization and tillage for a) continuous wheat, b) sorghum-wheat-soybean sampled after wheat, c) wheat-soybean, d) continuous sorghum, and e) sorghum-wheat-soybean sampled after sorghum. CT and NT indicate conventional and no tillage.

treatment (Table 6.1), indicating that under similar incubation conditions, decomposition of SOC in all treatments followed similar patterns.

Active and Slow Pool Sizes of SOM

In wheat management systems, the active pool size of SOC was affected by tillage (Fig. 6.3a). The active pool under NT was 34, 30, and 34% greater in CW, SWS, and WS, respectively, than with CT. Compared with CW, organic C in the active pool was 15 and 32% greater in SWS and WS, respectively, when averaged across tillage treatments, indicating that the active pool size increased with cropping intensity. A previous study (Chapter V) showed that the amount of POM increased with increasing cropping intensity. Particulate organic matter has been reported to represent a labile pool (Gregorich and Janzen, 1996). Therefore, a larger POM pool might also indicate a larger active pool. The active pool size was highly related to POM (Table 6.2), supporting our hypotheses on the relationships between labile pools separated by physical fractionation and active pools estimated by long-term incubation. In addition, N fertilization increased the active SOC pool following wheat (Fig. 6.3a). The proportion of SOC in the active pool was similar for all treatments and ranged from 4 to 5%. This proportion was similar in magnitude to the proportion of SOC as SMBC reported by Collins et al. (2000). These authors also observed that NT increased the proportion of SOC in the active pool.

In contrast to the active pool, a significant interaction between tillage and added N was observed for the slow SOC pool following wheat (Fig. 6.3b). Under no-till management, the slow pool of organic C was 21, 13, and 15% greater with than without

Table 6.1. Correlation matrix of long-term incubation curve fitting data (n = 14).

	CW1	CW2	CW3	CW4	SWS1	SW S2	SW S3	SW S4	WS1	WS2	WS3	WS4	SW S1	SW WS2	SW S3	SW S4	CS1	CS2	CS3	CS4
CW1†	---	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
CW2	1.0	---	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
CW3	1.0	1.0	---	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
CW4	1.0	1.0	1.0	---	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
SWS1‡	1.0	1.0	1.0	1.0	---	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
SWS2	1.0	1.0	1.0	1.0	1.0	---	**	**	**	**	**	**	**	**	**	**	**	**	**	**
SWS3	1.0	1.0	1.0	1.0	1.0	1.0	---	**	**	**	**	**	**	**	**	**	**	**	**	**
SWS4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---	**	**	**	**	**	**	**	**	**	**	**	**
WS1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---	**	**	**	**	**	**	**	**	**	**	**
WS2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---	**	**	**	**	**	**	**	**	**	**
WS3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---	**	**	**	**	**	**	**	**	**
WS4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---	**	**	**	**	**	**	**	**
SWS1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---	**	**	**	**	**	**	**
SWS2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---	**	**	**	**	**	**
SWS3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---	**	**	**	**	**
SWS4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---	**	**	**	**
CS1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	**	**	**	**
CS2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---	**	**
CS3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---	**
CS4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---

†1, 2, 3, 4 refer to conventional tillage (CT) without nitrogen fertilization, CT with 6.8 g N m^{-2}, no tillage (NT) without nitrogen fertilization, and NT with 6.8 g N m^{-2}, respectively. CS, CW, SWS, WS, refer to continuous sorghum, continuous wheat, soybean wheat-sorghum, and wheat soybean, respectively.

‡SWS refers to in sorghum system, otherwise in wheat system.

** denotes significance at P = 0.01.

Table 6.2. Correlation matrix of active and slow organic carbon pools in selected size fractions (n = 12).

	Unprotected POM†	Active Pool C‡	Microaggregate C§	Slow Pool C¶
Unprotected POMC	---	**	**	**
Active Pool C	0.896	---	**	**
Microaggregate C	0.962	0.822	---	**
Slow pool C	0.970	0.864	0.961	---

† Unprotected particulate organic matter C.
‡C in active pool obtained through curve fitting of C mineralization.
§Soil organic C in microaggregates (53 to 250 μm).
¶Slow pool C obtained through curve fitting of C mineralization.
** denotes significance at P = 0.01.

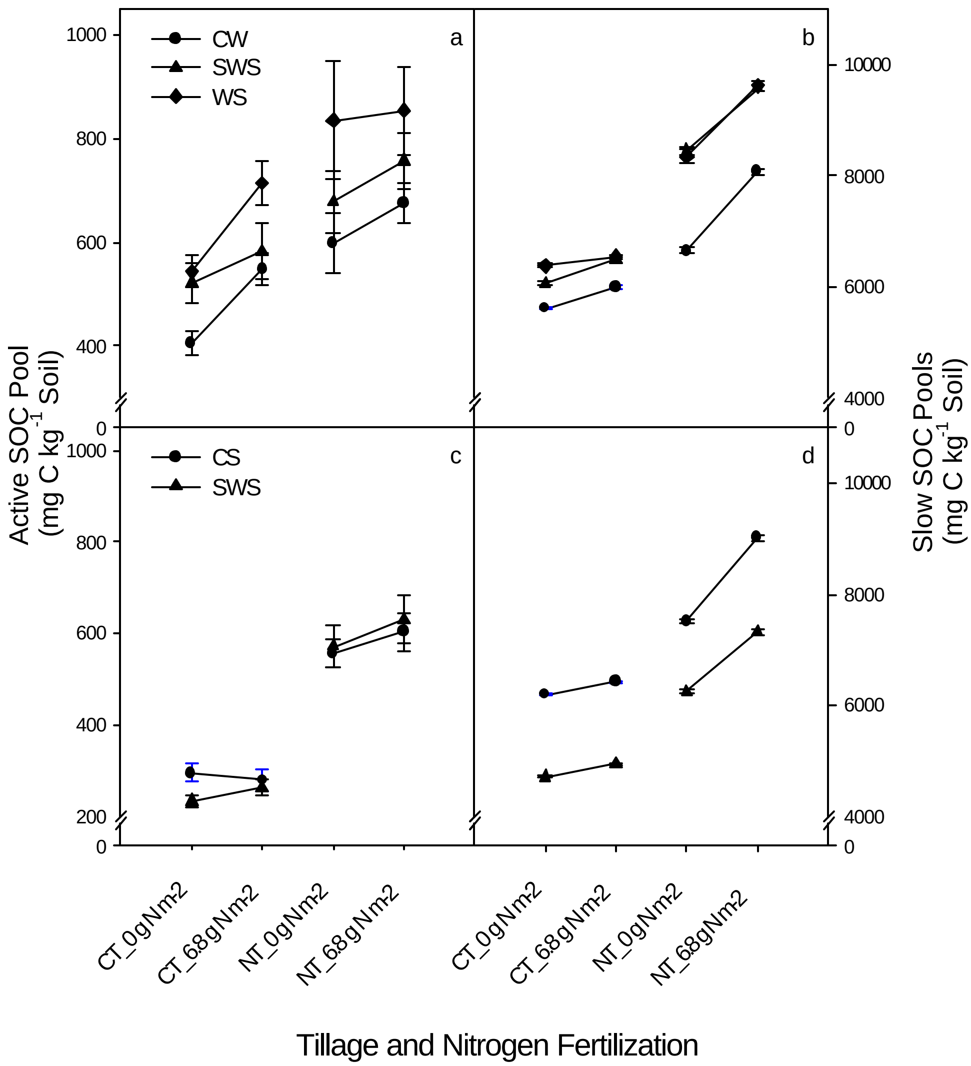

Figure 6.3. Active (a and c) and slow (b and d) pools of soil organic C (SOC) in wheat and sorghum systems as affected by cropping sequence, tillage, and N fertilization. CS, CW, SWS, and WS refer to continuous sorghum, continuous wheat, sorghum-wheat-soybean rotation, and wheat-soybean, respectively. CT and NT indicate conventional and no-tillage. Error bars represent standard errors.

N fertilization in CW, SWS, and WS, respectively. The effect of N fertilization under CT was smaller. Another significant interaction was observed between cropping sequence and tillage for slow pool SOC, with slow pool SOC under NT being 22% greater in both SWS and WS, respectively, compared with CW.

Different patterns for both active and slow SOC pools were observed for sorghum compared to wheat (Figs. 6.3c, d). The effect of cropping intensity on the active SOC pool was minimal. The most significant difference in active pool size resulted from tillage treatment, with the size in the active pool under NT being 43 and 63% greater for CS and SWS, respectively, compared to CT.

Only one significant interaction between tillage and fertilization was observed for the slow pool under sorghum, in that N fertilization increased pool size only under NT. The size of the active pool tended to be smaller under sorghum compared to wheat, but sizes of slow pools were similar for both crops. The slow pool tended to be 10 to 12 times larger than the active pool. The larger slow pool under NT may partly result from slower decomposition of this SOC fraction.

Mean Residence Time (MRT) of Both Active and Slow SOC Pools

Mean residence time of the active SOC pool ranged from 34 to 58 days. This was consistent with the MRT of the active pool reported by Collins et al. (2000). Compared to sorghum systems, the MRT of this active SOC pool was 5% longer in wheat systems. No tillage slowed the turnover of the active pool in both wheat and sorghum systems, but was significant only in the sorghum systems (Figs. 6.4a, c). Cambardella and Elliott (1992) assumed the effect could be due to differences in temperature, moisture, or

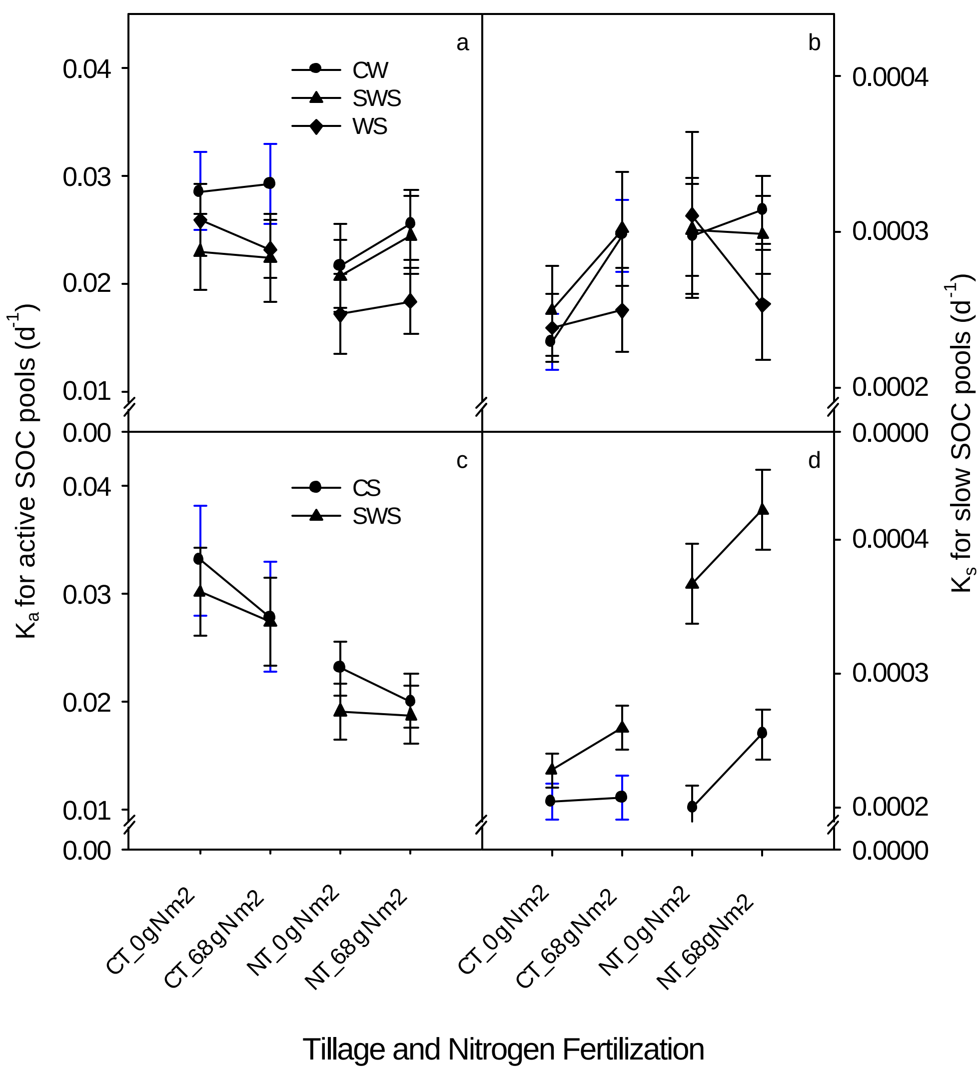

Figure 6.4. Decomposition rates of active (a and c) and slow (b and d) soil organic C (SOC) pools in wheat and sorghum systems as affected by cropping sequence, tillage, and N fertilization. CS, CW, SWS, and WS refer to continuous sorghum, continuous wheat, sorghum-wheat-soybean rotation, and wheat-soybean, respectively. CT and NT indicate conventional and no-tillage. Error bars represent standard errors.

substrate availability between CT and NT, all of which can affect decomposition rates. Since all soil samples were incubated under the same condition in our study, differences might be due to the larger pool size and/or more physical protection by aggregates with NT. Compared to CT, more SOC was protected by aggregates in NT according to our previous findings (Chapter V).

Mean residence time of the slow SOC pool in sorghum systems was 10% longer than in wheat systems. The MRT of the slow SOC pool under CT was 62, 8, 18, 10, and 15% greater in CS, SWS (after sorghum harvesting), CW, SWS (after wheat harvesting), and WS, respectively, than with NT, and averaged 11.3 years with CT and 9.4 years with NT (Fig. 6.4b, d). Our results contrast with those of Collins et al. (2000), where NT increased the MRT of both active and slow pools compared to CT.

One possible explanation for this result is that physically protected organic C in aggregates is normally labile. Using density fractionation and long-term incubation, Swanston et al. (2002) suggested that the recalcitrance of the heavy fraction (1.65 g ml^{-1}) was similar to that of the light fraction and, consequently, differences in their turnover rates may be due to physical protection or microbial accessibility. We observed a significant relationship between the slow SOC pools and unprotected POM-C (Table 6.2). Compared with CT, more C is physically protected or inaccessible to the microbial community under NT. However, after sampling, sieving, oven-drying, and wetting and drying cycles, aggregates, especially macroaggregates, are broken. Moreover, soils under CT were already more disturbed than NT due to tillage. Therefore, there might be less effect due to sample handling on the soil microbial community under CT. Collins et al. (2000) also reported that different pretreatment of soil samples (air-dried vs. no air-drying) could affect the MRT of the slow pool. Cambardella and Elliott (1992) reported that some POM had a half-life of almost 13 yrs and suggested it as a slow organic-matter pool.

In sorghum systems, we observed a significant difference in decomposition rate of the slow pool between CS and SWS under NT across N fertilization (Fig. 6.4d). The mean residence time in SWS was 75% less than in CS under NT, indicating a much shortened MRT with increased cropping intensity under NT. A similar tendency, however, was not observed in wheat systems. Moreover, the difference in SOC between CS and SWS was similar to that between CW and SWS under NT across N fertilization. The reason for these differences is not known.

Natural Abundance of ^{13}C in Size Fractions

The long-term experiment reported herein was initiated in 1982. Specific information on management at the field site prior to that time was not available. Since all treatments are located in close proximity and have the same soil series, we assumed an equal initial ^{13}C natural abundance for all treatments. Thereafter, differences in ^{13}C of soils resulted from different management strategies.

Size and density fractionation clearly described the effects of a plant community shift on the natural abundance of ^{13}C in different size fractions (Table 6.3). In the case of CW, the natural abundance of ^{13}C increased in the order of <53-μm fraction > ROC > microaggregate > POM. Results were similar to these observed by (Boutton et al., 1993), who proposed that organic matter turnover rates in their system appeared to decrease in the sequence sand > silt > clay. Therefore, a similar sequence of turnover rates of organic

Table 6.3. Natural abundance of ^{13}C of physical size fractions as well as resistant organic C (n=4).

Treatments		CS†	CW‡	SWS§	WS¶
FROC#	CT	-19.83a*	-22.89c	-20.96b	-23.33c
	NT	-19.49a	-23.19c	-21.37b	-23.51c
ROC$	CT	-19.38a	-23.29c	-20.78b	-23.97d
	NT	-18.51a	-23.60cd	-21.27b	-23.99c
Free fraction <53 um	CT	-17.80a	-22.94c	-19.83b	-23.39c
	NT	-16.90a	-22.97c	-19.93b	-23.12c
Fraction* <53 um	CT	-17.35a	-23.02c	-19.75b	-23.48c
	NT	-16.31a	-23.19c	-19.84b	-23.42c
Microaggregate	CT	-16.93a	-24.54c	-19.70b	-24.89c
	NT	-15.92a	-24.58c	-19.92b	-24.86c
PPOC%	CT	-15.65a	-28.49e	-20.42b	-28.27e
	NT	-15.32a	-27.10d	-20.29b	-26.51c
UPPOC&	CT	-14.24a	-28.70f	-18.97c	-28.57f
	NT	-14.93a	-27.88e	-20.66d	-27.70e

†Continuous sorghum.
‡Continuous wheat.
§Sorghum-wheat-soybean rotation.
¶Continuous wheat-soybean.
#Resistant organic carbon in <53-µm free fraction.
$Resistant organic carbon in <53-µm fraction from microaggregates.
%Protected particulate organic C (53 to 250 µm).
&Unprotected particular organic C (>250 µm).
*Means within each estimator across tillage treatment and cropping sequence followed by the same letter are not significantly different at P<0.05 (Fishers' protected LSD).

matter in different-size pools can be inferred. Compared to ^{13}C of the protected <53-µm fraction in CW, the ^{13}C value of the non-protected <53-µm fraction was less negative. Balesdent and Balanane (1992) observed an enrichment of ^{13}C associated with soil organic matter cropped to maize. In addition, using physical fractionation techniques, Golchin et al. (1997) proposed a conceptual model for the relationship between microaggregate-POM and its stability. They suggested that the ability of microaggregate-POM to form stable associations with soil mineral particles was related to the extent of POM decomposition. Therefore, as POM decomposition proceeds within microaggregates, the more labile portions of POM, such as protein and carbohydrates, are consumed by the decomposers leaving a POM core of organic matter which is biologically more recalcitrant. The latter was also consistent with differences in ^{13}C values of ROC between the protected and non-protected <53-µm fractions. The ROC in the protected <53-µm fraction in CW had lower ^{13}C values than that of the non-protected <53-µm free fraction. Lignin and some lipids are thought to be main components of resistant organic C. The ^{13}C values of lignin and lipids, however, are usually lower than the intact plant from which they are extracted (Balesdent and Mariotti, 1996; Boutton, 1996). Due to similar ^{13}C values of soybean compared to wheat, the same distribution was observed in the WS treatment.

In contrast with CW, a different pattern was observed for CS (Table 6.3). As mentioned before, we do not know the background information for this site prior to the long-term experiment. However, we may infer that the vegetation prior to this experiment was dominated by C_3 species with a similar ^{13}C value as wheat or soybean because sorghum is a C_4 crop and all ^{13}C values for CS increased compared to CW or WS in all size fractions. Therefore, the pattern of ^{13}C in size fractions in CS also could be explained with the same model of organic matter turnover as in the wheat system. Because POM is usually labile organic matter and has a short MRT (several months), this fraction was also most affected by wheat or sorghum residue input. The less the various fractions were affected by sorghum residue input, the more negative were the ^{13}C values observed. Results indicated that a greater fraction of SOC in the <53-μm fraction was derived from C_3 species than in the light fraction. Even in the case of POM, higher ^{13}C values of unprotected POM compared to protected POM were observed (Table 6.3). These results indicated that a portion of recent crop residues were also incorporated into more recalcitrant fractions, but a greater proportion was associated with more labile fractions.

A combined effect of C_3 and C_4 crops was observed in SWS. For labile fractions such as PPOM or UPPOM, ^{13}C values appeared to be more affected by the current crop (wheat). The ^{13}C value of ROC also appeared to reflect more effect from wheat or soybean than sorghum.

Tillage effects on ^{13}C values were observed primarily in the labile pool (Table 6.3). Compared to CT, greater ^{13}C values were observed in POM under NT, except in UPPOM in CS and in SWS. Different tillage treatments may affect the photosynthesis of crops. Fu et al. (1993) reported that soil water availability affected the ^{13}C values of plants. Soil moisture was higher under NT than CT in related studies (Franzluebbers et al., 1995b; Grant et al., 1997).

Compared with tillage, effects of N fertilization on ^{13}C values of POM were smaller (data not shown). Nitrogen application in wheat systems resulted in more negative ^{13}C values than without N, except UPPOM in SWS under NT. In contrast to wheat systems, the reverse pattern was observed in sorghum systems. Fu et al. (1993) also reported N effects on ^{13}C values similar to our result with CS. These authors also reported a significant N x species interaction. In contrast, several studies observed no effect of leaf N concentration on d ^{13}C (Hubick, 1990; White et al., 1990).

CONCLUSIONS

Tillage, cropping intensity, and N fertilization affected 1) sizes of active and slow pools of SOC as well as turnover rates, and 2) natural abundance of ^{13}C in different physical size fractions. The general significance of different treatments was in the order: tillage > cropping sequence > N fertilization. Four to 5% of SOC was in active pools with MRT around 50 days, 50% of SOC was in slow pools with an average MRT of 12 years, and the remainder was in the resistant pool with an assumed MRT of over 500 years. No tillage significantly increased both active and slow pool sizes of SOC compared to CT. Mean residence time was not significantly affected by tillage in wheat systems. Difference in ^{13}C concentration of crop residues significantly affected ^{13}C concentration of SOC in all size fractions, with greater differences in labile pools. Carbon turnover rates increased in the sequence: ROC < silt- and clay-associated C < microaggregate-C < POMC. Surprisingly, active and slow pool-C, POM, silt- and clay-associated C were highly related. Stable isotopic tracers, plus physical fractionation, improved our understanding of SOC dynamics.

CHAPTER VII

SUMMARY AND GENERAL CONCLUSIONS

Concerns about the effect of increasing concentrations of greenhouse gases in the atmosphere on global climate have increased research into the soil carbon (C) cycle with a focus on the potential for increasing organic C sequestration (Lal et al. 2004). Compared to other ecosystems, agroecosystems are highly manipulated. Therefore, best management strategies may potentially increase C storage in this ecosystem. This research dealt with quantifying labile and slow soil C and N pools as well as C turnover under CT and NT in different cropping sequences with and without N fertilizer addition.

Crop stover production was differentially affected by tillage, cropping sequence, and N fertilization for wheat and sorghum. Nitrogen addition significantly increased wheat stover yield regardless of tillage. NT also increased wheat stover yield compared to CT. No significant difference from N fertilization was observed for yield of sorghum stover. In contrast to wheat, NT also slightly decreased sorghum stover yield compared to CT.

Soil organic C (SOC) was highly affected by tillage, cropping sequence, and N fertilization in wheat systems. Two significant interactions in surface soil samples affecting SOC were tillage by N fertilization and tillage by cropping sequence. Under NT, SOC was significantly higher with than without N fertilization. The difference was insignificant under CT when averaged across all cropping sequences. Enhanced cropping intensity [wheat-sorghum/soybean (SWS) and wheat-soybean (WS)] significantly increased SOC under NT, but no such result was observed for CT. In general, SOC decreased with depth. SOC level is a balance of net input and net output. The greater input of plant residues with enhanced cropping intensity and slower decomposition under NT may explain higher SOC under NT than CT in wheat systems. However, the significant interaction between tillage and N addition for SOC could not be completely explained by differences in net input. Therefore, the decomposition rate of plant residue may be different under NT and CT. Significant interaction between tillage and N addition was also observed in the sorghum systems, although no significant difference in plant residue input was observed due to tillage treatment. This result may indicate that the magnitude of soil protection of residue input was greater in NT than the net input of plant residues. Similar results were also observed in soybean systems. Due to being coupled with SOC, TN usually mirrored trends of SOC as affected by all management practices. The C:N ratio of SOM under CT was greater than with NT through all soil depths in all crop systems. Differences, however, were only significant in surface soil.

Carbon sequestration rates in this study using the equation suggested by West and Post (2002) were negative compared with data collected in 1992, except in sorghum systems with N addition. Nitrogen addition tended to reduce the negative trend. One reason for negative sequestration rates was a decrease over time in the difference of SOC between CT and NT. Our results suggested that soil has a finite capability to protect or sequester SOC.

Soil microbial biomass (SMB) was more affected by tillage than by cropping intensity or N fertilization in all cropping systems. For example, in wheat systems, SMBC under NT was 18, 25, and 13% greater in CW, SWS, and WS, respectively, than for CT in surface soil. Lower SMBC under NT than CT was observed at a depth of 5 to 15 cm. At 15- to 30-cm depth, however, there was no consistent change between CT and

NT. Nitrogen fertilization also increased SMBC in all cropping systems, although it was not significant. Crop species also affected SMBC. Compared with continuous soybean, SMBC was greater for continuous wheat and sorghum regardless of tillage. The effect of tillage on the proportion of SOC as SMBC varied with cropping system. Unlike SOC or SMBC, this ratio changed minimally with depth. The proportion of SOC as SMBC in our study was about 5 to 8% for all depths. However, the proportion of SMBC as mineralizable C decreased with depth. Soil microbial biomass N as expected was highly related to SMBC and other SOC pools in wheat, sorghum, and soybean systems. Compared to SOC or SMBC, soil microbial biomass N decreased with soil depths faster in all studied crop systems.

Labile C pools showed similar patterns for sorghum, soybean, and wheat systems. SMBC, mineralizable-C, POM-C, and hydrolyzable C were significantly and positively correlated with each other and SOC. On average, SMBC was 5% of SOC, mineralizable C in a 24-day incubation was 3% of SOC, POM-C was 35% of SOC, and hydrolyzable C was 45% of SOC in this study. Although labile SOC pools increased as SOC increased, they exhibited significant differences in rates of increase. In addition, sensitivity of different labile C pools to change in SOC varied with CT or NT. Under CT, SMBC and hydrolyzable C were more affected by change of SOC; however, POM-C was more affected under NT.

Slow and resistant organic C pools were also highly correlated with SOC, and thus showed similar trends as affected by management practices in wheat, sorghum, and soybean systems. However, the proportion of SOC as mineral-associated C ranged from 0.61 to 0.95, and increased with soil depth, indicating that more C with depth occurred in stable form. Similar patterns were also observed for ROC as evidenced by high correlation ($r^2 = 0.85$) between mineral-associated and ROC pools, although the proportion of SOC as ROC decreased with depth.

Physical fractionation helped identify mechanisms of C storage under NT. A significant interaction between tillage and N fertilization was observed for protected particulate organic matter C (PPOM-C). PPOM-C under NT was significantly greater with than without N fertilization, except for WS. The proportion of SOC as PPOM-C was significantly greater under NT than CT. A similar result was observed for free POM-C. Carbon concentration for mineral-associated organic matter under NT was greater than CT regardless of protection. The proportion of mineral-associated C was greater under CT than NT, with values ranging from 23 to 39%. Similar trends were also observed in ROC. These findings indicated that NT stores more C not only through physical protection such as in macroaggregates and microaggregates, but also because changed environmental factors reduce decomposition.

These hypotheses were further supported by natural ^{13}C abundance determination. Tillage effects on ^{13}C values were observed primarily in the labile pool. Compared to CT, more positive ^{13}C values were observed in POM under NT, except in UPPOM in CS and in SWS. In the case of CW, the natural abundance of ^{13}C increased in the order: <53-µm fraction > ROC > microaggregate > POM. These results suggested that SOM decomposition increased with decreased particle size. More negative ^{13}C value of OM in protected than unprotected < 53-µm fraction may be similarly explained.

Long-term incubation results were well described by constrained two-pool exponential decay models. Both active and slow C pool sizes of SOC were estimated to be greater under NT in wheat and sorghum systems. Mean residence time (MRT) of the active C pool was longer under NT and ranged from 34 to 58 days. However, MRT for slow C pool was longer under CT than NT with a range from 9.4 to 11.3 years. These results may be partially explained by soil pretreatment such as being oven-dried as well as ground. Disturbed soil samples could release physically protected substrates, and thus accelerate their decomposition. However, the active C pool refers to substrate which is readily accessible to decomposers.

In summary, agricultural management practices such as NT, enhanced cropping intensity, and N fertilization increased soil C storage in both labile and slow pools, indicating that the studied agroecosystem could serve as a sink for sequestration of organic C. The potential for increased sequestration, however, appeared to be less than 20 years for NT in this soil and environment.

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